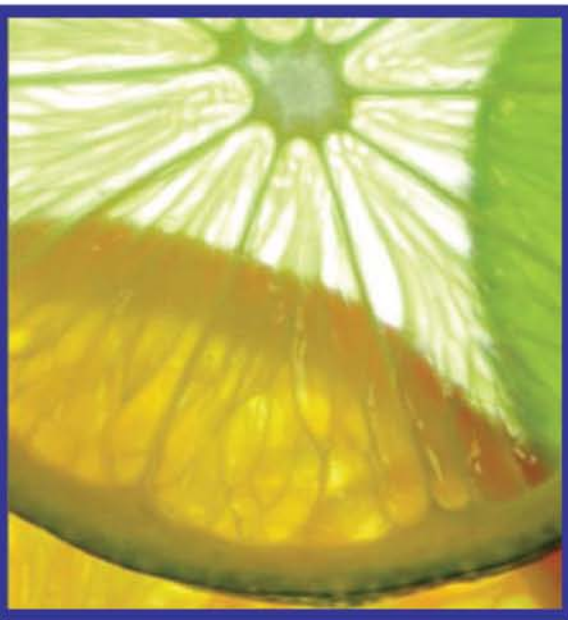
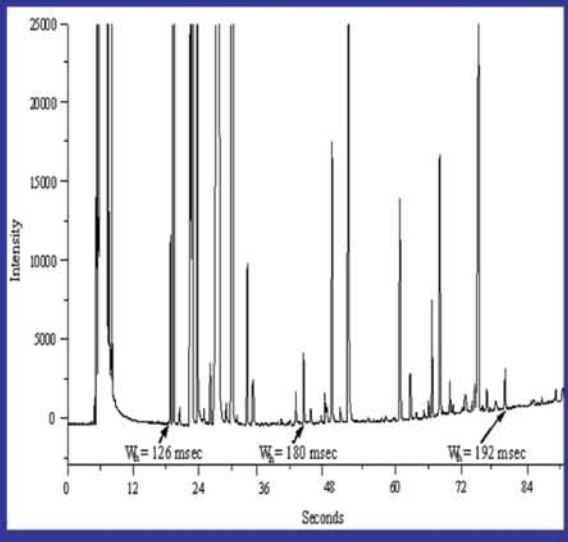
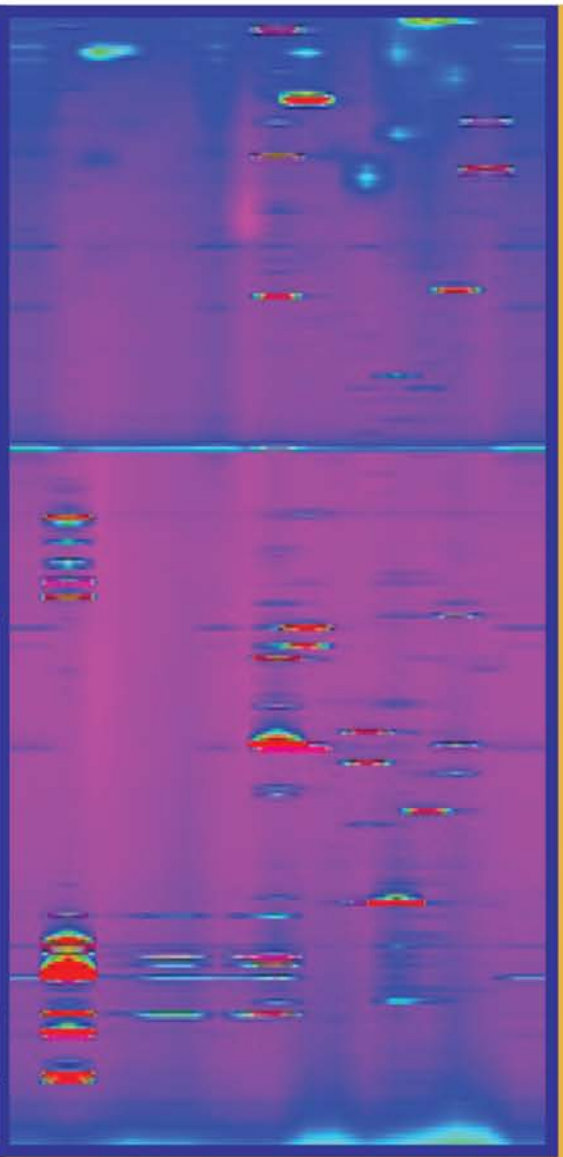
Medicinal and Aromatic Plants — Industrial Profiles

Citrus Oils

Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity







Edited by Giovanni Dugo and Luigi Mondello

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Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity

Medicinal and Aromatic Plants — Industrial Profiles

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Citrus Oils, edited by Giovanni Dugo and Luigi Mondello

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To the wonderful women of my family

To my grandmother, Francesca, who cheered and protected me. To my mother, Paola, who was always excessively proud of my limited success. To my wife, Anna, who loved and assisted me, always indulgent and understanding. To my daughters, Paola, Monica, and Laura, who showed to me how lucky a father can be. To the sweetest daughters of my daughters, Alice, Viola, and Laura, who, more than anything else, give a sense to my life and sweeten my old age.

Giovanni Dugo

To my wife, Paola, and to my children, Alice and Viola,

for their understanding and patience while I spent seemingly endless evenings and weekends working in my research laboratory.

To my parents

for their love and for believing in me and encouraging me in my career.

Luigi Mondello

Contents

Series Preface

There is increasing interest in industry, academia, and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information that is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved, such as forestry, agriculture, chemical, food, flavor, beverage, pharmaceutical, cosmetic, and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits, and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts, and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. A dealer's market report for an item may say "drought in the country of origin has forced up prices."

Natural products do not mean safe products, and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants that are approved for use in medicine must not be used in cosmetic products.

The assessment of "safe to use" starts with the harvested plant material, which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large-scale, contracted, mechanized cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but also for the plant's ability to overcome disease, climatic stress, and the hazards caused by mankind. Such methods as in vitro fertilization, meristem cultures, and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some end uses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically farmed medicinal plants, herbs, and spices. The Economic Union directive CVO/EU No. 2092/91 details the specifications for the obligatory quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology lead to medicinal potential. Examples are the muscle relaxants based on the arrow poison curare from species of *Chondrodendron*, and the antimalarials derived from species of *Cinchona* and *Artemisia*. The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay-linked fractionation of crude plant juices or extracts, compounds can be specifically targeted, which, for example, inhibit blood platelet aggregation, or have antitumor, antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be fully authenticated by a specialist.

The medicinal traditions of ancient civilizations such as those of China and India have a large armamentarium of plants in their pharmacopoeias that are used throughout southeast Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the world's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practice. It is noticeable that medical, pharmacy, and health-related schools throughout Europe and the United States are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamored of the single compound, magic-bullet cure. The high costs of such ventures and the endless competition from "me-too" compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, 11 (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western world.

The business of dietary supplements in the Western world has expanded from the health store to the pharmacy. Alternative medicine includes plant-based products. Appropriate measures to ensure their quality, safety, and efficacy either already exist or are being answered by greater legislative control by such bodies as the US Food and Drug Administration and the recently created European Agency for the Evaluation of Medicinal Products based in London.

In the United States, the Dietary Supplement and Health Education Act of 1994 recognized the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine, which in 1994 assisted the filing of several Investigational New Drug (IND) applications required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a *single* IND. A demonstration of the contribution to efficacy of each ingredient of each plant was not required. This was a major step toward more sensible regulations in regard to phytomedicines.

The citrus oils have a large industrial profile in beverages, household products, perfumes, medicines, and so forth. According to David A. Moyler, technical director of Fuerst Day Lawson (FDL) Ltd, London, UK, from November 2010 citrus oils, such as orange, lemon, and lime will come under a massive piece of European Union legislation called REACH covering their Registration, Evaluation, Authentication, and CHemical data. This legislation will apply to all companies handling more than 1000 tons of citrus oils per year. In 2013, this will apply to companies handling more than 100 tons of these oils and in 2018 the figure will drop to those companies handling 1 tonne. So this volume, Volume 49 and its accompanying earlier one, *Citrus: The Genus Citrus,* Volume 26, edited by Giovanni Dugo and Angelo Di Giacomo, are most relevant.

For Volume 49, I thank its editors Giovanni Dugo and Luigi Mondello for their dedicated work and the chapter contributors for their authoritative information. My thanks are also due to Barbara Norwitz, executive editor, life sciences for CRC Press and her staff for their unfailing help.

Roland Hardman, Bpharm, BSc (Chem), PhD (University of London), FRPharmS

Preface

This book follows a previous volume that deals with the historical, botanical, agronomical, technological, chemical–analytical, and biological–pharmacological aspects of citrus or their derivatives, mainly essential oils.

Not all of the subjects treated in the first volume are included here. In fact, we present those topics that have evolved in the last decade, such as composition of essential oils, including possible contaminants of different origin (agriculture, environment, and industry), and development of the analytical techniques applied in these fields. The last chapter is dedicated to the new information available on the pharmacological properties of citrus essential oils and their components.

Many of the authors who participated in the first book contributed again to this volume. Other scientists of great fame and recognized competence in this field and some young researchers whose scientific interest also focuses on the chemistry of citrus have also contributed to this volume.

The chapters relative to the different compositional aspects of the oils first briefly report for each oil the information given in the former book summarized in tables, and then the more recent information is discussed in detail. Concentrated oils and the composition of oils obtained from minor citrus species are discussed here in a new format compared to the previous book; two new aspects, the minor components of citrus essential oils and the carotenoid fraction, are also discussed in this book. The chapter on the adulteration of citrus essential oil, present in the former volume, is not included in this new book for the following reasons: the general and specific information on this topic were brilliantly treated in the former volume, and are still valid; to avoid the reiteration of information given in chapters focused on the composition of the volatile fraction, on the oxygen heterocyclic compounds, and on the chiral components of volatiles in citrus essential oils; the information given in these chapters can be sufficient for researchers and operators in this field to recognize contaminations and/or adulterations.

We hope that the results of our work together with that of the contributors to this book will be appreciated and will be useful to most of those who work in the fascinating field of citrus.

Editors

Giovanni Dugo is currently full professor of food chemistry at the University of Messina, Italy. His research activity is directed toward the development of innovative methods and toward the study of food matrices by using innovative methodologies such as multidimensional liquid chromatography (comprehensive LC), multidimensional gas chromatography (MDGC and comprehensive GC), ultrafast-GC and ultrafast-GC/MS, on-line SPME-GC/MS, micro-HPLC and micro-HPLC/API/MS, multidimensional HPLC and micro-HPLC, superheated HPLC, LC × GC; method validation by using pure standard compounds and complex food samples; exploitation of the developed methods for the study of food matrices such as essential oils, fruit juices of citrus and noncitrus origin, food lipids, wines, coffee, cheese, vegetable products; study of the following classes of compounds contained in the previously reported food products: triglycerides, fatty acids, sterols, tocopherols, monoterpenes, sesquiterpenes, coumarines, psoralens, polymethoxyflavones, carotenoids, anthocyanins, and other flavonoid-structured compounds, pesticides, paraffins, aromatic hydrocarbons, pyrazines, and so on; and the correlation of the results attained with the genuineness, quality, and the nutritional characteristics of the studied food samples.

Prof. Dugo's scientific activity, focused mainly on the development of separation methods and on the analysis of complex matrices, is reported in about 300 national and international papers; approximately 300 congress presentations; several scientific books and encyclopedia chapters; one book (as editor and author of some chapters) on the chemistry and technology of citrus products, and one on food toxicology.

Prof. Dugo has participated, chaired, and coordinated numerous committee and congress organization activities and research projects on food chemistry, advanced analytical techniques, and aromatic plants and citrus chemistry and technology. In 2005 he founded The Mediterranean Separation Science Foundation Research and Training Center in Messina co-chaired with Prof. Mondello. He was the director of the PhD school on Food Chemistry and Safety, University of Messina, from 2002 to 2004. Prof. Dugo is the coordinator of the Food Chemistry Group of the Italian Chemistry Society (SCI) and is a member of the board of *Journal Essential Oil Research*.

Prof. Dugo also held the following academic positions: vice-rector of the University of Messina and president of the evaluation board "Nucleo di Valutazione" of the University of Messina from 1995 to 1998; and vice-rector and delegate for the Scientific Research Activity of the same university from 2003 to 2007.

Prof. Dugo received the medals awarded by the Food Science Italian Society and the Flavor Science Italian Society; recently (2009) he was awarded the Liberti Medal by the Italian Society of Chemistry (SCI) for his contribution to the diffusion of science.

Luigi Mondello is full professor of analytical chemistry at the University of Messina, Italy and teaches the same course at the "Campus Biomedico" in Rome. He is the author of 200 scientific papers, 29 book chapters and 2 reviews; he is the co-editor of *Multidimensional Chromatography* (John Wiley & Sons); and he has been chairman and invited lecturer in national and international congresses and meetings. His research interests include chromatography techniques (HRGC, HPLC, HRGC/MS, HPLC/MS, OPLC) and the development of coupled techniques, such as LC–GC–MS, GC–GC, GC × GC, LC × LC, LC × GC, and their applications in the study of natural complex matrices in the fields of food, environmental, and biochemical science. Prof. Mondello has been a member of the organizing committees of national and international meetings. He is a permanent member of the scientific committee of the International Symposium on Capillary Chromatography

(ISCC), of the International Symposium on Essential Oils (ISEO), of the International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analyzers (HTC), of the Brazilian Symposium on Chromatography and Related Techniques (SIMCRO), of the Congresso Latino-Americano de Cromatografia e Técnicas Relacionades (COLACRO), a member of the Scientific Committee of the Workshop-Symposium on Analytical and Preparative Enantioseparation (Enantiosep '07). He has also been a member of the Scientific Committee of the 1st International Symposium on Separation Sciences, Pardubice, Czech Republic; co-chairman of the 34th International Symposium on Capillary Chromatography, Riva del Garda, Italy; member of the Scientific Committee of the 16th International Symposium on Separation Sciences, Rome, Italy; member of the Scientific Committee of the ExTEch on Advance in Extraction Technologies, Poznan, Poland; and the chairman of the 20th International Symposium on Solid Phase Microextraction; member of the steering committee of the Italian Separation Science Group of the Italian Chemical Society; member of the expert team of "Chromedia" (Chromatography Knowledge Base); editor of the *Journal of Separation Science* and *Flavour and Fragrance Journal,* both published by John Wiley & Sons; member of the Central Technical Committee of the National System for the Accreditation of the Laboratory (SINAL); member of the Advisory Board of LC-GC, Europe, Separation Science e Scientia Chromatographica; and reviewer for 42 different journals in the field of analytical chemistry and food chemistry. In February 2006 (York, U.K.), Prof. Mondello was awarded with the HTC-Award for the most outstanding and innovative work in the field of hyphenated chromatographic techniques by the Flemish Chemical Society. In May 2008 (Riva del Garda, Italy), he was awarded with the Silver Jubilee Medal for his considerable contribution to the development of separation sciences by the Chromatographic Society. In October 2008, during the Congresso Latino-Americano de Cromatografia e Técnicas Relacionades held in Florianòpolis, Brasil, the Instituto Internacional de Cromatografia awarded Prof. Mondello the COLACRO Medal for his contribution to the development and diffusion of chromatographic techniques.

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1 Composition of the Volatile Fraction of Citrus Peel Oils

Giovanni Dugo, Antonella Cotroneo,
Ivana Bonaccorsi, Alessandra Trozzi

CONTENTS

1.1 INTRODUCTION

Citrus essential oils are industrially cold extracted from the peels of sweet orange, lemon, mandarin, tangerine, grapefruit, Key lime, Persian lime, bitter orange, bergamot, and clementine using mechanical systems. Lime essential oils, however, are most commonly obtained by distillation (Guenther, 1949).

The cold-extraction process consists of three fundamental steps, regardless of the technology used:

- Mechanical action on the peel breaks the utricles and releases the oil.
- The oil is carried by streams of water, which in most cases is recycled.
- Separation, by centrifugation, of the essential oil from the aqueous emulsion.

The industrial transformation process of citrus fruit has been described in detail by Di Giacomo (2002a,b), Di Giacomo and Di Giacomo (2002), and Crupi and Rispoli (2002).

The volatile fraction of citrus essential oils ranges between 85% in Key lime oils and 99% in some sweet orange oils (Di Giacomo and Mincione, 1994). This fraction mostly consists of mono- and sesquiterpene hydrocarbons and their oxygenated derivatives, that is, alcohols, aldehydes, esters, ethers, and oxides, and also of linear hydrocarbons, alcohols, aldehydes, esters, and acids, and of phenolic compounds and their derivatives. In some cases, for example, mandarin, nitrogen esters such as methyl *N*-methyl anthranilate are present. These compounds contribute to the characteristic olfactory note of the oil (Wilson and Shaw, 1981). In citrus essential oil, volatile fractions also present trace amounts of heterocyclic nitrogen–containing components (e.g., pyrimidines and pyrazines) (Thomas and Bassols, 1992; Naef and Velluz, 2001) and sulfur-containing components (Demole et al., 1982). These play a very important role, contributing to the odor character of the oil. An important role for the olfactory notes is also played by unsaturated aliphatic hydrocarbons, aldehydes, and alcohols, which are mostly present at trace levels (Naef and Velluz, 2001).

Studies on the volatile fraction of citrus essential oils began as far back as the early nineteenth century, but it was only in the 1900s that any reliable results were obtained. The reference list is reported by Guenther (1949). Research in those days, using pioneer techniques, allowed to identify components that were mostly confirmed later on.

It was the advent of gas chromatography (GC), first on packed columns and then on capillary columns made first of stainless steel then glass, and finally of fused silica, that allowed the study of the composition of complex mixtures of volatile components, which is what the essential oils are. The

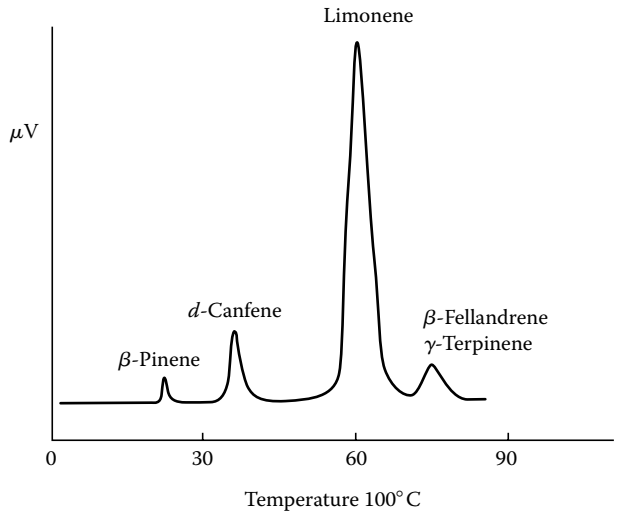

FIGURE 1.1 Gas chromatogram of a lemon essential oil. Column: tricresyl phosphate on celite; column temperature 100°C. (From Liberti, A., and Conte, G., 1956. Possibilita di applicazione della cromatografi a in fase gassosa allo studio delle essenze. *Atti I° Congresso Internazionale di Studi e Ricerche sulle Essenze*, Reggio Calabria, Italy, Marzo 1956.)

fascinating relationship between GC and citrus essential oils (a symbol of the southern Italian agro-industrial economy) was first studied in Messina, Sicily, in the mid-1950s, owing to the intuitive work of Professor Arnaldo Liberti. He was the first to apply the newly developed technique of GC, described only a few years before by James and Martin (1952), to research in this field. Professor Liberti presented the first chromatograms relative to bergamot and lemon oils in 1956 during the First International Congress on Essential Oils held in Reggio Calabria (Liberti and Conte, 1956). In these chromatograms four and five peaks were reported. Figure 1.1 shows the chromatogram of lemon oil obtained by Liberti and Conte (1956). Since then, the use of single-column GC in combination with flame ionization and mass selective detection has enabled the qualitative/quantitative determination of many citrus essential oil volatiles.

Using GC, Bernhard (1957) analyzed five monoterpene hydrocarbons standards occurring in cold-pressed lemon oil. Later, Bernhard (1958) separated five peaks in Californian lemon oil. Only two years later, the 5 peaks separated by Bernhard became 22, some of which were representative of more than one component. However, the 22 different compounds were fully or tentatively identified (Bernhard, 1960).

The preliminary gas chromatographic results obtained gave the information on the complexity of the essential oils and at the same time indicated the need for some kind of fractionation prior to the gas chromatographic analysis. In those days, fractionation was particularly necessary, given the limited efficiency of the columns used.

By separating on silica gel columns the hydrocarbons from the oxygenated compounds, Clark and Bernhard (1960) found numerous components in lemon oil, and Bernhard (1961) underlined the presence of about 50 components in sweet orange oil, most of which were also identified.

At the same time, in Great Britain, Slater (1961) reported the presence of 7 monoterpene hydrocarbons, 9 sesquiterpene hydrocarbons, and 24 oxygenated components in lemon and lime oils. A few years later, Kovats (1963) and Kugler and Kovats (1963) reported more than 100 components in lime and mandarin oils. Of these, 48 and 44 components were respectively identified.

In the same year Calvarano (1959) and Di Giacomo et al. (1962), in Italy, started research on the composition of citrus essential oils. Di Giacomo and Rispoli (1962) proposed using GC analysis to differentiate lemon oils extracted by the sponge method from those extracted by mechanical systems.

Since the 1960s, the capillary columns have been introduced for the analysis of essential oils (McFadden et al., 1963; Teranishi et al., 1963; MacLeod et al., 1966; Goretti et al., 1967; Di Giacomo et al., 1971). These columns have gradually replaced packed columns. Figures 1.2 and 1.3 show the chromatograms of lemon oil obtained on stainless steel capillary column by MacLeod et al. (1966) and on fused silica capillary column obtained by Dugo et al. (1999).

The availability of the bench-top mass spectrometer, coupled online with high-resolution gas chromatographs, allowed the identification of numerous minor components in citrus essential

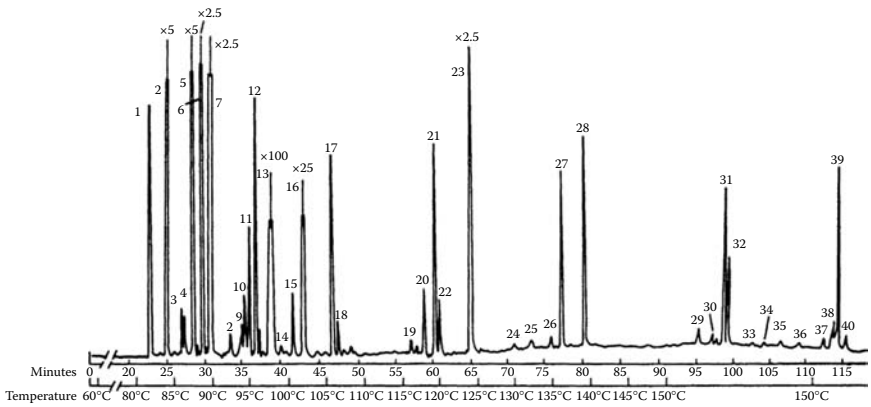

FIGURE 1.2 Gas chromatogram of lemon oil using a stainless steel capillary column. (From MacLeod, W.D., et al., *J. Food. Sc*i. 31, 591–594, 1966.)

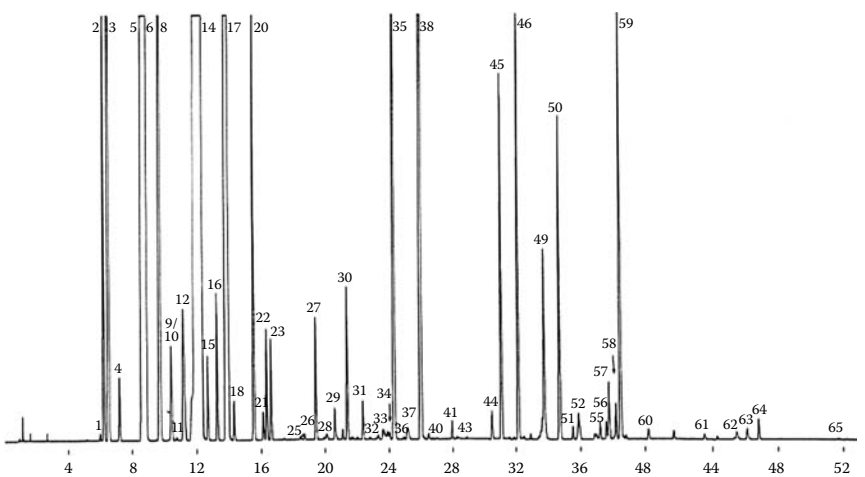

FIGURE 1.3 Gas chromatogram of a cold-pressed lemon essential oil. Column: fused silica capillary column (30 m × 0.25 mm × 0.25 μm), coated with SE-52; column temperature 45°C (6 min) to 200°C at 3°C/min. (From Dugo, G., et al., *Essenz. Deriv. Agrum*. 69, 79111, 1999.)

oils (Mazza, 1986, 1987a,b). It must be noted, however, that when identifying the components of a complex matrix, such as essential oils, it is not always possible to compare the commercially available mass spectra library with the experimental GC-MS data. The spectra interpretation could be confusing due to peak overlapping and due to the structural similarity of many of the components, particularly for those present at trace levels. To obtain more reliable information, mass data along with chromatographic retention data, such as linear retention indices (LRI), determined on two columns, one polar and one non-polar (Mondello et al., 1995a), were used. One application reported by these authors on bergamot essential oil proved that the identification of neryl acetate and α-bisabolol using GC/MS equipped with commercial library could yield unreliable results, while the use of LRI as interactive filter would simplify the identification of these components. A more recent application relative to the analysis of lemon oil (Mondello et al., 2004a) showed that univocal identification of the volatile fraction components can be achieved by the interactive use of LRI and the mass spectral data given by a conventional MS detector, even if ultrafast gas chromatography is applied. In this research, in fact, the entire volatile fraction was separated in 140 seconds.

Sebastiani et al. (1983), after testing five columns with different stationary phases (OV-1, SE-52, OV-17, UCON, and Carbowax) for the separation of lemon essential oil concluded that, regardless of the efficiency of the chromatographic system used, it was impossible to obtain a complete resolution of all the components of the essential oil. In particular, on the polysiloxane stationary phases, such as SE-52, some complete- or partial-peak overlaps were observed between octanal and α-phellandrene; 1,8-cineole and limonene; and nerol and citronellol. With polar polyglycol stationary phases, coelution was observed between monoterpene alcohols and esters, and sesquiterpene hydrocarbons. Therefore, in order to obtain satisfactory information on the composition of essential oils, it would be preferable to perform two different GC analyses, each on a different stationary phase, or to preseparate the oil, obtaining different fractions that are simpler in composition and avoiding peak coelution.

Off-line separation performed by open-column liquid chromatography prior to the GC analysis has been largely applied. Chamblee et al. (1985), by high performance liquid chromatography (HPLC), separated lime essential oil on three different silica columns in tandem, using as mobile phase an 8% ethyl-acetate solution in hexane in a ratio of 1:1 with methylene chloride. The separation allowed obtaining 18 peaks where the hydrocarbons were concentrated mostly under the first peak, and some under the second, along with oxygenated components that were also the components of all the other peaks.

Cotroneo et al. (1985, 1986a), and Dugo et al. (1987) used, for the separation of kumquat, lemon and bergamot oils, open-column liquid chromatography with neutral alumina activity II, with pure hexane, hexane/diethyl ether mixtures, and pure diethyl ether as mobile phase. These separations allowed fractionating the oil in classes of compounds, eluting in increasing polarity order: hydrocarbons, esters and ethers, carbonyl compounds and alcohols. Figures 1.4 and 1.5 show examples of these separations.

Mazza (1986) used open silica column liquid chromatography to separate bergamot oil into four fractions, eluting with a solvent system similar to that used by Cotroneo et al. (1985, 1986a) and Dugo et al. (1987).

Off-line methods are laborious, time consuming, and require sample manipulation, and contamination and loss of components can easily occur.

Since the 1990s, it has been possible to perform on-line LC pre-fractionation of the oil for the analysis of essential oils, so that the fractions can be directly analyzed by GC. This procedure does not require manipulations, and only sample dilution is necessary, permitting the analysis in a single run (Munari et al., 1990). This technique has been used for the analysis of aldehydes in sweet orange oil (Mondello et al., 1994a). Coupling the LC-GC system to a mass spectrometer detector useful information was obtained on bergamot oil (Mondello et al., 1994b), neroli oil (Mondello

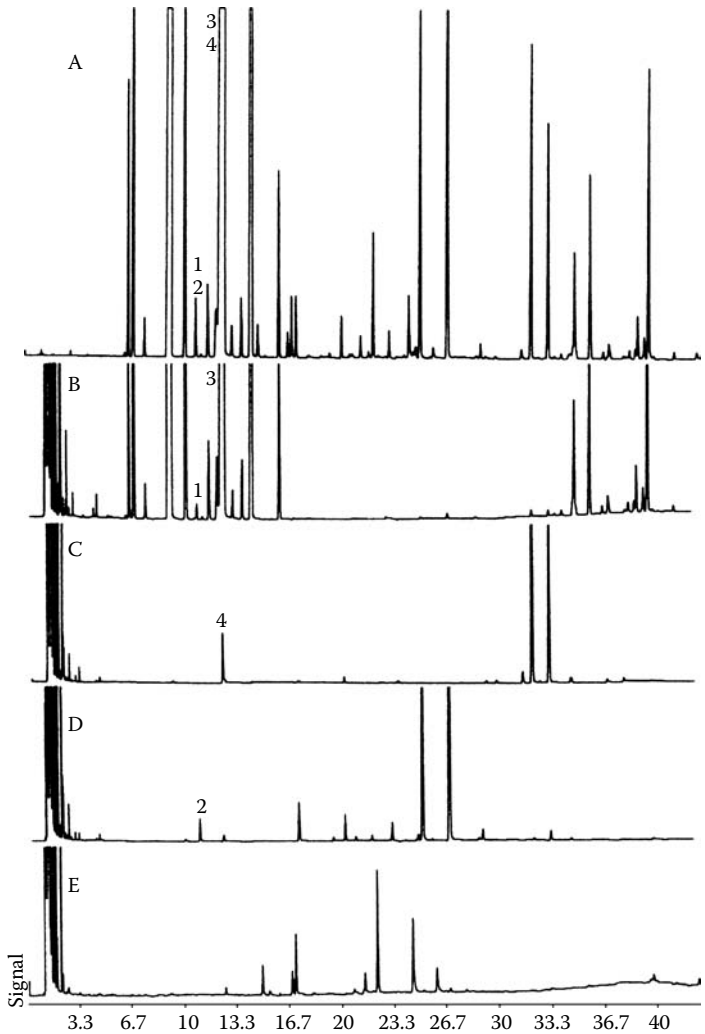

FIGURE 1.4 Gas chromatogram on a capillary column coated with SE-52 of a cold-pressed lemon oil and of the fraction obtained by separation on neutral alumina column: (A) whole oil (B) hydrocarbons (C) ethers and esters (D) carbonyl compounds (E) alcohols. (1) α-Phellandrene; (2) octanal; (3) limonene; (4) 1,8-cineole. Octanal and α-phellandrene and limonene and 1,8-cineole are coeluted in chromatogram A. These components are resolved in the chromatograms B, C, and D. (From Cotroneo, A., et al., *Flavour Fragr. J.* 1, 69–86, 1986a.)

et al., 1994c) and petitgrain oils (Mondello et al., 1996) and on the sesquiterpene hydrocarbons of different essential oils (Mondello et al., 1995b).

The multidimensional gas chromatography (MDGC) (Mosandl, 1995; Mondello et al., 1998a,b) with conventional capillary columns, along with Fast GC (David et al., 1999; Mondello et al., 2000), allowed obtaining reliable and rapid information on the composition of citrus essential oils. The former technique uses the high resolution of GC during both the preseparation and the final analysis of the transferred fractions; the latter allows the analysis of the volatile fraction and of some nonvolatile components of citrus essential oils with a speed gain of about five compared with the conventional GC, without resolution loss.

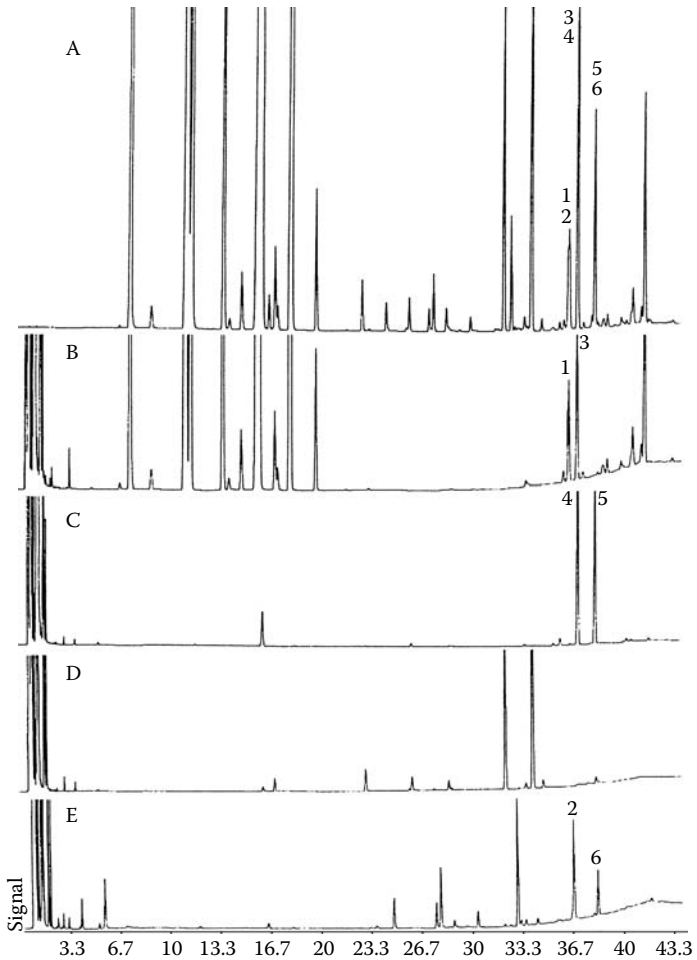

FIGURE 1.5 Gas chromatogram on a capillary column coated with Carbowax of a cold-pressed lemon oil and of the fraction obtained by separation on neutral alumina column: (A) whole oil, (B) hydrocarbons, (C) ethers and esters, (D) carbonyl compounds, and (E) alcohols. (1) β-caryophyllene; (2) nerol; (3) trans-α-bergamotene; (4) neryl acetate; (5) geranyl acetate; (6) geraniol. β-caryophyllene and nerol, trans-α-bergamotene and neryl avetate, geranyl acetate, and geraniol coeluted in chromatogram A. These components are resolved in the chromatograms B, C, D, and E. (From Cotroneo, A., 1985. Personal communication.)

During the recent years, the use of very short columns (5 m length and 50 μm internal diameter) allowed to completely resolve the whole volatile fraction of citrus essential oils in about 90 seconds, with a speed gain of 30 folds compared to the conventional GC separations (Mondello et al., 2004c). Figure 1.6 shows the ultrafast chromatogram of lime oil obtained by Mondello et al. (2004c).

Figures 1.1 through 1.6 illustrate the evolution of monodimensional gas chromatographic techniques and the consequent improvement of the information obtained by their use on the composition of the volatile fraction of citrus essential oils.

Recently a multidimensional GC system was developed (Mondello et al., 2006) that applies, for the transfer, an interface based on a pressure-balance mechanism (first described by Deans in 1968), with the advantage that on both the first and second dimension either conventional or fast capillary columns can be used. By using the latter type of columns it is possible to obtain reliable and reproducible quali-quantitative data in a very short time.

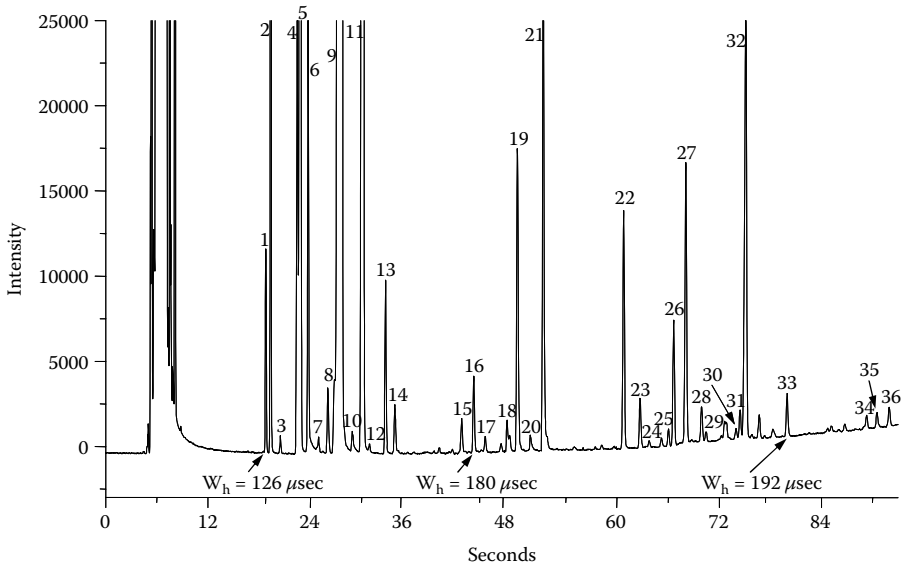

FIGURE 1.6 Ultrafast gas chromatogram of a lime essential oil on a fused silica capillary column (5 m × 0.05 mm × 0.05 μm). Time of analysis 93 seconds. (From Mondello, L., et al., *J. Sep. Sci.* 27, 699–702, 2004c. Printed with permission of John Wiley & Sons, Ltd.)

It can be asserted that the development of the chromatographic techniques along with the introduction of systems that are capable of interactively using the chromatographic retention information and the mass spectroscopy data for the identification of the components, made it possible to overcome almost completely the difficulties often encountered in the past in the analysis of very complex matrices such as citrus essential oils. It should also be noted that citrus essential oils represent an excellent matrix for the development of advanced chromatographic techniques and for their validation. For this purpose, particular emphasis should be laid upon comprehensive multidimensional (MD) techniques, which can be considered the most powerful separative systems available today. Mondello et al. (2005) applied the superior resolving power of comprehensive multidimensional GC to lemon essential oil using a polar column in the first dimension and an apolar column in the second dimension. The method avoided the peak overlapping that occurs in monodimensional separations. Shellie and Marriott (2002) reported a GC × enantio-GC method applied to the analysis of chiral components of bergamot oil. Mondello et al. (2008) recently applied comprehensive LC × GC chromatography in the separation of bergamot oil volatile components.

The development of the above-mentioned analytical methods not only allowed the characterization of many citrus oils but also gave accurate judgments on genuineness, geographic origin, possible contamination and adulteration.

The quantitative data obtained by the GC analysis of the volatile components of citrus essential oils are usually reported as the relative percent of the peak areas. In the literature, however, are found different papers where the wt% in the whole oil is reported for each component. These values are obtained by calibration with internal standard and response factors (RFs) (Staroscik and Wilson 1982a,b; Chamblee et al., 1991). The major differences between the two methods can be observed for lime and grapefruit cold-pressed oils. These oils in fact contain very high amounts of non-volatile residue. The complexity of the composition of citrus essential oils and the lack of commercially available pure standards make it impossible to determine the RFs of all components in an essential oil. Dugo et al. (1983) proposed a possible solution based on grouping the components

of essential oils in class of substances, so that the RF of hydrocarbons was assumed to equal one, and determining the RFs of the oxygenated classes of substances as reported below:

Class	RF
Aldehydes and ketones	1.24
Alcohols	1.28
Esters	1.40

Recently Costa et al. (2008) used nonane as internal standard, and determined for the different class of substances the following RFs:

Class	RF
Monoterpene hydrocarbons	1.03
Sesquiterpene hydrocarbons	0.98
Aromatic hydrocarbons	0.99
Aliphatic and monoterpene aldehydes and ketones	1.30
Aromatic aldehydes	1.27
Monoterpene alcohols	1.30
Aliphatic and monoterpene esters	1.59
Monoterpene ethers	1.28
Sesquiterpene oxides	1.53

The methods used for the quantitative analysis of essential oils have been revised by Bicchi et al. (2008).

The oldest data reported on the citrus essential oil components have been reviewed, as mentioned previously, by Guenther (1949). Most of those reported later, until 1979, were reviewed by Shaw (1977, 1979), and the majority of the literature published during the second half of the last century has been reviewed by Dugo et al. (2002). Moreover, since 1976 Lawrence (1976–2009) periodically reviewed the composition of citrus oils.

In this chapter, each citrus oil is discussed individually. The relative data published between 1979 and 1999 for each oil, which has already been revised by Dugo et al. (2002), is summarized in a table and in a schematic appendix containing useful information and comments. Following the most recent results on the composition of industrially cold-pressed oils of known origin and of commercial oils will be reported in detail in tables and discussed. Information on laboratory-extracted oils will be also reported. These results also include those relative to papers published before 1999 but not revised in the previous review by Dugo et al. (2002). The data published before 1979 can be found in the reviews by Shaw (1977, 1999) and by Dugo et al. (2002).

The data in the tables, drawn from the original papers, will represent the composition of a single sample, the mean values, or, when possible, variability ranges. These are expressed by two decimal figures, or by one if this was the approximation used in the original paper. If the minimum value reported in the variability range is zero, it means that the component was identified and quantitatively determined only in some of the samples analyzed in the original paper.

In the tables, the single components are grouped by chemical class, and each class is listed in alphabetical order. In the appendix to each table, the following information, when available, is provided: geographical origin, the production technology, the number of samples relative to the given data, the analytical technique, the component identification method, how the data are expressed (wt% or relative percentage of peak area). Those components identified by a single author and their content are also indicated in the appendix.

In both the text and in the tables the term tr (trace) indicates percentage equal or less than 0.05 if the results are reported to the first decimal figure; when two decimal figures are used the term indicates percentages equal or less than 0.005; the (+) symbol indicates a component present but not quantitatively determined; *cis*- and *trans*-linalol oxide, if not otherwise specified, are in the furanoid form; the asterisk (*) labels those components for which the correct isomer was not characterized in the original paper; the symbol t signs those components where the identification was tentative.

In the appendix, if the columns dimensions are reported in parentheses, the first number indicates the length (meters); the second indicates the internal diameter (millimeters); and the third indicates the film thickness of the stationary phase (micrometers).

1.2 BITTER ORANGE OIL (*CITRUS AURANTIUM* L.)

1.2.1 1979–1999

Table 1.1 summarizes the results published after 1979 on the composition of the volatile fraction of bitter orange oil, already revised by Dugo et al. (2002), for industrial, commercial, and laboratory-extracted oils.

1.2.2 1998–2009

1.2.2.1 Industrial Oils

Table 1.2 reports the results of most of the research published after the review by Dugo et al. (2002) on the composition of bitter orange oil. As can be seen in the table, the papers published on the composition of bitter orange oil are quite scant; only four were on industrial oils and three of these were limited to one sample each. One of these papers (Pino and Rosado, 2000) is relative to a Cuban oil characterized by a low content of limonene and by high contents of myrcene, γ-terpinene, β-bisabolene, and *trans*-α-bergamotene. Two papers (Mondello et al. 2003, 2004b), relative to an Italian oil, report data in agreement with those previously reviewed by Dugo et al., (2002). The results obtained by Dugo et al. (2010a) on Italian and Egyptian samples are also in good agreement with the values previously obtained for cold-pressed bitter orange oils. However, a wide range of variability is observed for the content of linalyl acetate in bitter orange oils.

Not included in Table 1.2 are the results obtained by Veriotti and Sacks (2002), on a commercial sample, and by Feger et al. (2001a). The composition of the oil analyzed by Veriotti and Sacks was not considered representative of bitter orange oil; the oil contained 1.66% of *p*-cymene, which is indicative, in our opinion, of inadequate storage. Moreover, the analytical technique applied, high-speed GC with time-of-flight MS (TOF/MS), is very complex and expensive. Its use is not justified by the results achieved by these authors, which are limited to the identification of few components, easily determinable by less complex and expensive techniques. The paper by Feger et al. (2001a) is interesting, since in an investigation on the germacrenes content on different citrus essential oils, they reported the presence in bitter orange oil of bicyclogermacrene (tr–0.01%); germacrene A (tr–0.01%); germacrene B (0.01%–0.02%); germacrene C (0.01%–0.02%); and germacrene D + valencene (0.07%–0.11%).

1.2.2.2 Laboratory Oils

Table 1.2 also reports the results of papers published after the review by Dugo et al. (2002) on the composition of bitter orange oil extracted in laboratory.

The results relative to these oils appear interesting. Kirbaslar and Kirbaslar (2003) and Gionfriddo et al. (2003) confirmed the existing data on Mediterranean bitter orange oil; Lota et al. (2001a) give information on the composition of oils extracted by numerous cvs. of C. *aurantium* cultivated in Corsica, France, and the results by Sawamura (2000) and by Song et al. (2000) are relative to the composition of some cvs. cultivated in Italy and Japan. Song et al. (2000)

TABLE 1.1
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils
	Hydrocarbons		
Aliphatic			
Tetradecane	–	–	0.02–0.15
Monoterpene			
Camphene	tr–0.01	–	tr–0.01
δ-3-Carene	tr–0.01	tr	0.01
p-Cymene	tr–0.01	0.1	tr–0.52
Limonene	91.54–94.34	93.50–93.6	86.06–95.49
Myrcene	1.60–3.10	1.8–2.44	0.04–2.25
(*E*)-β-Ocimene	0.14–0.38	0.09	0.14–0.75
(*Z*)-β-Ocimene	tr–0.05	–	tr–0.05
α-Phellandrene	tr–0.08	–	0.02
β-Phellandrene	tr	1.5	0.25–0.27
α-Pinene	0.29–0.89	0.5–0.71	0.21–1.17
β-Pinene	0.29–1.28	0.46–0.5	0.09–1.61
Sabinene	0.12–0.45	0.2–0.25	0.07–0.19
α-Terpinene	tr–0.02	–	0.01–0.22
γ-Terpinene	tr–0.13	tr–0.3	0.01–0.73
Terpinolene	tr–0.13	–	tr–0.72
α-Thujene	tr–0.02	–	tr–0.22
Sesquiterpene			
Bicyclogermacrene	tr	–	0.01
β-Bisabolene	tr–0.02	–	–
δ-Cadinene	tr–0.01	–	0.10
β-Caryophyllene	0.03–0.14	0.06–0.1	0.02–0.27
α-Copaene	+	–	tr–0.05
β-Elemene	tr–0.03	–	tr–0.01
δ-Elemene	0.02–0.05	–	0.01–0.05
(*E*)-β-Farnesene	0.01	–	–
(*Z*)-β-Farnesene	0.01–0.03	–	–
α-Humulene	tr–0.04	–	tr–0.04
Germacrene D	0.08–0.14	–	0.08–0.10
Valencene	tr–0.29	tr	0.01
	Aldehydes		
Aliphatic			
Decanal	0.11–0.20	0.17	0.09–0.20
(*E*,*E*)-2,4-Decadienal	tr–0.01	–	0.01
(*E*,*Z*)-2,4-Decadienal	tr–0.01	–	0.01
(*E*)-2-Decenal	0.02	–	0.01–0.02
Dodecanal	0.01–0.04	tr	tr–0.03
(*E*)-2-Dodecenal	0.01	–	–

continued

TABLE 1.1 (continued)
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils
Nonanal	0.01–0.11	tr	tr–0.08
Octanal	0.04–0.24	0.1	0.08–0.23
Tetradecanal	tr–0.03	–	0.01
Tridecanal	tr–0.01	–	0.01
Undecanal	tr–0.01	tr	tr–0.01
Monoterpene			
Citronellal	tr–0.02	–	tr–0.07
Geranial	0.01–0.10	0.06	tr–0.14
Myrtenal	tr–0.01	–	–
Neral	tr–0.05	tr	tr–0.10
Perilla aldehyde	tr–0.02	0.21	0.01–0.53
Sesquiterpene			
α-Sinensal	0–tr	–	–
β-Sinensal	tr–0.01	–	–
Ketones			
Monoterpene			
Camphor	tr–0.01	–	tr
Carvone	tr–0.02	0.06	0–0.03
Isopiperitone	0.01	–	0.02
Sesquiterpene			
Nootkatone	0–0.39	–	0–0.13
7-Epi-α-selinen-2-one	tr–0.02	–	0–0.02
Alcohols			
Aliphatic			
Decanol	tr–0.05	–	0.05
Nonanol	tr–0.03	–	–
Octanol	tr–0.18	0.10	0.33
Monoterpene			
Borneol	tr	–	tr
Carvacrol	–	–	0.01–0.02
Citronellol	tr–0.01	–	0–0.07
Geraniol	0.01–0.23	tr	tr–0.23
Linalol	0.10–0.37	0.1–0.23	0.15–3.24
Nerol	tr–0.04	–	tr–0.17
Terpinen-4-ol	tr–0.03	–	0.06–0.20
α-Terpineol	0.03–2.94	0.17–0.2	tr–0.66
Thymol	–	–	tr–0.12
Sesquiterpene			
Elemol	–	–	tr
Farnesol*	–	–	tr–0.55
(*E*,*E*)-Farnesol	tr–0.01	–	–

TABLE 1.1 (continued)
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils
(*Z*,*E*)-Farnesol	tr–0.01	–	–
(*E*)-Nerolidol	0.04–0.23	–	0.02–0.23
Esters			
Aliphatic			
Decyl acetate	tr–0.07	–	0.02
Dodecyl acetate	0.01	–	tr
Nonyl acetate	tr–0.01	–	0.01–0.05
Octyl acetate	0.01–0.05	–	0.05–0.11
Monoterpene			
Citronellyl acetate	tr–0.05	–	–
Geranyl acetate	tr–0.20	0.1–0.11	tr–0.38
Geranyl propanate	0.02	–	tr
Linalyl acetate	0.19–1.17	–	0.07–2.72
1,8(9)-Menthadien-10-yl acetate	tr–0.02	–	0.01
Neryl acetate	0.02–0.05	tr–0.09	0.01–0.09
α-Terpinyl acetate	tr–0.03	–	0.01–0.03
Ethers and oxides			
Monoterpene			
cis-Limonene oxide	tr–0.01	–	tr–0.05
trans-Limonene oxide	tr–0.01	–	tr–0.05
cis-Linalol oxide[a]	0.01	–	tr–0.15
trans-Linalol oxide[a]	0.01	tr	tr–0.29
Acids			
Aliphathic			
Decanoic acid	0.05	–	0.03

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined; [a] pyranoid or furanoid form.

Appendix to Table 1.1

- The results reported in Table 1.1 and in this appendix, for the different categories of bitter orange oils, are taken from the following original papers:
  - Cold-pressed industrial oils: Koketsu et al. (1983); Boelens and Sindreu (1988); Boelens and Jimenez (1989a); Boelens (1991); Boelens and Oporto (1991); Dugo et al. (1993, 1999); Dugo (1994). Were also included the qualitative results obtained by Micali et al. (1990), Lanuzza et al. (1991) and by Mondello et al. (1995b).
  - Commercial oils: Haubruge et al. (1989); Inoma et al. (1989).
  - Laboratory-extracted oils: Ashour and El-Kebeer (1983); Tuzcu et al. (1985); Lin et al. (1986); Caccioni et al. (1998); Boelens and Jimenez (1989b); Huang et al. (1990); Yang S. et al. (1992); Njoroge et al. (1994a); Boussaada (1995); Protopapadakis and Papanikolau (1998); Sawamura et al. (1999a).
- Ranges value reported in table refer to the papers where the compounds were identified; the minimum value equal to zero is used only if in the same paper the component was identified and quantitatively determined only in some of the samples analyzed.

continued

TABLE 1.1 (continued)
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1979–1999)

- Coelutions indicated by one or more authors in chromatographic separations of bitter orange oils:
  - Limonene + *p*-cymene + β-phellandrene; terpinolene + octanal; (*E*)-β-farnesene + (*Z*)-β-farnesene; citronellal + octyl acetate; citronellol + nerol; (*E*,*E*)-farnesol + (*Z*,*E*)-farnesol; *cis*- + *trans*-limonene oxide; *cis*- + *trans*-anhydrolinalol oxide; *cis*- + *trans*-linalol oxide.
- Ranges reported in Table 1.1 for some components in cold-pressed oils, coeluted in chromatographic separation reported by few authors ((*E*)-β-farnesene, (*Z*)-β-farnesene, citronellal, octyl acetate, (*E*,*E*)-farnesol, (*Z*,*E*)-farnesol, *cis*-limonene oxide, *trans*-limonene oxide), are determined considering the results where coelutions did not occur. The results relative to commercial and laboratory-extracted oils, due to the scant number of data relative to the former, and to the influence of the different extraction methods (cold pressing, solvent extraction, hydrodistillation) and to the different botanical and geographical origin of the fruits for the latter, include the variability ranges determined from the raw data even if coelution did occur.
- In addition to those listed in Table 1.1, in bitter orange oil were found the components listed below:
  - Cold-pressed oils: *trans*-α-bergamotene (0.01%–0.02%), germacrene B (0.01%), β-santalene (tr–0.01%), selinenes* (0.01%), β-sesquiphellandrene (0.01%), heptanal (0.01%), hexanal (0.03%), (*E*)-2-octenal (0.01%), dodecanol (0.01%), isopulegol (tr–0.01%), 1,8(9)-menthadien-10-ol (0.01%), *cis*-sabinene hydrate (tr–0.01%), neryl propanate (0.01%), myrcene oxide (0.01%), and trace amounts of linear chained hydrocarbons C_{21}–C_{23} and correspondent "iso" isomers C_{23}–C_{31}, *p*-cymenene, (*E*,*E*)-α-farnesene, α-muurolene, (*E*)-2-heptenal, (*E*)-2-hexenal, (*E*)-2-nonenal, (*E*)-2-tetradecenal, (*E*)-2-tridecenal, (*E*)-2-undecenal, piperitone^t, hexanol, *trans*-sabinene hydrate, undecyl acetate, *cis*- and *trans*-dehydrolinalol oxide, (*E*)-miroxide, perillene.
  - Commercial oils: *trans*-carveol (0.11%), limonene dioxide (0.09%), limonene oxide* (0.15%).
  - Laboratory-extracted oils: undecane (0.06%), β-cubebene (0.05%), γ-elemene^t (0.04%), farnesene* (0.08%–0.21%), β-farnesene* (0.01%), γ-muurolene (0.03%), linear chained alcohols C_{12}, C_{14}–C_{17}, C_{19}–C_{24} (2.89%), carveol* (0.05%), myrtanol (0.18%), *cis*-myrtenol (0.55%), perillyl alcohol (0.01%), β-terpineol* (0.02%), perillyl acetate^t (0.02%), α-ionone (0.04%), β-ionone (0.35%), and trace amounts of γ-cadinene, α-cubebene, naphthalene, *cis*-carveol, *trans*-pinocarveol, thujyl alcohol, α-bisabolol, cedrol, heptyl acetate, bornyl acetate, *p*-menth-1-en-9-yl acetate.
- The results reported in Table 1.1 relative to industrial cold-pressed oils refer to Italian, Spanish and one Brazilian oil. Variability ranges are quite narrow, particularly those of the principal components, therefore they can represent the composition of bitter orange oil. It should be highlighted the high value of valencene (0.29%) reported by Koketsu et al. (1983) for a Brazilian oil, considering that this component is present at trace levels in all the other oils; the high values of nootktatone and α-terpineol in Spanish oils compared to the Italian oils.
- The results relative to commercial oils are in good accordance with those of secure origin. Among these results the data reported by Chouchi et al. (1996) were not included due to the high number of anomalous values reported by these authors such as the low content of limonene (82.12%), the high value of β-pinene (2.53%) and *p*-cymene (2.07%), this latter probably due to bad storage condition.
- Laboratory-extracted oils show, mostly for certain components, wide ranges of variability. This could be probably due to the botanical origin of the fruits and the different extraction techniques applied. It should be mentioned the high value of α-terpinene (4.25%, not included in the table) reported by Huang et al. (1990) and by Yang, S. et al. (1992) in an oil extracted from oranges cultivated in China; the high value of tetradecanal (0.49%, not included in the table) determined by Tuzcu et al. (1985) in an oil of Turkish origin.
- Among the laboratory-extracted oils were not included those analyzed by El-Samahy et al. (1982), since limonene (38.95%) and linalol (18.85%) content were mainly similar to that of bergamot oil than to bitter orange oil, and the values of camphene (14.02%) and α-pinene (22.44%) appeared unusual for citrus peel oils; the sample analyzed by Kusunose and Sawamura (1980) for the low value of limonene (73.8%) and the high value of myrcene (24.3%) unusual for bitter orange oils; the oil analyzed by Zhu et al. (1995) that presented a very different composition from that commonly observed for bitter orange oil: high amounts of linalol (20.69%) and of linalyl acetate (30.72%).

TABLE 1.2
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1998–2009)

	Industrial Oils			Laboratory Oils						
				Cold Extracted						Distilled
	1	**2,3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
				Hydrocarbons						
Monoterpene										
Camphene	–	0.01	tr	tr	tr	tr–0.01	0–tr	–	tr–0.01	tr
δ-3-Carene	–	tr	tr	–	–	–	–	0.01–0.02	tr	–
p-Cymene	–	b	tr–0.01	tr	tr	0–tr	0–0.5	–	–	–
Limonene	86.2	93.26[b]	93.43–96.52	92.53–94.29	92.5	92.03–96.28	72.9–95.1[c]	93.68–94.32	90.86–92.06	90.6
Myrcene	4.9	1.90	1.38–1.56	1.89–1.93	2.2	1.64–1.73	1.0–1.7	1.73–1.86	1.38–1.64	1.6
(*E*)-β-Ocimene	–	0.62	0.09–0.54	0.16–0.25	0.3	0.01–0.53	0–0.4	0.01–0.25	0.19–0.24	tr
(*Z*)-β-Ocimene	–	0.01	–	–	tr	tr–0.01	0–tr	0–tr	0.01	0.2
α-Phellandrene	–	0.20[a]	0.01–0.02	0.03	–	tr	0–tr	0.01–0.13	0.03–0.08	–
β-Phellandrene	–	–	–	0.25	–	tr	0.2–0.5	–	–	0.3
α-Pinene	0.9	0.62	0.33–0.38	0.32–0.52	0.6	0.28–0.57	0.2–0.9	0.39–0.45	0.34–0.40	0.4
β-Pinene	0.6	1.01	0.10–0.59	0.24–0.78	0.4	0.03–1.05	0.3–4.0[d]	0.40–0.57	0.64–0.86	0.3
Sabinene	0.1	0.29	0.09–0.17	0.17–0.22	0.2	0.19–0.30	0.1–0.7	0.14–0.21	0.17–0.22	0.1
α-Terpinene	–	tr	–	–	–	–	–	0–0.01	tr	tr
γ-Terpinene	0.7	0.08	tr–0.01	tr–0.16	–	0–0.02	0–4.4[e]	0.10–0.21	0.01–0.16	tr
Terpinolene	0.1	0.01	tr	tr	tr	tr–0.03	0–0.2	0–0.01	tr–0.01	0.6
α-Thujene	–	0.01	tr–0.02	tr	–	–	0–0.1[f]	–	tr	–
Sesquiterpene										
trans-α-Bergamotene	0.3	0.02	–	–	–	–	–	–	0.01–0.02	–
Bicyclogermacrene	–	0.01	tr	–	tr	0–0.28	–	–	–	–
β-Bisabolene	0.5	0.01	–	0.06–0.29	–	–	–	–	tr	–
γ-Cadinene	–	–	–	–	–	0–0.01	–	–	tr	–

continued

TABLE 1.2 (continued)
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1998–2009)

	Industrial Oils			Laboratory Oils						
				Cold Extracted						Distilled
	1	**2,3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
δ-Cadinene	–	–	–	0.02	–	0–0.03	–	–	–	–
β-Caryophyllene	–	0.06	0.04	0.07–0.12	tr	tr–0.18	0–0.9	0.06–0.16	0.05–0.08	tr
β-Elemene	–	–	–	0.02–0.09	0.1	0–0.03	–	–	–	–
δ-Elemene	–	0.03	tr	–	–	0–0.03	–	–	–	–
(*E,E*)-α-Farnesene	tr	–	–	0.02–0.03	–	–	–	–	–	–
(*E*)-β-Farnesene	–	–	tr–0.01	–	tr	0–0.02	–	–	–	–
α-Humulene	–	–	tr	0.04–0.09	tr	0–0.05	0–tr	0–tr	–	–
Germacrene B	–	–	tr	–	–	0–0.01	–	–	–	–
Germacrene D	0.2	0.12	0.02–0.06	–	0.1	0.01–0.47	0.1–2.1[g]	–	0.06–0.15	–
Aldehydes										
Aliphatic										
Decanal	tr	0.13	0.07–0.09	–	0.2	0.01–0.10	0–0.4	0.16–0.22	0.10–0.19	–
(*E*)-2-Decenal	–	–	0.01	–	tr	0–0.01	–	–	–	–
Dodecanal	0.1	0.02	tr–0.02	0.02–0.08	–	tr–0.02	–	0.07–0.15	0.01–0.03	–
Nonanal	–	0.03	0.01	tr	tr	0.01–0.02	0–tr	–	0.01–0.03	–
Octanal	–	0.20[a]	0.05–0.07	–	0.6	tr–0.16	0–0.2	0.08–0.16	0.10–0.19	–
Undecanal	–	0–0.1	tr–0.01	tr–0.03	tr	0–0.01	–	–	tr–0.01	–
Monoterpene										
Citronellal	–	tr	tr	tr–0.03	0.1	0–0.04	–	–	tr–0.01	–
Geranial	0.1	0.05	0.03–0.04	0.06–0.16	0.1	0–0.08	0–0.1	0.08–0.12	0.05–0.10	–
Neral	tr	0.03	0.01–0.02	0.02–0.05	tr	0.01–0.04	0–0.1	–	0.03–0.05	–
Perilla aldehyde	–	0.02	0.01	–	0.1	tr–0.02	–	tr	0.01–0.02	–
Sesquiterpene										
α-Sinensal	–	–	–	–	–	0–tr	–	–	tr	–

Ketones										
Monoterpene										
Carvone	–	–	0.01–0.03	–	tr	0–tr	–	–	–	–
Sesquiterpene										
Nootkatone	–	0.01	tr–0.03	–	–	–	–	–	tr	–
Alcohols										
Aliphatic										
Octanol	–	–	tr–0.02	0.09–0.14	–	0–tr	–	–	0.01–0.02	–
Monoterpene										
cis-Carveol	–	–	tr–0.02	–	–	0–0.05	–	–	–	–
trans-Carveol	–	–	tr–0.03	–	tr	0–0.15	–	–	–	–
Citronellol	–	–	–	tr	–	0–0.01	–	–	–	–
Geraniol	–	0.01	–	0.06–0.07	tr	–	0–0.3	–	–	0.1
Linalol	0.3	0.32	0.06–0.30	0.16–0.37	0.2	0.15–1.04	tr–5.2[h]	0.33–0.46	0.28–0.53	1.5
Nerol	–	–	–	0.03–0.04	–	0–0.01	0–tr	–	tr	0.1
Perylla alcohol	–	–	tr–0.01	–	–	0–0.02	–	–	–	–
cis-Sabinene hydrate	–	tr	–	0–tr	–	–	–	–	–	–
trans-Sabinene hydrate	–	–	–	tr–0.06	–	tr–0.01	–	–	tr	–
Terpinen-4-ol	0.1	tr	tr	0.10–0.40	–	–	0–tr	–	tr–0.01	0.1
α-Terpineol	0.4	0.03	0.04–0.06	0.05–0.12	–	0.04–0.05	0–0.9	0.06–0.15	0.03–0.05	0.6
Thymol	–	–	–	tr	tr	–	–	–	–	–
Sesquiterpene										
Elemol	–	–	–	–	tr	0–tr	–	–	–	–
(*E*)-Nerolidol	–	0.09	0.03–0.06	0.10–0.32	–	0–0.08	0–3.2[i]	0.03–0.07	–	0.1
(*Z*)-Nerolidol	0.2	–	–	–	–	–	–	–	0.03–0.06	–
Spathulenol	–	–	tr	–	–	0–0.11	–	–	–	–
Esters										
Aliphatic										
Decyl acetate	–	–	tr–0.03	–	0.1	0–0.02	–	0–0.02	–	–

continued

TABLE 1.2 (continued)
Percentage Composition of the Volatile Fraction of Bitter Orange Oils (1998–2009)

	Industrial Oils			Laboratory Oils						
				Cold Extracted						Distilled
	1	**2,3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Dodecyl acetate	–	–	tr	–	tr	0–tr	–	–	–	–
Heptyl acetate	–	–	tr	–	–	0–tr	–	–	–	–
Nonyl acetate	–	0.01	–	–	tr	0–0.01	–	–	tr–0.01	–
Octyl acetate	tr	0.04	0.02–0.03	–	tr	0–0.07	0–0.2	0.02–0.07	0.03–0.06	–
Monoterpene										
Citronellyl acetate	–	0.01	–	tr–0.03	–	0–0.01	–	–	0.01	–
Geranyl acetate	0.5	0.12	0.07–0.08	0.16–0.34	0.2	tr–0.13	0–0.6	0.04–0.13	0.06–0.12	0.2
Linalyl acetate	–	1.04	0.08–0.81	0.32–0.73	1.4	–	0–5.0[j]	1.17–1.32	0.71–1.26	0.2
Neryl acetate	0.5	0.03	0.01–0.02	0.04–0.11	tr	0–tr	0–0.2	0–0.03	0.02–0.03	0.1
Perillyl acetate	–	–	0.01	–	tr	–	–	–	–	–
α-Terpinyl acetate	tr	tr	tr–0.01	–	0.1	–	0–tr	0–0.08	tr–0.01	–
Ethers and oxides										
Monoterpene										
cis-Limonene oxide	tr	0.01	tr–0.08	–	tr	0–0.04	–	–	tr–0.01	–
trans-Limonene oxide	tr	tr	tr–0.08	–	tr	0–0.03	0–0.1	–	0.01–0.05	–
cis-Linalol oxide	–	–	–	–	–	–	0–0.04	–	–	0.2

Notes: tr, traces; *, correct isomer not characterized; [a] α-phellandrene + octanal; [b] limonene + *p*-cymene; [c] limonene values lower than 90% were reported for the following cvs.: Sae Algérie (89.8%), Bouquetier de Nice (89.2%), Doux (87.6%), Gou tou (84.2%), Espagne (72.9%); [d] β-pinene values higher than 1% are reported for the following cvs.: Apepu, A fleurs ferrando, Tunisian (1.1%), Alibert de Cors (1.5%), Commune de Tuléar (1.6%), Sae Algérie (1.3%), Bouquetier de Nice à fruits plates (2.4%), Luisi, Bouquet de fleurs, Espagne (1.2%), Corsigliese (1.4%), Gou tou (4.0%); [e] γ-terpinene values higher than 0.5% are reported for the following cvs.: Bouquetier de Nice (2.1%), Gou tou (4.4%); [f] α-thujene is present only in the cv. Gou tou; [g] germacrene D values higher than 0.4% are reported for the cvs.: Gou tou (0.8%), Espagne (2.1%); [h] linalol values higher than 1% are reported for the following cvs.: Bouquetier de Nice (1.5%), Doux (1.1%), Espagne (5.2%); [i] (*E*)-nerolidol values higher than 0.3% are reported for the following cvs.: Doux (0.6%), Espagne (3.2%); [j] linalyl acetate values higher than 1.5% are reported for the following cvs.: Doux amer (1.8%), Sae Algérie (3.9%), Bouquetier de Nice à fruits plates (1.8%), A fleurs ferrando (2.8%), Granito (2.7%), Doux (1.8%), Espagne (5.0%).

Appendix to Table 1.2

1. Pino and Rosado (2000). Cuba; one commercial sample cold-pressed; GC/MS on capillary column (25 m × 0.32 mm × 0.25 μm) coated with BP-1; IDENT home-made MS library; relative percentage of peak areas. Pino and Rosado also found dodecanol (0.2%), cis-piperitol (0.3%), terpinen-4-yl acetate (0.7%), and trace amounts of (*Z*)-β-damascenone.

2 and 3. Mondello et al. (2003, 2004b). Sicily, Italy, one sample; conventional GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX-5 MS; Fast GC/FID on capillary column (10 m × 0.1 mm × 0.1 μm) coated with RTX-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. In the table are reported the results obtained with conventional method; the results obtained with fast method were very similar. More information is reported in the Chapter 11. Mondello et al. also found (*Z*)-β-farnesene (0.02%), and trace amounts of tetradecanal, 2,3-dimethyl-3-(4-methyl-3-pentenyl-2)-norbornanol.

4. Dugo et al. (2010a). Range of the values of one commercial oil from Italy and two cold-pressed oils from Egypt. GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made. LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found 4,8-dimethyl-nona-1,3(*E*),7-triene (tr–0.01%), (*E,Z*)-2,6-dodecadienal (tr–0.01%), *trans*-*p*-mentha-2,8-dien-1-ol (tr–0.03%), caryophyllene oxide* (tr–0.01%), and trace amounts of (*E*)-2-dodecenal, (*E*)-2-tetradecenal, decanol, nonanol, limonene-10-ol, (*E*)-2-hexyl acetate.

5. Huang and Wu (1998). China; ranges relative to the composition of cold-pressed oils of the following cvs.: Morocco, Bangan, Daidai; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-17 and OV-101; relative percentage of peak areas; Huang and Wu also found γ-muurolene (0–0.03%), (*Z*)-3-hexenol (0.02%), and in one or more of the analyzed samples trace amounts of farnesal*, hexanol, thymol.

6. Song et al. (2000). Japan; one laboratory sample cold extracted from the cv. Daidai; GC/FID on capillary columns coated with Thermon 600T (50 m × 0.25 mm × 0.25 μm) and DB-5 (30 m × 0.25 mm × 0.25 μm); GC/MS on capillary column (30 m × 0.32 mm × 0.25 μm) coated with HP-5; GC-O on capillary column (30 m × 0.53 mm × 50 μm) coated with Supelcowax 10; LRI on Thermon 600T and on DB-5 are reported; wt%. Song et al. also found nerolidol* (0.1%), ethyl acetate (0.3%), and trace amounts of tridecanal, camphor, β-terpineol*, isomenthol, thujl alcohol, α-bisabolol, cedrol, geranyl propanate, *p*-menth-1-en-9-yl acetate.

7. Sawamura (2000). Ranges relative to the composition of cold-pressed oils of the cv. Bittersweet orange (from Italy) and the cvs.: Daidai, Kabusu, Sour orange, Tokosu (from Japan); GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T. Sawamura also found 3,3-dimethyl-1-octene (0%–0.01%), aromadendrene (0%–0.01%), α-copaene (0%–0.01%), γ-elemene (0%–0.05%), α-farnesene* (0%–0.01%), valencene (0%–0.02%), 2-dodecenal* (0%–0.01%), pentadecanal (0%–0.02%), β-sinensal (0%–0.01%), bicyclopentan-2-one (0–0.01%), hexadecanol (0%–0.01%), *trans*-*p*-mentha-2,8-dien-9-ol (0%–0.02%), *p*-mentha-1,8-dien-9-ol (0%–0.03%), T-cadinol (0%–0.01%), geranyl isobutyrate (0%–0.22%), and trace amounts in one or more of the analyzed samples of δ-2-carene, 2,5,6-trimethyl-1,3,6-heptatriene, β-cubebene, α-muurolene, (2,4)-decadienal*, 2,7-dimethyl-2,6-octadien-1-ol, *p*-menth-1-en-9-ol, δ-cadinol, *cis*-carvyl acetate, octyl propyl ether, solanone. Moreover Sawamura found in three of the cvs. analyzed (Daidai, Kabusu, Sour orange) respectively 0.52%, 2.36%, and 1.27% of linalyl anthranilate a compound that, as far as the writers now, unusual in citrus peel essential oils.

8. Lota et al. (2001a). Corsica, France; ranges relative to the composition of cold-pressed oils of bitter oranges of the following cvs.: Apepu, Brazilian, Doux amer, Menton, Alibert de Corse, Commune de Tuléar, Sae Algérie, Bouquetier de Nice à fruits plats, A fleurs ferrando, Granito, Bouquetier de Nice, Doux, Sans épine, Luisi, Santucci, De Floride, Algerian, Alibert hybride 12, Corsigliese, Tunisian, Bouquet de Fleurs, Maroc, Petit Pierre, Australian, Gou tou, Espagne; GC/FID and GC/MS on capillary columns (50 m × 0.22 mm × 0.25 μm) coated with BP-20 and BP-1, MS libraries: NIST and Wiley; LRI on BP-20 and BP-1 columns are reported; ^{13}C-NMR spectroscopy; relative percentage of peak areas. Lota et al. also found (Z)-α-bisabolene (0%–0.1%).

9. Kirbaslar and Kirbaslar (2003). Turkey; three laboratory samples cold-pressed (two from Antalya and one from Marmaris); GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 1.0 μm) coated with OPTIMA 5; MS libraries: Wiley and NBS; relative percentage of peak areas. Kirbaslar and Kirbaslar also found isopulegol (0–tr).

10. Gionfriddo et al. (2003). Calabria, Italy; eight samples rasped in laboratory; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; MS library: NIST 1.7; relative percentage of peak areas.

11. Boussaada and Chemli (2006). Tunisia; one laboratory sample extracted by hydrodistillation; GC/FID and GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with BP-1 and BP-20; MS library: Wiley 275; LRI on BP-20 and BP-1 columns are reported; relative percentage of peak areas. Boussaada and Chemli also found farnesene* (0.1%), *trans*-linalol oxide (0.2%), and trace amounts of farnesol*.

reported on the aromagrams of a Daidai oil and the FD factors and relative flavor activities of 31 odorants.

Not included in Table 1.2 are the results obtained by Giampieri et al. (2002) relative to a hydrodistilled oil obtained from fruits spontaneously grown in Sicily and those reported by de Gonzalez et al. (2002) relative to an oil from Venezuela. The sample analyzed by Giampieri et al. (2002) presented a very unusual composition for a bitter orange oil, in fact, not only for the low content of limonene (78.80%), but also for the presence of guaiacol (2.15%), thymol (1.42%), and phenol (1.60%). The research by de Gonzalez et al. (2002) was limited to the quantitative determination of main components: limonene (90.04%); myrcene (1.74%); (*E*)-β-ocimene (0.94%); α-pinene (0.64%); β-pinene (3.25%); sabinene (0.52%); and linalol (2.87%).

1.3 GRAPEFRUIT OIL (*CITRUS PARADISI* MACF.)

1.3.1 1979–1999

Table 1.3 summarizes the results published after 1979 on the composition of the volatile fraction of grapefruit, already revised by Dugo et al. (2002), for both cold-pressed industrial and laboratory-extracted oils.

1.3.2 1998–2009

1.3.2.1 Industrial Oils

In the last decade, the literature has provided poor information on the composition of the whole volatile fraction of industrially processed grapefruit oils. To our knowledge, there are only a few results available, and these are reported in Table 1.4. Three papers report quantitative results relative to a single sample (Oberhofer et al., 1999; Pino and Sanchez, 2000; Viuda-Martos et al., 2009); one is limited to a qualitative investigation for the identification of the aroma impact compounds, many of which were indicated for the first time in a grapefruit oil (Lin and Rouseff, 2001). Finally, Feger et al. (2001b) reported the average composition of a not precisely indicated number of samples of white and pink grapefruits of different geographical origins. These findings are in good agreement with the literature data previously reviewed (Dugo et al., 2002).

The results reported by Oberhofer et al. refer only to olfactory impact components. Oberhofer et al. also evaluated the odor differences and the composition variation of the head space of the oils, at room temperature and after heating to 65°C, on a tube placed at the top of a commercial aroma lamp, prior to and after the reduction of 50% of the volume of the oil. Heated oils showed slight variation of monoterpene aldehydes and alcohols, and of sesquiterpene hydrocarbons. After the reduction of the volume, limonene drastically decreased while monoterpene aldehydes and alcohols increased. The same paper reports results relative to other oils, mainly citrus. The behavior of the components variation after heating is often different for the same components in different oils.

Pino and Sanchez (2000) reported the composition of a grapefruit oil along with that of concentrated oils (two, five, and ten folds) by vacuum distillation. The concentrated oils, compared to the whole oil, obviously present lower levels of monoterpene hydrocarbons, higher amounts of oxygenated compounds and of sesquiterpene hydrocarbons.

Viuda-Martos et al. (2009) reported the composition of a Spanish oil. In addition to the articles included in the table, other papers were published on the composition of the volatile fraction of industrial grapefruit oil. Feger et al. (2001a) in an investigation on the germacrenes content of different citrus essential oils reported the presence in white grapefruit oil from Cuba and in pink grapefruit oil from Florida, USA of bicyclogermacrene (0.02%–0.04%); germacrene A (0.02%–0.03%); and germacrene D (0.07%–0.11%). Steuer et al. (2001) and Shultz et al. (2002), in research carried out using spectroscopic methods for the classification of different essential oils, and the determination of major monoterpene hydrocarbons content, reported the average

TABLE 1.3
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1979–1999)

	Cold-Pressed Oils		Laboratory-Extracted Oils		
			Italian Oils	Other Oils	
	wt%	% Peak Areas	% Peak Areas	wt%	% Peak Areas
Hydrocarbons					
Monoterpene					
Camphene	–	tr–0.01	tr	0–tr	–
δ-3-Carene	–	tr–0.03	tr	–	0.04–0.27
p-Cymene	0.02	0.02–0.32	0.18	0–0.01	–
Limonene	83.40–84.34	92.20–95.40	92.16–93.70	76.14–83.11	89.08–92.95
Myrcene	1.37–3.67	1.82–2.63	1.76–2.09	1.32–1.29	1.89–1.95
(*E*)-β-Ocimene	–	0.13–0.31	0.21–0.37	–	–
(*Z*)-β-Ocimene	–	0.01	tr–0.02	–	–
α-Phellandrene	–	tr–0.20	0.03–0.08	–	0.06–0.19
β-Phellandrene	–	tr–1.3	tr–0.18	–	–
α-Pinene	0.38	0.53–0.75	0.33–0.52	0.42–0.47	0.60–0.63
β-Pinene	0.02–0.05	tr–0.26	0.02–0.07	0.02–0.04	0.06–0.09
Sabinene	0.42–1.08	0.38–1.12	0.36–1.20	0.27–0.60	0.54–0.85
α-Terpinene	–	tr–0.01	0.01–0.04	–	–
γ-Terpinene	0.01–0.12	tr–0.3	0.01–0.08	0.08–0.12	0.04–0.17
Terpinolene	–	tr–0.02	tr–0.31	0.01	–
α-Thujene	–	0.01	tr–0.01	–	–
Sesquiterpene					
trans-α-Bergamotene	–	tr–0.01	–	–	–
Bicyclogermacrene	–	0.03	–	–	–
β-Bisabolene	–	0.02–0.41	–	–	–
γ-Cadinene	–	tr–0.01	–	–	0.11–0.12
δ-Cadinene	0.12	0.08–0.13	–	0.15	–
β-Caryophyllene	0.24–0.25	0.18–0.46	0.17–0.34	–	0.16–0.18
α-Copaene	0.06	0.08–0.16	0.04–0.08	0.10–0.12	0.08–0.09
β-Copaene	0.04	0.06	–	–	–
α-Cubebene	–	+	0.06–0.08	–	–
β-Cubebene	–	+	0.07	0.11–0.16	–
β-Elemene	0.02	0.05–0.09	–	0–0.01	–
(*E,E*)-α-Farnesene	–	tr–0.01	0.02	–	–
(*Z*)-β-Farnesene	–	0.01–0.03	–	–	–
Germacrene D	–	0.06	–	–	–
α-Humulene	0.07	0.03–0.04	tr–0.03	–	0.02–0.03
α-Muurolene	–	+ [t]	–	0.01–0.03	–
Valencene	tr	tr	–	0.02–0.04	–
Aldehydes					
Aliphatic					
Decanal	0.39–0.46	0.17–0.38	0.22–0.30	0.45–0.53	0.47–0.51

continued

TABLE 1.3 (continued)
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1979–1999)

	Cold-Pressed Oils		Laboratory-Extracted Oils		
			Italian Oils	Other Oils	
	wt%	% Peak Areas	% Peak Areas	wt%	% Peak Areas
Dodecanal	0.02–0.22	tr–0.06	tr–0.02	–	0.07–0.11
Dodec-2-en-1-al*	–	–	0.02–0.05	–	–
Nonanal	0.07–0.12	0.01–0–07	0.05–0.07	0.05–0.06	0.09–0.15
Octanal	0.47–0.79	0.04–0.32	0.23–0.58	0.24–0.45	0.51–0.76
Undecanal	–	0–0.02	tr–0.01	0.01	–
Monoterpene					
Citronellal	0.08–0.13	tr–0.08	0.05	0.12–0.14	0.03–0.06
Geranial	0.06–0.11	tr–0.10	0.07–0.13	0.07–0.13	–
Neral	0.04–0.11	tr–0.06	0.05–0.08	0–0.06	0.03–0.05
Perilla aldehyde	0.02–0.07	tr–0.01	–	–	–
Sesquiterpene					
β-Sinensal	0.02–0.03	0.01	–	–	–
Ketones					
Monoterpene					
Carvone	0.02–0.06	0.01–0.21	0.08	–	–
Sesquiterpene					
Nootkatone	0.02–0.24	0.02–0.84	0.07–0.74	0.07–0.10	0.28–0.64
Alcohols					
Aliphatic					
Decanol	–	tr	0.01–0.08	–	–
Nonanol	–	0.09–0.18	tr–0.02	–	–
Octanol	0.04–0.09	tr–0.08	0.01–0.30	–	0.01–0.05
Monoterpene					
Carveol*	–	–	0.05–0.10	–	–
trans-Carveol	–	tr–0.24	0.03–0.10	–	–
Citronellol	–	tr–0.06	–	0.03	–
Geraniol	tr	tr	tr–0.05	0.01–0.02	–
Isopulegol	–	–	0.05–0.06	–	–
Linalol	0.10–0.14	tr–0.16	0.12–0.45	0.17–0.30	0.04–0.10
Nerol	–	tr	0.01–0.07	–	–
cis-Sabinene hydrate	–	0.01	0.01	–	–
Terpinen-4-ol	–	tr–0.01	0.07–0.18	0.24–0.40	–
α-Terpineol	0.04–0.05	tr–0.05	0.07–0.20	0.05–0.10	0.01–0.05
Sesquiterpene					
Elemol	0.04	0.04[t]	–	0.02–0.04	–
Esters					
Aliphatic					
Decyl acetate	–	0.01–0.10	tr–0.01	0–0.02	–
Octyl acetate	0.05	0.01–0.07	–	0.04–0.09	–

TABLE 1.3 (continued)
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1979–1999)

	Cold-Pressed Oils		Laboratory-Extracted Oils		
			Italian Oils	Other Oils	
	wt%	% Peak Areas	% Peak Areas	wt%	% Peak Areas
Monoterpene					
trans-Carvyl acetate	–	tr[t]	tr	–	–
Citronellyl acetate	0.06	tr–0.01	0.01	–	–
Geranyl acetate	0.04	0.02–0.13	0.05–0.13	0.10–0.15	–
Linalyl acetate	–	tr–0.06	–	–	–
Neryl acetate	0.02	tr–0.02	tr–0.01	–	–
α-Terpinyl acetate	–	0.01	tr–0.04	–	–
	Ethers and oxides				
Monoterpene					
cis-Limonene oxide	0.09	tr–0.02	–	–	–
trans-Limonene oxide	0.04	tr–0.02	–	–	–
cis-Linalol oxide	–	–	0.76	–	0.01–0.05
trans-Linalol oxide	–	–	0.21–0.55	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined.

Appendix to Table 1.3

- The results reported in Table 1.3 and in this appendix, for the different categories of grapefruit oils are taken from the following original papers:
  - Industrial cold-pressed essential oils (wt%): Wilson and Shaw (1980, 1984); Wilson et al. (1981); Myers (1988).
  - Industrial cold-pressed essential oils (relative percentage of peak areas): Cappello et al. (1981); Koketsu et al. (1983); Inoma et al. (1989); Haubruge et al. (1989); Boelens (1991); Schultz et al. (1992); Dugo et al. (1999). The qualitative studies carried out by Mondello et al. (1995b) and by Chamblee et al. (1997) have also been included.
  - Laboratory-extracted Italian oils: Dugo Giacomo et al. (1990); Ruberto et al. (1993, 1997b); Caccioni et al. (1998).
  - Other laboratory oils (wt%): Sawamura et al. (1991).
  - Other laboratory oils (relative percentage of peak areas): Sun and Petraceck (1999). Some of the results presented by these authors are affected by the conditions under which fruits have been subjected before oil extraction (waxing and time and temperature storage).
- Coelutions indicated by one or more authors in chromatographic separations of grapefruit oils:
  - β-Pinene + sabinene; limonene + *p*-cymene + β-phellandrene; α-phellandrene + octanal; citronellal + octyl acetate; geraniol + geranyl acetate; *cis*-limonene oxide + *trans*-limonene oxide. Ranges reported in Table 1.3, for some components in cold-pressed oils, coeluted in chromatographic separation reported by few authors (*p*-cymene, α-phellandrene, β-phellandrene, β-pinene), are determined considering the results where coelutions did not occur. It should be highlighted the high value of myrcene (3.76%) reported by Wilson and Shaw (1980) and by Wilson et al. (1981) probably due to the coelution of myrcene with other compounds.
- In addition to those reported in Table 1.3 in grapefruit oils were determined the components listed below:
  - Cold-pressed oils: hexadecanal (0.01%), 6-methyl-5-epten-2-one (0.07%), *cis*-carveol (0.12%), *cis*-β-terpineol (0.01%), (*E,E*)-farnesol (0.01%), (*E,Z*)-farnesol (0.01%), *p*-mentha-1,8(9)-dien-10-yl-acetate (tr–0.01%); limonene dioxide (0.18%); limonene oxide* (0.28%), and trace amounts of (*E,E*)-2,4-decadienal, (*E,Z*)-2,4-decadienal, (*E*)-2-hexenal, tetradecanal, α-sinensal, (*Z*)-3-hexenol, germacrene D-4-ol[t], (*E*)-nerolidol, ethyl butyrate, *trans*-hexenyl butyrate*, methyl *N*-methyl anthranilate, perillene; caryophyllene oxide*; also not quantitatively determined germacrene B.
  - Laboratory oils (Italy): hex-1-en-3-ol (0.01%), linalol oxide* (0.66%), and trace amounts of *trans*-sabinene hydrate.
  - Others laboratory oils: ocimene* (0.19%–0.38%), α-caryophyllene (0.04%–0.06%), 2-undecenal* (0.01%–0.03%), *cis-p*-mentha-2,8-dien-1-ol (0.02%–0.24%), nerolidol* (0.01%–0.02%), nonyl acetate (0.03%–0.06%), *trans*-linalol oxide (0.07%–0.33%).

TABLE 1.4
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1998–2009)

	Industrial Oils					Laboratory-Extracted Oils						
						Cold-Pressed			Solvent	Distilled		
	1	2	3	4	5	6	7	8	9	10a	10b	11
						Hydrocarbons						
Monoterpene												
Camphene	–	–	–	–	–	tr	0–tr	–	tr	tr	tr	–
p-Cymene	1.42	–	–	–	–	tr	0–0.01	–	–	–	–	–
Limonene	93.52	84.8	+	93.4–94.5[b]	96.2	92.12	93.61–94.41	92.5	94.87	93.0	92.2	94.2
Myrcene	1.58	6.9	+	1.87– 1.92	1.5	1.82	1.76–1.82	2.6	1.75	1.8	2.1	–
(*E*)-β-Ocimene	–	–	–	–	–	0.43	0.34–0.47	–	0.18	0.2	0.2	0.2
(*Z*)-β-Ocimene	–	–	–	–	–	–	0.01–0.02	–	0.01	tr	–	–
α-Phellandrene	–	–	–	–	–	0.02	tr	tr	–	0.1	tr	–
β-Phellandrene	–	–	–	b	–	0.23	tr	–	0.12	–	tr	–
α-Pinene	0.54	1.7	–	0.48–0.55	0.5	0.39	0.48–0.58	0.2	0.53	0.4	0.3	0.7
β-Pinene	–	0.1	–	0.02–0.04	tr	0.68	0.06–0.08	0.6	0.03	tr	tr	–
Sabinene	0.29	–	–	0.28–0.43	0.4	0.22	1.30–1.54	0.4	0.42	1.2	0.4	0.2
α-Terpinene	tr	–	–	–	–	–	–	tr	–	tr	–	–
γ-Terpinene	tr	–	–	–	–	0.02	0–tr	0.1	0.01	tr	tr	0.4
Terpinolene	tr	–	–	–	0.1	tr	tr–0.01	–	0.01	tr	0.3	–
α-Thujene	–	–	–	–	tr	tr	–	–	0.01	tr	tr	0.1
Sesquiterpene												
cis-α-Bergamotene	–	–	–	–	–	–	–	–	0.02	–	–	–
trans-α-Bergamotene	–	–	–	–	–	–	–	–	tr	–	–	–
Bicyclogermacrene	–	0.1	–	0.02–0.04	–	–	0–tr	–	–	–	–	–
δ-Cadinene	tr	0.4	–	0.09–0.15	–	0.15	0.03–0.08	0.2	0.11	–	–	0.1
β-Caryophyllene	0.17	1.1	–	0.28–0.32	0.2	0.57	0.27.0.32	0.4	0.25	0.3	0.3	0.2
α-Copaene	–	0.4	–	0.09–0.14	–	0.15	0–0.07	0.1	–	0.1	0.1	0.1

α-Cubebene	tr	–	–	0.04–0.05[c]	–	–	–	–	–	–	0.1	–
β-Cubebene	–	0.5	–	0.09–0.15[d]	–	–	0.05–0.07	0.1	–	–	–	0.1
β-Elemene	–	–	–	0.09–0.15[d]	–	0.16	0.01–0.02	–	–	–	–	–
(*E*)-β-Farnesene	–	–	–	–	–	–	0.01–0.06	–	0.04	–	–	–
Germacrene D	–	0.3	–	0.06–0.10	tr	–	0.04–0.05	–	0.06	–	–	0.1
α-Humulene	tr	0.2	–	0.04–0.05	–	0.07	tr–0.03	tr	0.04	tr	tr	–
Aldehydes												
Aliphatic												
Decanal	–	1.4	+	0.30–0.53	0.2	tr	0.14–0.21	0.2	0.16	0.2	0.2	0.1
Dodecanal	–	–	+	–	–	0.03	0.01–0.02	–	–	tr	tr	–
2-Dodecenal*	–	–	–	–	–	–	–	–	–	tr	tr	–
Nonanal	–	0.5[a]	+	0.06–0.10	tr	tr	0.01–0.03	tr	0.03	0.1	0.1	–
Octanal	–	–	+	0.34–0.51	0.5	–	0.03–0.44	0.2	0.34	0.2	–	0.5
Undecanal	–	–	–	–	–	0.02	0–tr	–	–	–	tr	–
Monoterpene												
Citronellal	tr	0.2	+	0.06–0.10	–	0.06	0.03–0.08	0.1	0.04	–	–	0.1
Geranial	0.11	0.4	+	0.07–0.10	–	0.08	0.04–0.08	0.1	0.07	0.1	0.1	–
Neral	0.15	0.2	+	0.03–0.05	–	0.07	0.02–0.07	0.1	0.04	0.1	–	–
Perilla aldehyde	–	–	–	–	–	0.06	0–0.01	0.1	–	–	–	–
Sesquiterpene												
β-sinensal	–	–	+	–	–	0.05	0–0.02	–	0.04	–	–	–
Ketones												
Sesquiterpene												
Nootkatone	–	tr	+	tr–0.37[e]	–	0.06	0.01–0.04	0.2	0.18	0.5	0.7	–
Alcohols												
Aliphatic												
Octanol	–	–	+	0.03–0.05	–	–	tr–0.03	–	–	0.1	0.3	–

continued

TABLE 1.4 (continued)
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1998–2009)

	Industrial Oils					Laboratory-Extracted Oils						
						Cold-Pressed			Solvent	Distilled		
	1	2	3	4	5	6	7	8	9	10a	10b	11
Monterpene												
Citronellol	–	–	+	–	–	0.04	0–0.01	–	0.02	–	–	–
Geraniol	–	–	+	–	–	0.11	–	tr	0.03	tr	tr	–
Linalol	0.12	0.5[a]	+	0.07–0.10	–	0.09	0.07–0.16	0.2	0.14	0.4	0.3	–
Nerol	tr	–	+	–	–	0.08	0–tr	–	–	tr	0.1	–
trans-Sabinene hydrate	–	–	–	–	–	tr	0.01–0.03	–	–	–	–	–
α-Terpineol	–	0.2	–	0.03–0.08	tr	0.05	0.03–0.07	0.1	0.08	0.2	0.2	0.1
Terpinen-4-ol	–	–	+	–	–	tr	0–tr	–	–	0.1	0.1	–
Sesquiterpene												
Elemol	–	0.2	–	–	–	–	0.01–0.02	–	–	–	–	–
(*E*)-Nerolidol	–	tr	–	–	–	0.02	0–0.01	–	–	–	–	–
Esters												
Aliphatic												
Octyl acetate	–	–	–	0.03–0.07	–	–	0–0.04	tr	–	–	–	–
Monoterpene												
Citronellyl acetate	–	–	–	–	–	tr	0–0.01	tr	–	–	–	–
Geranyl acetate	–	–	–	0.04–0.05[c]	–	0.06	0.05–0.15	0.1	0.10	0.1	0.1	–
Neryl acetate	–	0.1	–	–	–	tr	–	0.1	tr	tr	tr	–
Ethers and oxides												
Monoterpene												
1,8-Cineole	–	–	+	–	–	–	–	–	0.24	–	–	–
cis-Limonene oxide	–	0.1	–	–	0.1	–	0–tr	–	–	–	–	–
trans-Limonene oxide	–	0.1	–	–	tr	–	0–0.06	–	–	–	–	–

Notes: tr, traces; *, correct isomer not characterized; +, identified but not quantified; [a] nonanal + linalol; [b] limonene + β-phellandrene; [c] α-cubebene + geranyl acetate; [d] β-cubebene + β-elemene; [e] nootkatone is present at trace levels in Cuban white grapefruit oils, it ranges from 0.28% to 0.37% in the other oils analyzed.

Appendix to Table 1.4

1. Oberhofer et al. (1999). One sample of commercial oil acquired in Italy; GC/FID on capillary columns coated with HP-5 (25 m × 0.32 mm × 0.52 μm), OV-1 (25 m × 0.25 mm × 0.3 μm), Carbowax (25 m × 0.25 mm × 0.3 μm); GC/sniffing technique on capillary column (25 m × 0.53 mm × 0.3 μm) coated with FSOT-RSL-150; GC/IR/MS on capillary columns coated with RSL-200 (30 m × 0.52 mm × 0.25 μm) or with Stabilwax (60 m × 0.32 mm × 0.25 μm); NBS and Wiley MS libraries; EPA-REVA and Robertet IR libraries; LRI on OV-1 are reported; relative percentage of peak areas. Oberhofer et al. also found pulegone (0.17%).
2. Pino and Sanchez (2000). Cuba; one sample; GC/MS on capillary column (25 m × 0.32 mm × 0.25 μm) coated with DB-1; IDENT database homemade MS library. Authors did not indicate how the results were expressed. From the limonene content (84.8%) it is possible to assume that the results were expressed as wt%; Pino and Sanchez also found trace amounts of germacrene D-4-ol.
3. Lin and Rouseff (2001). One commercial oil acquired in Florida; GC/FID and GC-O on capillary columns coated with DB-Wax (30 m × 0.32 mm × 0.5 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; LRI on DB-Wax and on DB-1 columns are reported. Lin and Rouseff also found (*E*,*E*)-2,4-decadienal, (*E*)-2-decenal, (*Z*)-2-decenal, *trans*-4,5-epoxy-(*E*)-2 decenal, (*E*,*E*)-2,4-nonadienal, (*Z*)-2-nonenal, (*Z*)-4-nonenal, *trans*-4,5-epoxy-(*E*)-2-nonenal), 2(or5)-ethyl-4-hydroxy-5(or2)-methyl-3(2H)-furanone, eugenol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, β-ionone, 4-mercapto-4-methyl pentan-2-ol, wine lactone, 4-vinylguaiacol, undecanoic acid, methional.
4. Feger et al. (2001b). Several samples (number not indicated) of white grapefruit oil from Cuba, Israel, Florida, and of pink grapefruit oils from Florida; GC/FID and GC/MS on capillary columns coated with DB-1 (66 m × 0.25 mm × 0.5 μm), OV-1701 (30 m × 0.25 mm × 0.25 μm); home-made GC/MS library; relative percentage of peak areas; the analyses on OV-1701 were performed to prevent peak overlapping (octanal/myrcene, *p*-cymene/limonene/β-phellandrene, bicyclogermacrene/α-muurolene, dodecanal/decyl acetate/*p*-mentha-1(2),8-dien-10-yl acetate). Feger et al. also found germacrene A (0.02%–0.03%) and germacrene C (0–tr). The quantification of germacrenes A and B as well as δ- and γ-elemene was carried out with a gentle GC method to minimize Cope rearrangement during analysis.
5. Viuda-Martos et al. (2009). Spain; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 × 0.25 μm) coated with HP-5MS; Wiley 229 MS library; LRI on HP-5MS are reported; relative percentage of peak areas; Viuda-Martos et al. also found trace amounts of β-ocimene*, carvone, *trans*-carveol.
6. Huang and Wu (1998). China; Marsh grapefruit; one sample cold-pressed; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-17 and OV-101; relative percentage of peak areas; Huang and Wu also found γ-elemene (0.05%), α-farnesene* (0.05%), γ-muurolene (0.11%), hexanol (0.02%), and trace amounts of β-farnesene*, valencene, (*Z*)-3-hexenol, *cis*-sabinene hydrate, thymol.
7. Sawamura (2000). Japan; three sample cold-pressed from the cvs.: Marsh, Redblush, Star Ruby; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25μm) coated with Thermon 600T; relative percentage of peak areas. Sawamura also found β-bisabolene (0%–0.02%), α-muurolene (0%–0.01%), bicyclopentan-2-one (0%–0.01%), carvone (0%–0.04%), 2,7-dimethyl-2,6-octanediol (tr–0.01%), hexadecanol (0%–0.01%), 2-octen-4-ol* (tr–0.01%), *trans*-carveol (0%–0.01%), δ-cadinol (0%–0.01%), α-muurolol (0%–0.01%), decyl acetate (0.01%), *cis*-carvyl acetate (0%–0.04%) and in one or more of the analyzed samples, trace amounts of γ-cadinene, octadecanal and nonyl acetate.
8. Kirbaslar et al. (2006). Antalya, Turkey; one sample laboratory cold-pressed; GC/FID and GC/MS on capillary columns coated with DB-5 (60 m × 0.25 mm × 0.25 μm) and Innowax (30 m × 0.25 mm × 0.25 μm); MS libraries: Wiley and NIST; relative percentage of peak areas.

continued

TABLE 1.4 (continued)
Percentage Composition of the Volatile Fraction of Grapefruit Oils (1998–2009)

9. Gancel et al. (2002). Corsica, France; Star Ruby grapefruit; one laboratory sample extracted with pentane: ether (1:1); GC/FID and GC/MS on capillary column coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); LRI on DB-Wax and on DB-1 columns are reported; the results were expressed as mg/g of fresh fruit, but for a direct comparison are here reported the relative percentage determined from the original paper. Gancel et al. also found β-bisabolene (0.03%), and trace amounts of hexanal, (*E*)-2-hexenal.
10. Ruberto et al. (1999). Sicily, Italy; two samples laboratory extracted by steam distillation (a) Duncan grapefruit (b) not specified cv.; GC/FID and GC/MS on capillary column (25 m × 0.2 mm × 0.33 μm) coated with HP-1; MS libraries: Wiley and NBS; relative percentage of peak areas. Ruberto et al. also found in sample (a) carveol* (0.1%), linalol oxide* (0.7%), and trace amounts of decanol, nonanol, isopulegol, decyl acetate, α-terpinyl acetate; in sample (b) decanol (0.1%), carvone (0.1%), *cis*-linalol oxide (0.8%), *trans*-linalol oxide (0.3%), and trace amounts of δ-3-carene, α-farnesene*, 1-hexen-3-ol, nonanol, carveol, isopulegol, decyl acetate, carvyl acetate, terpinyl acetate*. The same results were previously reported by Ruberto et al. (1997b).
11. Karioti et al. (2007). Nigeria; one sample laboratory extracted by hydrodistillation; GC/FID on capillary column (30 m × 0.32 mm × 0.25 μm) coated with Supelcowax-10; GC/MS on capillary columns coated with HP Innowax (30 m × 0.25 mm × 0.50 μm) and with HP-5 MS (30 m × 0.25 mm × 0.25 μm); MS libraries: Wiley, NBS and Adams; LRI on HP-5 MS and on HP Innowax are reported; relative percentage of peak areas.

amount in 26 oils of grapefruit, determined by GC, of limonene (94.8%); myrcene (1.8%); and of total aldehydes (1.3%). Veriotti et al. (2002) analyzed one sample of grapefruit oil by fast gas chromatography and TOF/MS. Their results do not appear reliable, nor representative of grapefruit oil due to the identification of unusual components such as δ-2-carene, δ-3-carene-2-ol, and verbenene and especially due to the identification in the sesquiterpene zone of the chromatogram of one ocimene.*

1.3.2.2 Laboratory Oils

Table 1.4 also reports the results of articles published after the review by Dugo et al. (2002) on the composition of laboratory-extracted grapefruit oils. Three are relative to manually cold-pressed peel oils (Huang and Wu, 1998); Sawamura et al., 2000; Kirbaslar et al., 2006), one on a solvent-extracted oil (Gancel et al., 2002) and two on oils extracted by distillation techniques (Ruberto et al., 1999; Karioti et al., 2007).

These oils generally present a composition sufficiently similar among themselves, at least for the components identified and quantitatively determined, and are in good agreement with the oils industrially extracted.

It is, however, surprising that Sawamura (2000) is able to separate enantiomer pairs, using a conventional polar stationary phase.

Not included in Table 1.4 are the results relative to the composition of two oils from Venezuela obtained by distillation analyzed by de Gonzalez et al. (2002); these oils are characterized by the high level of octanal (3.58% and 2.22%) and for the unusual presence of octyl formate (0.78% and 0.95%).

1.4 KEY LIME OIL (*CITRUS AURANTIFOLIA* [CHRISTM.] SWING.) AND PERSIAN LIME OIL (*CITRUS LATIFOLIA* TAN.)

1.4.1 1960–1999

Table 1.5 summarizes the results relative to articles published from 1979 to 1999 on the volatile fraction of lime oils, already revised by Dugo et al. (2002). The data relative to the composition of Key lime type A, Key lime type B, Persian lime, commercial lime oils, and laboratory-extracted Key and Persian lime oils are reported separately. If not otherwise specified, the results are expressed as relative percentage of peak areas.

1.4.2 1998–2009

1.4.2.1 Key Lime Industrial Oils

The investigations on the composition of the whole volatile fraction of Key lime industrially cold-pressed published in this time range are quite scant. The data available are relative to a commercial oil (Kubeczka and Formáček, 2002), to the study by Dugo et al. (2010b) on two samples of Key lime type A and two samples of type B of secure Mexican origin, and to a study on sesquiterpene hydrocarbons by Feger et al. (2000). These data are reported in Table 1.6.

In particular, the qualitative and quantitative results by Dugo et al. (2010b) can be considered significant of the cold-pressed Key lime oils and in good agreement with those previously reviewed by Dugo et al. (2002). The analyses were carried out on a slightly polar column (SLB-5MS). The components were identified by GC-MS using LRI obtained from literature and determined on pure standards, and with MS libraries, one commercial, the other built in a laboratory, allowing the interactive use of MS data with chromatographic data (Mondello et al., 1995a).

Other papers are available on the composition of Key lime oils. Veriotti and Sacks (2001), reported the results of a qualitative study on a commercial oil performed by high-speed GC and GC and TOF/MS. Their results are absolutely improbable, based on acritical comparison with MS spectra of commercial libraries, and must therefore be rejected since they do not contribute to the

TABLE 1.5
Percentage Composition of the Volatile Fraction of Lime Oils (1979–1999)

	Cold-Pressed Key Lime Oils				Cold-Pressed Persian Lime Oils	Commercial Oils	Laboratory Oils		
	Type A		Type B				Key Lime Oils		Persian Lime Oils
	wt%	% Peak Areas	wt%	% Peak Areas			Hydrodistilled	Cold-Pressed	Cold-Pressed
					Hydrocarbons				
Aliphatic									
Decane	–	0–tr	–	tr	tr	–	–	–	–
Dodecane	–	tr–0.01	–	0.03	0.01–0.02	–	–	–	–
Nonane	–	0.01–0.02	–	0.02	tr	–	–	–	–
Tridecane	–	0.01–0.03	–	0.01	tr	–	–	–	–
Monoterpene									
Camphene	0.10	0.10–0.12	0.10	0.09–0.11	0.06–0.11	0–0.5	0.16	–	0.1
δ-3-Carene	–	tr–0.02	–	tr–0.02	tr–0.01	–	0.48	–	tr
p-Cymene	0.38	0.23–0.62	0.17	0.27–1.95	0.11–10.43	0.1–1.3	–	0.1	0.1
Limonene	43.89	49.28–50.01	38.40	47.87–49.38	51.47–59.81	43.7–56.2	39.28	50.5	52.2
Myrcene	1.09	1.17–1.30	1.04	1.18–1.22	0.89–1.76	0.8–1.6	1.00	1.4	1.3
Allo-ocimene	–	tr	–	–	tr	–	–	–	–
(*E*)-β-Ocimene	0.31	0.38–0.40	0.37	0.34	0.08–0.17	–	0.47	–	tr
(*Z*)-β-Ocimene	–	0.13–0.14	–	0.13	0.04–0.09	0.1–0.3	0.14	–	tr
α-Phellandrene	0.04	0.02–0.04	0.04	0.03–0.05	tr–0.05	–	0.08	–	tr
β-Phellandrene	–	–	–	–	tr	–	–	–	tr
α-Pinene	2.28	2.23–2.70	2.10	2.16–2.44	1.96–5.03	2.7–3.1	1.45	3.6	3.2
β-Pinene	19.50	19.95–25.45	17.42	19.53–24.33	11.02–16.04	4.3–23.0	28.44	13.4	13.0
Sabinene	3.10	3.04	3.19	3.28	0.91–2.07	0.4–4.2	–	–	2.0
α-Terpinene	0.14	0.17–0.35	0.15	0.09–0.16	tr–0.33	0.1–0.4	0.03	0.3	0.3
γ-Terpinene	7.39	7.10–8.04	7.59	6.19–8.23	12.55–15.65[f]	2.5–16.6	0.79	17.7	17.0
Terpinolene	0.35	0.37–0.49	0.38	0.31–0.45	0–0.70	0.5–0.7	0.43	0.7	0.7

Thuja-2,4(10)-diene	–	tr–0.01	–	tr	tr	–	–	–	–
α-Thujene	0.38	0.35–0.43	0.36	0.37–0.39	0.46–0.60	–	0.07	–	tr
Tricyclene	–	0.01	–	0.01	tr–0.01	–	–	–	–
Sesquiterpene									
α-Bergamotene*	–	–	–	–	–	–	0.41	–	0.8
cis-α-Bergamotene	0.12	0.08–0.10	0.11	0.09	0.07–0.08	–	–	–	–
trans-α-Bergamotene	1.15	1.13–1.58	1.01	1.20–1.35	0.86–1.34	–	–	–	–
(*Z*)-α-Bisabolene	0.05	0.06–0.20	0.07	0.15	0.04–0.16	–	–	–	–
β-Bisabolene	1.54	2.11–3.79	1.27	1.97–3.08	0.24–2.25	1.5–2.3[i]	tr	–	0.8
(*E*)-γ-Bisabolene	–	tr	–	tr	tr	–	–	–	–
(*Z*)-γ-Bisabolene	–	tr	–	tr	tr	–	–	–	–
β-Caryophyllene	0.82	0.93–1.19	0.89	0.98–1.16	0.07–0.72	0.1–0.9	0.84	–	0.3
α-Elemene	–	0.08	–	–	0.09	–	tr	–	–
β-Elemene	–	0.12–0.22	–	0.16	0.05–0.09	–	0.45	–	0.1
δ-Elemene	0.06[t]	0.25–0.37	0.08[t]	0.07	0.03–0.12	–	0.44	–	0.1
(*E*,*E*)-α-Farnesene	1.24[t]	1.55–3.79	1.33[t]	1.89–3.08	0.44–2.25	–	–	–	0.2
(*E*)-β-Farnesene	0.11[t]	0.11–0.16	0.10[t]	0.12	0.10–0.12	–	1.48	–	0.1
(*Z*)-β-Farnesene	+	0.01–0.02	+	0.01	tr–0.01	–	0.41	–	–
Germacrene B	0.14	0.43–0.57	0.39	0.33	0.10–0.19	–	–	–	0.1
Germacrene D	–	0.26–0.49	–	0.16	0.06–0.12	–	–	–	0.1
α-Humulene	0.14[t]	0.10–0.14	0.13[t]	0.11	0.04–0.06	–	0.12	–	tr
β-Santalene	–	0.04–0.06	–	0.05	0.04–0.05	–	0.03	–	tr
α-Selinene	–	0.08–0.13	–	0.08	0.03–0.07	–	–	–	–
Aldehydes									
Aliphatic									
Decanal	0.25	0.20–0.22	0.29	0.14–0.24	0.04–0.09	–	0.41	tr	0.1
Dodecanal	0.17	0.10–0.18	0.16	0.08–0.12	tr–0.25	–	0.10	–	–
Hexadecanal	–	0.04–0.07	–	0.04–0.06	0.06–0.12	–	–	–	0.1
Nonanal	0.02	0.02–0.03[b]	0.02	0.02[b]	0.01–0.05[b]	–	–	–	tr
Octanal	0.04	0.05–0.06[c]	–	0.04[c]	0–0.05[c]	–	tr	tr	tr

continued

TABLE 1.5 (continued)
Percentage Composition of the Volatile Fraction of Lime Oils (1979–1999)

	Cold-Pressed Key Lime Oils				Cold-Pressed Persian Lime Oils	Commercial Oils	Laboratory Oils		
	Type A		Type B				Key Lime Oils		Persian Lime Oils
	wt%	% Peak Areas	wt%	% Peak Areas			Hydrodistilled	Cold-Pressed	Cold-Pressed
Pentadecanal	–	tr–0.01	–	0.01	tr–0.01	–	–	–	–
Tetradecanal	0.04	0.04–0.06	0.04	0.04–0.05	0.01–0.05	–	–	–	tr
Undecanal	0.04	0.02–0.04	0.05	0.02–0.03	0.01–0.07	–	–	–	tr
Tridecanal	–	0.01	–	–	0.01	–	–	–	tr
Monoterpene									
Citronellal	0.03	0.01–0.03	0.05	0.03–0.04	0.03–0.06	–	0.10	0.1	0.1
Geranial	2.66	1.71–2.36[d]	3.65	1.97–2.99[d]	1.81–3.93	0.3–4.9	2.05	2.1	2.3
Neral	1.73	1.06–1.43	2.31	1.15–1.82	0.48–1.72	0.5–1.0	5.30	1.0	1.2
Perilla aldehyde	–	1.71–2.00[d]	–	1.97[d]	1.81–2.77[d]	–	–	–	tr
Ketones									
Aliphatic									
6-Methyl-5-hepten-2-one	0.02	0.01–0.03	0.02	0.01–0.02	tr–0.07	–	–	–	–
Monoterpene									
cis-Pinocamphone	–	0.02	–	0.02	tr	–	–	–	–
Piperitone[t]	–	tr	–	tr	tr	–	–	–	–
Alcohols									
Aliphatic									
Octanol	0.02[g]	0.02[g]	–	+	0.05–0.09	–	0.11	–	tr
Monoterpene									
Borneol	0.02	0.01–0.04	0.03	0.02	0.01–0.03	–	–	–	tr
Citronellol	–	+	–	–	tr–0.05	–	–	tr	–

Endo-fenchol	–	tr–0.02	–	tr	tr–0.02	–	–	–	–
Geraniol	0.04	0.03–0.07	0.07	0.04–0.07	0.02–0.10	0–0.2	7.50	0.1	tr
Linalol	0.21	0.15–0.18	0.25	0.16–0.17	0.11–0.24	0.1–0.3	1.40	tr	0.2
cis-p-Menth-2-en-1-ol	–	0.01–0.02	–	0.01	tr–0.01	–	–	–	–
trans-p-Menth-2-en-1-ol	–	tr–0.01	–	0.05	tr	–	–	–	tr
Nerol	0.03	0.02–0.05	0.05	0.04–0.09	tr–0.20	–	–	0.1	–
trans-Pinocarveol	–	0.01	–	tr	tr–0.02	–	–	–	–
cis-Sabinene hydrate	–	0.02–0.05	–	0.01	0.03–0.05	–	–	–	0.1
trans-Sabinene hydrate	0.05	0.02–0.04	0.08	0.01	0.04–0.07	–	–	–	0.1
α-Terpineol	0.23	0.22–0.35	0.29	0.21–0.30	0.16–0.37	0.1–0.4	2.39	0.3	0.4
Terpinen-4-ol	0.23	0.22–0.70	0.09	0.04–0.14	0.04–0.17	0.7–1.3[h]	2.01	tr	–
Sesquiterpene									
α-Bisabolol	0.11	0.07–0.10	0.08	0.10	0.07–0.10	–	–	–	tr
α-Cadinol	–	+	–	–	–	–	–	–	0.1
Campherenol	0.05	0.05–0.07	0.04	0.07	0.05–0.08	–	–	–	–
Norbornanol[a]	0.08[t]	0.05–0.08	0.06[t]	0.07	0.05–0.07	–	–	–	–
Spathulenol	–	0.02	–	–	0.03	–	–	–	tr
Esters									
Monoterpene									
Bornyl acetate	0.04	0.01	0.04	0.02	tr–0.01	–	–	–	tr
Citronellyl acetate	0.01	tr	–	–	0.02–0.10	–	–	–	tr
Geranyl acetate	0.29	0.21–0.29	0.31	0.25	0.07–0.63	0.1–1.8	0.59	0.7	1.0
Linalyl acetate	–	–	–	–	tr–0.12	–	–	–	tr
Neryl acetate	0.10	0.07–0.09[e]	0.10	0.07[e]	0.36–1.53	1.5–2.3[i]	0.21	–	1.4
Ethers and oxides									
Monoterpene									
Dehydro-1,8 cineole[t]	–	0–tr	–	tr	tr	–	–	–	–

continued

TABLE 1.5 (continued)
Percentage Composition of the Volatile Fraction of Lime Oils (1979–1999)

	Cold-Pressed Key Lime Oils				Cold-Pressed Persian Lime Oils	Commercial Oils	Laboratory Oils		
	Type A		Type B				Key Lime Oils		Persian Lime Oils
	wt%	% Peak Areas	wt%	% Peak Areas			Hydrodistilled	Cold-Pressed	Cold-Pressed
1,8-Cineole	+	tr	–	tr	–	0.3–0.5	–	–	–
cis-Limonene oxide	–	0.01	–	0.01	0.01	–	0.11	–	–
trans-Limonene oxide	–	tr	–	tr	tr	–	tr	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined; [a] 2,3-dimethyl–3-(4-methyl-3-pentenyl)-2-norbornanol; [b] values reported by Haro and Faas (1985) for nonanal (respectively 0.41% for Key lime type A, 0.45% for Key lime type B and 0.63% for Persian lime oil) were not included, due to a possible co-elution with terpinolene; [c] values reported by Haro and Faas (1985) for octanal (respectively 1.17% for Key lime type A and 1.22% for Key lime type B, and 1.35% for Persian lime oil) were not included, due to a possible co-elution with myrcene; [d] geranial + perylla aldehyde, perilla aldehyde is probably present only at trace levels; [e] values reported by Haro and Faas (1985) for neryl acetate (respectively 0.47% for Key lime oil type A and 0.51% for Key lime oil type B) were not included, due to a possible coelution with a not identified component; [f] variability ranges reported by Koketsu et al. (1983) (1.30%–8.46%) were not included in the table; [g] octanol +1,4-cineole; [h] bergamotene*+ terpinen-4-ol; [i] bisabolene* + neryl acetate.

Appendix to Table 1.5

- The results reported in Table 1.5 and in this appendix, for the different categories of lime oils, are taken from the following original papers:
  - Industrial cold-pressed Key lime oils (wt%): Clark and Chamblee (1992).
  - Industrial cold-pressed Key lime oils (relative percentage of peak areas): Haro and Faas (1985); Dugo P. et al. (1997); Dugo et al. (1999). The qualitative results reported by Clark et al. (1987); Mondello et al. (1995b); Chamblee et al. (1985,1997) were also included.
  - Industrial cold-pressed Persian lime oils: Koketsu et al. (1983); Haro and Faas (1985); Lancas et al. (1988); Dugo P. et al. (1997); Dugo et al. (1999).
  - Commercial oils: Alessandro et al. (1985).
  - Laboratory hydrodistilled Key lime oil: Jantan et al. (1996).
  - Laboratory cold-pressed Key lime oil: Sawamura et al. (1999a).
  - Laboratory-extracted Persian lime oil: Edwards and Marr (1990); Njoroge et al. (1996), Sawamura et al. (1999a).
- Coeluitions indicated by one or more authors in chromatographic separations of lime oils:
  - δ-3-Carene + 1,4-cineole; limonene + 1,8-cineole; limonene + 1,8-cineole + β-phellandrene; myrcene + octanal; β-pinene + sabinene; γ-terpinene+octanol; terpinolene + octanal; terpinolene + nonanal; β-bisabolene + (*E*,*E*)-α-farnesene; citronellal + octyl acetate; geranial + perilla aldehyde; neryl acetate + unknown hydrocarbon; *trans*-α-bergamotene + terpinen-4-ol; octanol + 1,4 cineole.

- The maxima values of β-pinene are affected by the contribute, due to sabinene, of about 3% for cold-pressed Key lime oil and of about 2% for cold-pressed Persian lime oil; the values of β-bisabolene are often affected by a variable contribute due to (*E*,*E*)-α-farnesene; the maxima values of *p*-cymene in cold-pressed Persian lime oils could be explained by the inappropriate storage conditions of the samples, in genuine oil *p*-cymene is generally lower then 1%; the high values of α-pinene in cold-pressed Persian lime oils are reported by Koketsu et al. (1983), the maximum value of α-pinene reported for these oils in other papers is 2.25%.
- In addition to those listed in Table 1.5, in lime oils were found the components listed below:
  - Cold-pressed Key lime oil type A: β-copaene[t] (0.33%), heptanal (0.02%), β-bisabobol (0.01%), selin-11-en-4-α-ol[t] (0.01%), *cis*-sesquisabinene hydrate (0.03%), *trans*-pinocarvyl acetate (0.01%), and trace amounts of α-santalene, *cis*-piperitol, (*E*,*Z*)-farnesol, decyl acetate, 1,4-cineole, also not quantitatively determined, 3-hexanone, (*E*)-epi-β-santalene, farnesal*, *cis*-and *trans*-carveol; *p*-cymen-8-ol, γ-isogeraniol, isopiperitol, *p*-menth-3-en-1-ol, myrtenol, sabinol, β-terpineol*, farnesol*, verbenol, dodecyl acetate.
  - Cold-pressed Key lime oil type B: β-copaene[t] (0.30%).
  - Cold-pressed Persian lime oil: nonanol (0.12%–0.30%), β-bisabolol (0.01%), (*E*,*Z*)-farnesol (0.01%), *cis*-sesquisabinene hydrate (0.03%), and trace amounts of α-santalene, carvacrol, selin-11-en-4-α-ol[t], decyl acetate, α-terpinyl acetate, caryophyllene oxide*.
  - Commercial oils: fenchol* (0%–0.46%).
  - Laboratory-extracted Key lime oils: elemol (0.07%), α-eudesmol (0.06%), β-eudesmol (0.06%), (*Z*)-nerolidol (0.61%), sabinyl acetate (0.10%), hexadecanoic acid (0.06%), and trace amounts of nootkatone, isopulegol.
  - Laboratory-extracted Persian lime oil: β-santalol* (0.05%), nonyl acetate (0.01%), and trace amounts of pentadecane, (*E*)-α-bisabolene, α-cubebene, β-cubebene, γ-elemene, heptadecanal, octadecanal, isopiperitone, (*E*)-2-dodecenol, *trans*-*p*-mentha-2,8-dien-1-ol, thymol, γ-eudesmol, (*E*)-nerolydol, viridiflorol, geranyl formate, *p*-mentha-1,8-dien-10-yl acetate, perillyl acetate, (*E*,*E*)-farnesyl acetate*, decanoic acid, undecanoic acid, cynnamyl alcohol*.
- Among the laboratory-extracted oils were not included those analyzed by El-Samahy et al. (1982) for an Egyptian oil and by Khurdiya and Maheshwari (1988) for two Indian oils which presented a composition absolutely incompatible with that of lime oils. High values of monoterpene alcohols reported by Jantan et al. (1996) for a Key lime oil obtained by hydrodistillation in laboratory from fruits cultivated in Malaysia should be highlighted; these values are surely due to the extraction technique; in this paper it was also reported a particularly high value of neral (5.30%). Also not reported in the table are the results of Yang, R.-H. et al. (1992); these authors in a paper on the odor quality of different citrus peel oils determined the composition of the hydrocarbon and oxygenated fraction of Japanese Key lime oil obtained by solvent extraction. In addition to the compounds present in Table 1.5 and in his appendix Yang, R.-H. et al. found α-caryophyllene, α-cedrene, γ-muurolene, (*E*,*E*)-2,4-decadienal, 2,6-dimethyl-5-hexanal, camphor, fenchone, decanol, perillyl alcohol, hexyl acetate, octyl acetate, methyl geranate.
- In a paper of McHale (1980) are also reported the following information on the composition of the volatile fraction of ecuelled lime oil: myrcene (1.2%), limonene (41.8%), β-phellandrene (0.6%), α-pinene (2.9%), β-pinene (17.8%), sabinene (3.1%), γ-terpinene (7.2%), terpinolene (0.4%), *trans*-α-bergamotene (0.6%), β-bisabolene (1.0%), caryophyllene* (0.5%), β-sesquiphellandrene (probably misidentification of (*E*,*E*)-α-farnesene 1.0%), linear chained aldehydes C_8–C_{16} (1.0%), geranial + neral (6.2%), α-terpineol (0.3%), other alcohols (fenchol*, linalol, terpinen-4-ol, 0.3%), geranyl acetate + neryl acetate (0.5%) and not quantitatively determined camphene, *p*-cymene, isoterpinolene, α-terpinene, δ-elemene, α-, and β-humulene.
- The results published from 1979 to 1999, even if based on a scant number of papers, provide a good description of the real composition of industrial cold-pressed Key and Persian lime oils. The composition of the two oils is very similar and only some differences appear in the quantitative composition: β-pinene is higher in Key (17%–22%) than in Persian lime oil (11%–16%) and γ-terpinene is higher in Persian (13%–15%) than in Key lime oil (6%–8%); limonene represents at the most the 50% of the whole volatile fraction in Key lime but its content is higher in Persian lime.

TABLE 1.6
Percentage Composition of the Volatile Fraction of Key Lime Oils (1998–2009)

	Industrial						Laboratory											
							Cold-Pressed				Solvent		Distilled					
	1	2a		2b		3a	3b	4	5	6	7	8	9	10a	10b	11,12(a)	11,12(b)	13a	13b
								Hydrocarbons											
Aliphatic																			
Tridecane	–	0.01	–	0.01	–	–	–	–	–	–	tr	–	–	–	–	–	–	0.3	0.2
Monoterpene																			
Camphene	0.11	0.07	0.08	0.09	0.07	–	–	0.06	0.06	tr–0.2	tr	0.09	–	0.09	–	0.1–0.2	0.1–0.2	0.2	0.3
δ-3-Carene	–	tr	–	tr	–	–	–	tr	–	–	–	–	–	–	–	–	–	–	–
p-Cymene	0.33	0.24	0.11	0.33	0.10	–	–	0.19	0.10	0.2–5.2[a]	0.1	0.09	0.2	4.42	7.98	–	–	–	–
p-Cymenene	–	tr	–	0.01	–	–	–	–	–	0–0.01	–	–	–	–	–	–	–	–	–
Limonene	48.24	51.14	50.29	50.96	49.60	–	–	50.17	50.44	39.9–66.8[b]	71.0	45.35	30.5	45.31	70.70	33.0–35.3	45.1–47.0	21.0	21.3
Myrcene	1.26	1.01	1.05	1.07	1.04	–	–	1.22	1.42	0.8–1.5	1.6	1.10	1.0	0.24	1.24	0.6–0.7	1.5–1.6	1.6	1.4
(*E*)-β-Ocimene	0.33	0.22	0.29	0.30	0.28	–	–	0.49	–	0.2–0.5	0.1	0.35	0.2	0.24	0.21	–	–	–	0.2
(*Z*)-β-Ocimene	0.12	0.09	0.12	0.13	0.12	–	–	0.31	0.04	0.1–0.3	0.3	0.16	–	–	–	–	–	0.6	–
α-Phellandrene	0.04	0.03	0.03	0.05	0.03	–	–	tr	tr	0–0.1	0.1	–	–	–	–	0.4–0.5	–	0.3	0.3
β-Phellandrene	0.46	–	–	–	–	–	–	0.25	tr	0–0.5[c]	–	0.37	–	–	–	–	–	–	–
α-Pinene	2.46	2.09	1.96	1.92	1.91	–	–	2.09	3.61	1.1–2.1	1.3	2.51	1.4	1.37	0.84	1.2–1.4	2.4–2.5	1.9	1.4
β-Pinene	21.10	20.17	21.16	18.25	20.10	–	–	12.49	13.41	6.1–19.2[d]	3.7	19.28	7.9	15.65	0.91	13.5–15.1	21.7–23.8	13.1	8.4
Sabinene	3.06	2.45	2.43	2.31	3.02	–	–	1.87	2.31	1.0–5.4[e]	0.9	2.65	1.3	1.44	0.14	–	–	–	–
α-Terpinene	0.20	0.20	0.19	0.23	0.13	–	–	tr	0.30	0.1–0.3	0.2	0.11	0.3	–	–	–	–	0.9	0.9
γ-Terpinene	8.12	9.29	7.66	9.79	8.68	–	–	15.64	17.72	6.9–15.5[f]	11.9	12.20	19.2	3.54	4.13	7.9–8.4	9.6–9.9	8.3	8.9
Terpinolene	0.41	0.38	0.33	0.52	0.33	–	–	0.07	0.73	0.4–1.1	0.6	0.36	0.8	0.32	0.25	0.5–0.7	0.5–0.7	2.5	8.5
α-Thujene	0.42	0.33	0.27	0.31	0.29	–	–	0.56	–	0.2–0.5	–	0.60	0.4	0.19	0.20	–	–	–	–
Sesquiterpene																			
Aromadendrene	–	–	–			–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
α-Bergamotene*	–	–	–			–	–	1.25	0.83	–	–	–	1.7	–	–	–	–	–	–

cis-α-Bergamotene	0.12	0.07	–	0.06	–	0.08	0.08	–	–	–	–	0.09	–	–	–	–	–	–	–
trans-α-Bergamotene	1.12	1.12	1.14	0.86	0.93	1.15	0.96	–	–	0.3–1.0	–	1.08	–	0.66	0.55	–	–	–	4.2
trans-β-Bergamotene	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8	–
Bicyclogermacrene	–	–	0.15	tr	0.12	–	–	–	–	–	0.1	–	–	0.20	0.18	–	–	–	–
(*E*)-α-Bisabolene	–	0.03	–	0.03	–	0.05	0.05	–	–	–	–	–	–	–	–	–	–	0.4	0.2
(*Z*)-α-Bisabolene	–	0.14	–	0.12	–	0.12	0.12	–	–	–	–	–	0.2	–	–	–	–	0.2	0.1
β-Bisabolene	1.78	1.85	1.83	1.35	1.50	1.70	1.47	1.86	–	0.4–2.1	–	2.07	2.8	1.16	0.88	–	–	–	–
(*E*)-γ-Bisabolene	–	–	0.04	0.02	0.03	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	tr
(*Z*)-γ-Bisabolene	–	–	–	tr	–	0.02	0.02	–	–	–	–	–	–	–	–	–	–	–	–
δ-Cadinene	–	–	–	–	–	–	0.1	0.03	–	–	–	–	–	–	0.07	–	–	0.2	0.2
β-Caryophyllene	1.02	0.79	0.96	0.73	0.97	0.95	0.89	0.93	0.32	0–0.5	0.5	0.90	0.7	0.64	0.06	–	–	0.1	0.4
α-Copaene	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	0.04	–	–	0.1	–
γ-Curcumene	–	0.03	–	0.02	–	0.02	0.01	–	–	–	–	–	–	–	–	–	–	–	–
β-Elemene	0.17	0.16	0.29	0.19	0.27	0.07	0.05	0.14	0.09	0–0.8	tr	–	0.3	0.38	0.06	–	–	–	0.4
γ-Elemene	–	0.03	–	0.06	–	–	–	0.07	–	–	–	–	0.1	–	–	–	–	0.3	0.2
δ-Elemene	0.31	0.02	0.56	0.39	0.46	0.05	0.03	0.16	0.10	–	–	–	0.5	0.31	0.12	–	–	0.9	0.4
(*E*,*E*)-α-Farnesene	1.03	1.00	1.27	1.06	1.20	1.25	1.32	0.17	0.13	0–1.9[g]	–	1.28	1.4	0.58	–	–	–	6.4	4.8
(*E*)-β-Farnesene	–	0.10	0.11	0.06	–	0.11	0.10	–	0.07	0–0.1	–	0.10	–	–	–	–	–	0.7	0.3
(*Z*)-β-Farnesene	–	–	–	0.03	0.09	–	–	–	–	–	–	0.08	–	0.09	0.08	–	–	–	–
Germacrene A	-	–	–	–	–	0.36	0.40	–	–	–	–	0.70	–	–	–	–	–	–	–
Germacrene B	–	0.54	0.55	0.52	0.45	0.80	0.75	–	0.09	–	–	1.04	0.3	0.57	–	–	–	–	–
Germacrene C	–	–	–	–	–	0.53	0.52	–	–	–	–	0.50	–	–	–	–	–	–	–
Germacrene D	0.30	0.26	0.07	0.27	0.06	0.36	0.35	–	0.05	0.1–0.5	0.5	0.63	0.2	0.55	0.03	–	–	0.5	0.2
α-Humulene	0.11	0.09	0.11	0.10	0.11	0.12	0.11	0.05	tr	0–0.1	0.1	0.14	–	0.11	–	–	–	–	–
β-Santalene	–	0.04	0.04	0.03	0.03	0.06	0.05	–	0.03	–	–	–	–	–	–	–	–	0.1	0.2
α-Selinene	–	0.06	–	0.07	–	0.07	0.07	–	–	–	–	0.12	–	0.10	0.06	–	–	–	–
7-Epi-α-selinene	–	–	–	0.01	–	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–
β-Selinene	–	0.04	–	0.02	–	0.03	0.03	–	0.03	–	–	–	–	–	–	–	–	–	0.1
β-Sesquiphellandrene	–	–	0.01	–	–	0.01	tr	–	–	–	–	–	–	–	–	–	–	–	–
Aldehydes																			
Aliphatic																			
Decanal	0.20	0.17	0.24	0.18	0.22	–	–	0.08	0.03	0–0.3	0.1	0.11	0.2	–	–	0.4	0–0.1	1.7	0.6
Dodecanal	–	0.10	–	0.09	–	–	–	0.03	0.02	0–tr	tr	–	–	–	–	–	–	–	0.8

continued

TABLE 1.6 (continued)
Percentage Composition of the Volatile Fraction of Key Lime Oils (1998–2009)

	Industrial							Laboratory											
								Cold-Pressed				Solvent		Distilled					
	1	2a		2b		3a	3b	4	5	6	7	8	9	10a	10b	11,12(a)	11,12(b)	13a	13b
Hexadecanal	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–	–	0.2	0.1
Nonanal	0.04	0.02	0.03	0.03	0.03	–	–	0.14	tr	0–0.1	–	0.02	–	–	–	–	–	–	–
Octadecanal	–	–	–	–	–	–	–	–	tr	–	–	–	–	–	–	–	–	–	tr
Octanal	0.03	0.03	0.05	0.04	0.03	–	–	–	tr	0–0.1	0.1	0.03	–	0.97	–	–	–	–	–
Tetradecanal	–	0.04	–	0.03	–	–	–	–	0.01	–	–	0.02	–	–	–	–	–	0.4	–
Undecanal	–	0.02	0.02	0.02	0.02	–	–	0.02	0.01	–	–	0.03	–	–	–	–	–	–	–
Monoterpene																			
Citronellal	–	0.01	tr	0.03	0.03	–	–	0.06	0.08	tr–0.3	0.6	0.05	–	–	–	–	–	0.5	–
Geranial	2.43	1.90	2.29	2.63	2.88	–	–	2.77	–	0.8–6.1[h]	0.2	2.22	5.9	5.21	0.39	3.0–3.6	0.7–0.9	0.9	1.4[q]
Neral	1.36	1.12	1.36	1.61	1.82	–	–	1.72	0.98	0.4–3.1[i]	0.1	1.40	3.8	1.16	0.12	3.6–4.2	1.8–2.2	0.9	1.4[q]
Perilla aldehyde	–	0.02	0.01	0.02	0.02	–	–	0.05	0.03	–	tr	–	–	–	–	–	–	–	–
Sesquiterpene																			
β-Sinensal	–	–	–	–	–	–	–	–	–	–	tr	–	–	0.11	0.16	–	–	–	–
Ketones																			
Aliphatic																			
6-Methyl-5-hepten-2-one	–	0.03	tr	0.03	0.10	–	–	–	–	–	–	–	–	0.06	–	–	–	–	–
Monoterpene																			
Carvone	–	–	–	tr	–	–	–	–	0.01	–	–	–	–	–	–	–	–	–	–
Alcohols																			
Aliphatic																			
Decanol		0.01	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
(*Z*)-3-Hexenol	–	–	–	–	–	–	–	tr	–	–	–	–	–	0.06	–	–	–	–	–
Octanol	–	–	–	tr	0.06	–	–	–	–	–	tr	–	–	–	0.25	–	–	–	–

Monoterpene																		
Citronellol	–	0.01	0.01	–	–	–	–	0.04	0.03	0–0.1	0.1	0.05	–	–	0.04	1.2	0.4–0.6	–	–
p-Cymen-8-ol	–	–	–	0.02	–	–	–	–	–	–	–	–	–	0.11	0.06	–	–	–	–
Endo-fenchol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0–1.2	0.5–0.6	–	–
Fenchol*	–	tr	–	0.04	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	0.7
Geraniol	0.05	0.07	tr	0.07	tr	–	–	0.09	0.14	0–0.3	–	0.18	–	1.49	0.26	5.9–7.3	0.9–1.4	0.7	1.2
Linalol	0.16	0.15	0.17	0.24	0.22	–	–	0.22	0.19	0.2–1.4[j]	0.1	0.32	0.2	0.82	2.18	1.5–1.6	0.5–0.6	–	5.5
Nerol	0.04	0.04	–	0.01	–	–	–	0.14	0.09	0–0.1	tr	0.03	–	4.03	0.31	–	–	–	–
trans-Piperitol	–	tr	–	–	–	–	–	–	–	–	tr	–	–	–	–	–	–	–	–
cis-Sabinene hydrate	–	0.04	–	0.05	–	–	–	0.10	0.07	–	–	–	–	–	–	–	–	–	–
trans-Sabinene hydrate	–	0.03	–	tr	–	–	–	tr	0.06	0–0.1	–	–	–	–	–	–	–	–	–
α-Terpineol	0.26	0.27	0.22	0.80	0.31	–	–	0.44	0.29	0.3–4.7[k]	0.4	0.49	0.7	2.04	1.67	1.9–2.0	0.7–1.1	11.7	14.1
β-Terpineol*	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	0.03	–	–	0.8	0.8
γ-Terpineol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.30	–	–	–	0.6	0.5
Terpinen-4-ol	0.23	0.30[p]	0.41[p]	0.33	0.06	–	–	0.04	0.04	0–1.7[l]	–	0.22	–	1.39	0.68	3.3–3.9	1.2–1.5	2.7	3.3
Thymol	–	–	–	–	–	–	–	0.02	–	–	–	–	–	0.06	0.12	–	–	–	–
Sesquiterpene																			
α-Bisabolol	–	0.08	0.07	0.06	0.06	–	–	–	0.05	–	–	0.09	–	–	–	–	–	0.3	0.2
Elemol	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	0.05	–	–	–	–
γ-Eudesmol	–	–	–	–	–	–	–	–	–	–	–	0.04	–	0.20	0.26	–	–	–	–
(*Z*,*E*)-Farnesol	–	–	–	–	–	–	–	–	–	–	tr	–	–	0.11	0.09	–	–	–	–
(*E*)-Nerolidol	–	–	–	–	–	–	–	–	tr	–	tr	–	–	–	–	–	–	–	–
Spathulenol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	0.05	–	–	–	–
Esters																			
Aliphatic																			
Decyl acetate	–	–	0.13	tr	0.12	–	–	–	tr	–	–	–	–	–	–	–	–	–	–
Dodecyl acetate	–	–	–	0.01	–	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–
Monoterpene																			
Bornyl acetate	–	tr	–	–	–	–	–	–	–	–	–	0.02	–	0.06	0.11	–	–	–	–
Citronellyl acetate	–	0.01	tr	0.01	–	–	–	0.04	0.04	0–0.1	tr	tr	–	0.08	–	–	–	–	tr
Geranyl acetate	0.35	0.18	0.19	0.25	0.19	–	–	0.61	0.71	0.5–2.1[m]	tr	0.41	0.5	0.29	0.06	2.5–3.0	0.6–0.7	0.1	0.2
Geranyl formate	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	0.10	–	–	–	–

continued

TABLE 1.6 (continued)
Percentage Composition of the Volatile Fraction of Key Lime Oils (1998–2009)

	Industrial							Laboratory											
								Cold-Pressed				Solvent		Distilled					
	1	2a		2b		3a	3b	4	5	6	7	8	9	10a	10b	11,12(a)	11,12(b)	13a	13b
Neryl acetate	0.39	0.16	0.07	0.14	0.12	–	–	0.99	–	0–0.9[n]	tr	0.07	2.2	–	–	1.6–1.7	0.9–1.2	0.1	0.2
α-Terpenyl acetate	–	–	tr	–	–	–	–	–	–	–	–	–	–	0.13	0.30	–	–	–	–
Ethers and oxides																			
Monoterpene																			
1,8-Cineole	0.05	–	–	–	–	–	–	–	–	0–0.5[c]	–	0.03	0.4	–	–	–	–	–	–
Limonene dioxide	–	–	–	–	–	–	–	–	0.02	–	tr	–	–	–	–	–	–	–	–
cis-Limonene oxide	–	–	tr	0.01	–	–	–	–	tr	0–tr	tr	–	–	0.13	0.32	–	–	–	–
trans-Limonene oxide	–	–	tr	tr	tr	–	–	–	–	0–tr	tr	–	–	0.06	0.17	–	–	–	–
trans-Linalol oxide	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–	–	–	0.7	–
Thymol methyl ether	–	–	–	–	–	–	–	–	–	0–1.2[o]	tr	–	–	–	–	–	–	–	–
Sesquiterpene																			
Caryophyllene oxide*	–	tr	0.01	0.01	–	–	–	–	–	0–tr	–	–	–	0.06	0.13	–	–	–	–

Notes: tr, traces; * correct isomer not characterized; t, tentative identification; [a] *p*-cymene content is present at high values in the cvs. Mexicaine (2.3%), Kirk (5.2%), Ambilobe (1.3%), in the cvs. Antillaise and Nouvelle Calédonie his content is 0.6% and 0.2%, respectively; [b] limonene is present at highest value (66.8%) in the cv. Mexicaine and at lowest value (39.9%) in the cv. Kirk, in the other cvs. ranges from 46.4% to 49.9%; [c] β-phellandrene + 1,8-cineole, β-phellandrene is absent in the cv. Kirk where 1,8-cineole is 0.6%, 1,8-cineole is absent in the cvs Ambilobe and Antillaise; [d] β-pinene is present at the lowest value (6.1%) in the cv. Mexicaine, in the other cvs. ranges from 14.7% to 19.2%; [e] sabinene is present at the higher value 5.4% in the cv. Mexicaine, in the other cvs. ranges from 1.0% to 3.1%; [f] γ-terpinene is present at the lowest value (6.9%) in the cv. Mexicaine, in the other cvs. ranges from 9.6% to 15.5%; [g] (*E*,*E*)-α-farnesene is absent in the cv. Mexicaine, in the other cvs. ranges from 0.4% to 1.9%; [h] geranial is present at the lowest value (0.8%) in the cv. Mexicaine, in the other cvs. ranges from 2.8% to 6.1%; [i] neral is present at the lowest value (0.4%) in the cv. Mexicaine, in the other cvs. ranges from 1.5% to 3.1%; [j] linalol is present at the highest value (1.4%) in the cv. Kirk, in the other cvs. ranges from 0.2% to 0.4%; [k] α-terpineol is present at the highest value in the cv. Kirk (4.7%) in the other cvs. ranges from 0.3% to 0.6%; [l] terpinen-4-ol is absent or present at trace levels in the cvs. Antillaise, Ambilobe, Nouvelle Calédonie, in the other cvs. is present at percentage of 0.9% (cv. Mexicaine) and 1.7% (cv. Kirk); [m] geranyl acetate is present at highest values in the cvs. Kirk (1.8%) and Ambilobe (2.1%), in the other cvs. range from 0.5% to 0.8%; [n] neryl acetate is absent in the cvs. Antillaise and Nouvelle Calédonie in the other cvs. ranges from 0.5% to 0.9%; [o] thymol methyl ether is present only in the cv. Mexicaine; [p] isogeranial + terpinen-4-ol; [q] geranial + neral.

Appendix to Table 1.6

1. Kubeczka and Formáček (2002). One commercial sample; GC/FID on capillary column (50 m) coated with VG 11; ^{13}C-NMR; relative percentage of peak areas. Kubeczka and Formáček also found calamenene (0.40%).
2. Dugo et al. (2010b). Mexico; (a) two samples of Key lime oil type A, (b) two samples of Key lime oil type B; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found in samples (a) tricyclene (0.03%, 0.01%), valencene (0, 0.07%), (*E*,*E*)-farnesal (0.01%, 0), *cis*-pinocamphone (0.01%, tr), campherenol (0, 0.02%), 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol (0, 0.04%), *cis*-sesquisabinene hydrate (0.01%, 0), *trans*-sesquisabinene hydrate (0, 0.01%) and in one or both the two samples trace amounts of decane, 4,8-dimethyl nona-1,3(*E*),7-triene, α-fenchene, allo ocimene, thuja-2,4(10)-diene, pinocarvone, piperitone, 6-methyl-5-hepten-2-ol, borneol, *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol, *cis*-*p*-menth-2-en-1-ol, *trans*-pinocarveol, and in samples (b) decane (0.01%, 0), α-fenchene (0.01%, 0), tricyclene (0.01%, 0.01%), β-sesquisabinene (0.01%, 0), valencene (0.01%, 0.06%), (*Z*)-isocitral (0.02%, 0), (*E*,*E*)-farnesal (0.02%, tr), *cis*-pinocamphone (0.02%, tr), borneol (0.04%, 0), *cis*-*p*-mentha-2,8-dien-1-ol (0.01%, 0), 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol (tr, 0.04%), *cis*-sesquisabinene hydrate (0.01%, tr), *trans*-sesquisabinene hydrate (tr, 0.02%), isobornyl acetate (0.01%, 0), *trans*-pinocarvyl acetate (0.01%, 0), (*Z*)-nerolidyl acetate (0.01%, 0), 2,3-benzopyrrole (0.01%, 0), and in one or both the two samples trace amounts of thuja-2,4(10)-diene, piperitone, *trans*-*p*-menth-2-en-1-ol, octyl acetate, methyl geranate.
3. Feger et al. (2000). Several samples of Key lime oils type A (a) and B (b) from Mexico. Enrichment of sesquiterpene fraction by vacuum distillation; GC/MS of the enriched fraction on capillary columns coated with OV-1701 (30 m × 0.25 mm × 0.25 μm) and DB-1 (33 m × 0.25 mm × 0.5 μm) and (60 m × 0.25 mm × 0.5 μm); GC/FID of the crude oil on the same capillary columns; relative percentage of peak areas. Feger et al. also found in samples (a) and (b) α-santalene (0.01%), epi-β-selinene (0.03%), and trace amounts of epi-β-santalene.
4. Huang and Wu (1998). China; one sample of laboratory cold-pressed oil; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-17 and OV-101; relative percentage of peak areas. Huang and Wu also found β-farnesene* (0.55%), γ-muurolene (0.22%), and trace amounts of hexanol.
5. Sawamura (2000). Japan; one sample from the cv. Mexicaine; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T; relative percentage of peak areas. Sawamura also found δ-muurolene (0.03%), pentadecanal (0.01%), farnesal* (0.01%), hexadecanol (0.06%), β-bisabolol (0.01%), (*Z*)-nerolidol (0.01%), verbenol (0.02%), neryl acetone (0.02%), and trace amounts of α-santalene, solanone, nootkatone, (*E*,*E*)-farnesol.
6. Lota et al. (2002). Corsica, France; range of the composition of the oils cold-pressed from the cvs.: Nouvelle Calédonie, Antillaise, Ambilobe, Kirk, Mexicaine; GC/FID and GC/MS analyses on capillary column (50 m × 0.22 mm × 0.25 μm) coated with DB-1; GC/FID analysis was also performed on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; LRI on DB-1 and DB-20 columns are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found *trans*-*p*-menth-2-en-1-ol (0%–0.01%), santal-10-en-1-ol (0–tr), *cis*-linalol oxide (0–tr).
7. Minh Tu et al. (2002). Vietnam; one sample; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; LRI on DB-Wax are reported; relative percentage of peak areas. Minh Tu et al. also found bergamotene* (0.5%), cadinene* (0.1%), β-cubebene (0.1%), α-farnesenes* (1.6%), β-farnesenes* (0.1%), menthone (0.1%), *p*-menth-2-en-1-ol* (0.1%), cineole* (0.3%), and trace amounts of 2,7-dimethyl-1,6-octadiene, tetradecane, bisabolene*, α-cedrene, α-cubebene, santalene, sesquiphellandrene*, cumin aldehyde, camphor, *cis*-carveol, eugenol, perilla alcohol, cedrenol, bisabolol*, (*Z*)-nerolidol, nonyl acetate, linalyl acetate, *cis*-linalol oxide, nonanoic acid.
8. Gancel et al. (2002). Corsica, France; one sample extracted with pentane: ether (1:1) from the cv. Star Ruby; GC/FID and GC/MS on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm). The results of this paper are expressed as micrograms per gram of fresh peels, the percentage composition was calculated based on the original data. Gancel et al. also found isopinocamphone (0.02%) and trace amounts of hexanal, (*E*)-2-hexenal.
9. Craske et al. (2005). Australia; one sample solvent extracted with CH_2Cl_2; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5MS; NBS 75K MS library; relative percentage of peak areas.

continued

TABLE 1.6 (continued)
Percentage Composition of the Volatile Fraction of Key Lime Oils (1998–2009)

10. Zollo Amvam et al. (1998). Yaoundè, Cameroon; one sample each hydrodistilled from the cvs.: (a) Mexicaine, (b) Likeland; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 and Carbowax 20M; GC/MS on capillary column (25 m × 0.22 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found cadina-1,4-diene (0.41%), α-cadinene (0.07%), myrtenal (0.07%), β-sinensal (0.11%), isoeugenol (0.06%), sabinene hydrate* (0.10%), terpinen-1-ol (0.11%), T-cadinol (0.16%), β-eudesmol (0.33%), (*Z*,*Z*)-farnesol (0.08%), globulol (0.12%), α-pinene oxide (0.06%) in sample (a); alloaromadendrene (0.04%), α-cadinene (0.12%), γ-cadinene (0.07%), santalene* (0.04%), verbenone (0.14%), dodecanol (0.17%), undecanol (0.11%), terpinen-1-ol (0.14%), T-cadinol (0.15%), cubenol (0.14%), β-eudesmol (0.53%), (*E*,*E*)-farnesol (0.03%), (*Z*,*Z*)-farnesol (0.09%), globulol (0.17%), nerolidol* (0.09%), myrtenyl acetate (0.04%), linalol oxide* (0.13%), α-pinene oxide (0.07%) in sample (b).
11 and 12. Venkateshwarlu and Selvaray (2000), Selvaray et al. (2004). India; hydrodistillation; Kagzi lime; (a) dark green fruits, (b) yellow fruits; GC/FID on capillary columns coated with Carbowax 20M (25 m × 0.2 mm × 0.2 μm) and HP-101 (50 m × 0.32 mm); LRI on HP-101 column are reported; relative percentage of peak areas.
13. Afolayan and Asekun (2008). South Africa; hydrodistillation; (a) ripe fruits, (b) rotten fruits; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with a stationary phase slightly polar (probably 5-phenyl, 95-methylpolysiloxane); LRI on the used stationary phase are reported; relative percentage of peak areas. Afolayan and Asekun also found heptadecane (0.2%), eicosane (0.1%), tricosane (1.0%), 1,4,8-*p*-mentha-triene (0.1%), α-gurjunene (0.2%), 3-cyclohexen-1-acetaldehyde (0.1%), *p*-menth-3-en-1-ol (0.2%), butyl acetate (0.6%), linoleic acid (0.2%), palmitic acid (0.4%) in sample (a); heptadecane (0.1%), hexadecane (0.3%), eicosane (0.2%), nonane (0.3%), α-gurjunene (0.2%), 3-cyclohexen-1-acetaldehyde (0.2%), cyclofenchol (0.9%), myrtenol (0.2%), palmitic acid (0.4%), butyl acetate (0.8%) in sample (b).

knowledge of the composition of lime oils but instead provide mistaken information. It is the opinion of the writers that these results could hardly induce researchers expert on citrus oils to use such a complex and expensive technique as the one proposed by Veriotti and Sacks (2001).

Steuer et al. (2001), in a study for the classification of citrus oils by NIR spectroscopy determined in lime oils (distilled and cold-pressed) by GC the following components: limonene (49.9%); myrcene (1.3%); α-pinene (1.4%); β-pinene (4.2%); sabinene (0.5%); γ-terpinene (12.3%); terpinolene (6.2%); and total aldehydes (1.7%).

In their investigation on germacrenes content in some citrus essential oils, Feger et al. (2001a) determined in Key lime oil type A and B the following contents expressed as relative peak areas: germacrene A (0.36%–0.40%); germacrene B (0.78%–0.90%); germacrene C (0.50%–0.59%); germacrene D (0.30%–0.41%).

1.4.2.2 Key Lime Laboratory Oils

Table 1.6 also reports the composition of some laboratory-extracted oils, obtained by manual pressure and by solvent extraction, from fruits of different cultivars and geographical origins. There are great differences in the results, making it difficult to compare them.

The high values of *p*-cymene, γ-terpinene, monoterpene aldehydes and alcohols present in some of the oils analyzed by Lota et al. (2002) should be noted, along with that of limonene in the cv. Mexicaine grown in Corsica, France, highlighted by the same authors, and the low value of β-pinene determined in the same cv.; the very high value of limonene and the low values of β-pinene, geranial, and neral found by Minh Tu et al. (2002) in an oil from Vietnam; the low values of limonene and β-pinene, and the high value of γ-terpinene found by Craske et al. (2005) in an oil from Australia; the high value of limonene and the low values of β-pinene, geranial, and neral found by Zollo Amvam et al. (1998) in the cv. Likeland cultivated in Cameroun and the low values of γ-terpinene, which they found in both the cvs. analyzed; low value of limonene and high values of monoterpene esters determined by Venkateshwarlu and Selvaray (2000) and by Selvaray et al. (2004) in oils obtained from green fruits, probably due to the stage of ripening; and the very low values of limonene and the very high values of sesquiterpene hydrocarbons present in the oils from South Africa analyzed by Afolayan and Asekun (2008). These last authors asserted that although some qualitative and quantitative differences exist among ripe and rotten fruits, these do not influence the quality of the oils. As was predicted, the oils obtained by distillation had a higher content of monoterpene alcohols. The oils analyzed by Huang and Wu (1998) and by Sawamura (2000), mainly because of their content of β-pinene and γ-terpinene, seem to be extracted from the fruits of Persian lime, rather than Key lime. The same observation can be valid for samples analyzed by Sawamura (1999a) and by Njoroge et al. (1996), which are also included in Table 1.5.

The results of Indian researchers who studied the compositional variation of Key lime oils in function of ripening degree (Venkateshwarlu and Selvaray, 2000) also include data on the composition of Persian lime oil (seedless lime), and that of a hybrid obtained from the former specie with Key lime (Selvaray et al., 2002). These authors also studied the influence of treatment with ethylene and acetylene on the green fruits of Kagzi lime on the composition of the essential oil (Selvaray et al., 2004). During the natural ripening process of the fruits, an increase of limonene, myrcene, α- and β-pinene, and a decrease of monoterpene aldehydes, alcohols and esters was noticed. However, a content of monoterpene hydrocarbons and alcohols similar to that determined in the oil extracted from green fruits and a content of carbonyl compounds similar to that of naturally ripened fruits was observed in the oil obtained from fruits ripened by ethylene. In the oil obtained from fruits treated with acetylene, the content of monoterpene alcohols was similar to that of the oil obtained from green fruits; the content of carbonyl compounds was close to that of the oil obtained from naturally ripened fruits; and monoterpene hydrocarbons and esters content was, for both treated fruits, half-way between green and naturally ripened fruits.

Chisholm et al. (2003) studied the most intense odorants of lime-oil laboratory solvent extracted (hexane:diethyl ether 1:1) and one obtained by distillation from a suspension of 5 ml of oil in 200 ml

of a 5% water solution of citric acid, kept for 5 hours under reflux. Many of the sesquiterpene hydrocarbons, not all identified, showed significant odor activity. They listed the components in decreasing odor intensity describing each odor character. The odor of both oils was dominated by geranial, neral, and linalol and by numerous aliphatic and monoterpene aldehydes.

Thao et al. (2007), characterized several citrus oils, manually extracted from fruits cultivated in Vietnam, by the determination of the isotopic ratio of monoterpene hydrocarbons. The monoterpene hydrocarbons in the analyzed lime oils ranged in the following intervals expressed as relative percent of peak areas: limonene 38.66%–62.99%; myrcene 1.31%–1.91%; α-phellandrene 0.05%–0.08%; β-phellandrene 0.43%–0.82%; α-pinene 1.75%–3.18%; β-pinene 3.26%–14.75%; sabinene 0.82%–4.33%; γ-terpinene 7.43%–16.55%; and terpinolene 0.40%–0.94%.

Chienthavorn and Insuan (2004) performed a comparative study on the extraction of Key lime oil by superheated water (SWE) and by some traditional methods such as water distillation and solvent sonication. They concluded that from the results obtained that the SWE method allows to obtain better quality oils, and to modify the composition of the extracted oils by changing the experimental parameters, such as temperature, flow, time, and type and quantity of modifiers added to the water (ethanol and methanol).

1.4.2.3 Persian Lime Industrial Oils

Information on the composition of the entire volatile fraction of Persian lime industrial oils in the last decade is limited to the results relative to samples analyzed by Franceschi et al. (2004) (in the original paper the sample is indicated as lemon oil, its composition however gives reason to believe that it was Persian lime oil), by Mondello et al. (2004c) and by Dugo et al. (2010b); in literature are also available the results obtained within an investigation on sesquiterpene fraction of a commercial Brazilian oil deeply carried out by Feger et al. (2000). These results are reported in Table 1.7. They appear to be in good agreement with each other and with those previously reviewed by Dugo et al. (2002) summarized in Table 1.5.

In addition to the results reported in Table 1.7, in literature are available the results published by Feger et al. (2001a), relative to an investigation on germacrenes content in some citrus essential oils, who determined in high quality Brazilian Persian lime oil the following contents: germacrene A (0.12%–0.18%); germacrene B (0.16%–0.19%); germacrene C (0.09%–0.12%); and germacrene D (0.07%–0.16%).

1.4.2.4 Persian Lime Laboratory Oils

Table 1.7 also shows the results relative to the composition of Persian lime extracted in laboratory by cold extraction and by distillation. Many of these oils are characterized by lower content of limonene compared to industrial oils, such as the oil analyzed by Zollo Amvam et al. (1998) and those extracted from the cvs. Tahiti and De Perse by Lota et al. (2002) present high content of *p*-cymene. The oils obtained by hydrodistillation also present higher amounts of alcohols than those that are cold extracted. The oil obtained from seedless lime, analyzed by Selvaray et al. (2002), has lower amounts of neral and geranial.

Atti-Santos et al. (2005) also reported the results relative to the oil extracted by supercritical CO_2 from the same matrix used for the hydrodistilled oil. They considered the oil obtained by supercritical CO_2 of better quality than that obtained by hydrodistillation because of the shorter time of extraction, higher yields, and composition that is closer to what has been reported for commercial oils.

However, it should be mentioned that the unusual value of myrcene (0.01%) also reported by Sawamura (2000) is probably a misprint.

Not included in Table 1.7, nor in the previous review by Dugo et al. (2002), is the paper by Asano (1997) on four oils obtained from fruits cultivated in Thailand (Menawa lime, Large Tahitian lime, Old Tahitian lime, Young Tahitian lime) that presented very important differences among them. Their composition is reported in detail by Lawrence (2003).

TABLE 1.7
Percentage Composition of the Volatile Fraction of Persian Lime Oils (1998–2009)

	Industrial Oils				Laboratory Oils				
					Cold Extracted		Hydrodistilled		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
				Hydrocarbons					
Monoterpene									
Camphene	–	–	0.05	0.04–0.06	0.05	tr–0.1	0.05	0.1	–
p-Cymene	–	–	b	0.08–0.30	0.05	0.2–5.3	5.54	1.4	1.0
Limonene	–	58.64	59.37[b]	54.20–58.20[h]	55.47	40.3–46.5	46.41	49.2	47.5
Myrcene	–	1.51	1.26	1.26–1.30	0.01[i]	1.1–1.3	1.25	1.3	1.2
(*E*)-β-Ocimene	–	–	0.07	0.06–0.11	–	0.1–0.2	0.10	–	–
(*Z*)-β-Ocimene	–	–	–	h	0.05	0–0.1	–	–	–
α-Phellandrene	–	–	0.05[a]	0.04	1.41	tr–0.1	–	–	–
β-Phellandrene	–	–	–	–	tr	0.5–0.6[g]	–	–	–
α-Pinene	–	2.13	1.78	1.99–2.05	2.98	1.3–2.4	1.64	1.4	1.9
β-Pinene	–	11.17	10.44	10.01–11.82	11.59	11.1–13.7	11.18	9.2	12.4
Sabinene	–	1.79	1.45	1.70–1.92	2.10	1.6–2.1	1.28	–	1.6
α-Terpinene	–	–	0.24	0.20–0.23	0.28	0.2–0.4	–	–	0.3
γ-Terpinene	–	14.82	14.29	12.84–14.61	14.50	17.3–21.5	12.54	12.1	12.3
Terpinolene	–	0.70	0.55	0.49–0.51	0.60	0.7–0.9	0.63	0.7	0.6
α-Thujene	–	0.60	0.55	0.48–0.51	–	0.4–0.7	0.44	–	0.5
Sesquiterpene									
cis-α-Bergamotene	0.07	–	0.07	0.06–0.08	–	–	–	–	0.3
trans-α-Bergamotene	1.01	1.26	1.01	0.67–0.98	–	1.0–1.6	0.56	–	–
(*E*)-α-Bisabolene	0.05	–	–	0–0.03	–	–	–	–	–
(*Z*)-α-Bisabolene	0.12	–	0.12	0.11–0.18	–	–	–	–	–
β-Bisabolene	1.47	2.18	1.73[e]	1.48–1.67	–	1.5–2.4	0.85	–	1.8
(*E*)-γ-Bisabolene	0.01	–	–	0–0.01	–	–	–	–	–
(*Z*)-γ-Bisabolene	0.02	–	–	0–0.02	–	–	–	–	-
β-Caryophyllene	0.48	0.65	0.46	0.29–0.61[k]	0.35	0.3–0.5	0.28	–	–
γ-Curcumene	0.02	–	–	0.01–0.02	–	–	–	–	–
β-Elemene	0.02	–	–	0.04–0.06	0.11	0–0.2	0.08	–	–
δ-Elemene	tr	–	–	0.03–0.07	0.14	–	0.07	–	–
(*E*,*E*)-α-Farnesene	0.22	–	1.73[e]	0.21–0.23	0.16	0–0.4	0.15	–	–
(*E*)-β-Farnesene	0.10	–	0.16[d]	0.09–0.10	0.06	0–0.1	–	–	–
(*Z*)-β-Farnesene	–	–	–	0–0.01	–	–	0.05	–	–
Germacrene B	0.17	–	0.15	0.09–0.12	0.13	–	0.08	–	–
Germacrene D	0.08	–	–	0–0.05	0.07	tr–0.2	0.06	–	–
α-Humulene	0.04	–	0.16[d]	0.03–0.05	0.04	0–0.2	–	–	–
β-Santalene	0.06	–	0.04	0.01–0.04	0.02	–	–	–	–
α-Selinene	0.02	–	0.08	0–0.02	–	–	–	–	–
				Aldehydes					
Aliphatic									
Decanal	–	–	0.06	0.05–0.06	0.03	tr–0.1	–	0.1	–

continued

TABLE 1.7 (continued)
Percentage Composition of the Volatile Fraction of Persian Lime Oils (1998–2009)

	Industrial Oils				Laboratory Oils				
					Cold Extracted		Hydrodistilled		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Dodecanal	–	–	–	0.01–0.04	0.02	–	–	–	–
Nonanal	–	–	–	0.01	tr	0–tr	–	–	–
Octanal	–	–	0.05[a]	0.01	0.01	0–tr	–	–	–
Undecanal	–	–	–	0.01–0.02	0.01	0–tr	–	–	–
Monoterpene									
Citronellal	–	–	–	0.03–0.06	0.04	tr–0.2	–	–	–
Geranial	–	2.09	1.70	1.79–2.20	5.14	2.7–4.0	3.89	0.4	6.4
Neral	–	1.26	0.99	1.09–1.35	1.73	1.6–2.3	1.56	0.4	4.7
Perilla aldehyde	–	–	–	0.02–0.03	0.02	–	–	–	–
Sesquiterpene									
β-Sinensal	–	–	–	–	–	0–0.1	0.07	–	–
Ketones									
Aliphatic									
6-Methyl-5-hepten-2-one	–	–	–	0–0.01	–	0–tr	0.09	–	–
Monoterpene									
Piperitone	–	–	0.08[c]	–	–	0–tr	–	–	–
Alcohols									
Monoterpene									
Citronellol	–	–	–	0.09–0.16[j]	0.02	–	0.05	–	–
Geraniol	–	–	0.08[c]	0.03–0.04	0.02	tr	1.55	4.3	0.3
Linalol	–	0.17	0.19	0.13–0.14	0.31	0.4–0.5	0.74	1.0	1.3
Nerol	–	–	0.13	0.09–0.16[j]	0.06	0–tr	3.10	3.0	0.7
cis-Sabinene hydrate	–	–	0.04	0.04	0.06	–	–	–	–
trans-Sabinene hydrate	–	–	–	0–0.05	0.08	0.1	–	–	–
α-Terpineol	–	–	0.23	0.21–0.25	0.35	0.4–0.6	1.47	1.9	2.2
Terpinen-4-ol	–	–	0.14	0.05–0.07	0.04	tr–0.1	0.95	0.8	1.2
Sesquiterpene									
α-Bisabolol	–	–	0.09	0–0.08	0.05	–	–	–	–
Campherenol	–	–	0.05	0–0.05	–	–	–	–	–
Norbornanol[f]	–	–	0.06	0–0.05	–	–	–	–	–
Esters									
Monoterpene									
Bornyl acetate	–	–	–	0–tr	–	–	0.05	–	–
Citronellyl acetate	–	–	–	0.01	tr	0–0.1	0.05	–	–
Geranyl acetate	–	–	0.19	0.14–0.23	0.36	0.7–2.4	0.38	0.3	0.3
Neryl acetate	–	1.05	0.82	1.01–1.11	–	1.2–2.0	–	2.5	–
α-Terpenyl acetate	–	–	–	–	–	0–tr	1.56	–	–

TABLE 1.7 (continued)
Percentage Composition of the Volatile Fraction of Persian Lime Oils (1998–2009)

	Industrial Oils				Laboratory Oils				
					Cold Extracted		Hydrodistilled		
	1	2	3	4	5	6	7	8	9
				Ethers and oxides					
Monoterpene									
1,8-Cineole	–	–	b	h	–	0.5–0.6[g]	–	–	–
cis-Limonene oxide	–	–	–	0–0.01	–	0–tr	0.09	–	–
trans-Limonene oxide	–	–	–	0–0.01	–	0–0.1	0.05	–	–
Sesquiterpene									
Caryophyllene oxide*	–	–	–	0–0.01	–	0–0.1	–	–	–

Notes: tr, traces; *, correct isomer not characterized; [a] α-phellandrene + octanal; [b] limonene + *p*-cymene + 1,8-cineole; [c] piperitone + geraniol; [d] (*E*)-β-farnesene + α-humulene; [e] β-bisabolene + (*E*,*E*)-α-farnesene; [f] 2,3-dimethyl-3-(4-methyl-3-penthenyl)-norbornanol; [g] β-phellandrene + 1,8-cineole; [h] limonene + (*Z*)-β-ocimene + 1,8-cineole; [i] this very low value maybe due to a printing error; [j] citronellol + nerol; [k] β-caryophyllene + α-santalene.

Appendix to Table 1.7

1. Feger et al. (2000). Several samples of Persian lime oils from Brazil. Enrichment of sesquiterpene fraction by vacuum distillation; GC/MS of the enriched fraction on capillary columns coated with OV-1701 (30 m × 0.25 mm × 0.25 μm) and DB-1 (33 m × 0.25 mm × 0.5 μm). GC/FID of the crude essential oil on the same capillary columns; relative percentage of peak areas. Feger et al. also found germacrene A (0.12%), germacrene C (0.11%), α-santalene (0.02%), β-selinene (0.01%), and trace amounts of epi-β-santalene, 7-epi-α-selinene, β-sesquiphellandrene.
2. Franceschi et al. (2004). Brazil; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coeted with HP-5MS; Wiley MS library; relative percentage of peak areas.
3. Mondello et al. (2004c). One commercial sample; conventional GC/FID on capillary column (30 m × 0.25 × 0.25 μm) coated with RTX5-MS; fast GC/FID on capillary column (5 m × 0.05 mm × 0.05 μm) coated with SE-52; relative percentage of peak areas. In the table are reported the results obtained with conventional method; the results obtained with fast method were very similar. More information is reported in Chapter 11. Mondello et al. also found α-elemene (0.05%).
4. Dugo et al. (2010b). One cold-pressed oil from the Mexico, two commercial samples; GC/FID and GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found tricyclene (0.03%), *trans*-β-bergamotene (0%–0.05%), γ-elemene (0.01%), β-selinene (0.01%–0.02%), tetradecanal (0%–0.02%), (*E*)-isocitral (0%–0.01%), borneol (0.01%), *cis*-sesquisabinene hydrate (tr–0.01%), and trace amounts of δ-3-carene, α-fenchene.
5. Sawamura (2000). Japan, one sample; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T; Sawamura also found aromadendrene (0.03%), α-bergamotene* (0.71%), δ-muurolene (0.02%), farnesal* (0.01%), carvone (0.01%), hexadecanol (0.06%), (*Z*)-nerolidol (0.01%), verbenol (0.01%), neryl acetone (0.02%), limonene dioxide (0.02%), and trace amounts of δ-2-carene, dodecyl acetate.
6. Lota et al. (2002). Corsica, France; range of the composition of the oils cold-pressed from the cvs.: El Kseur, Bearss, De Perse, IAC, Tahiti; GC/FID and GC/MS on capillary column (50 m × 0.22 × 0.25 μm) coated with DB-1; GC/FID analysis was also performed on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; LRI on DB-1 and DB-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found bicyclogermacrene (0%–0.4%), santal-10-en-2-ol (0%–tr), linalyl acetate (0%–tr).

continued

TABLE 1.7 (continued)
Percentage Composition of the Volatile Fraction of Persian Lime Oils (1998–2009)

7. Zollo Amvam et al. (1998). Yaoundè, Cameroon; one sample hydrodistilled; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 and Carbowax 20M; GC/MS on capillary column (25 m × 0.23 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found verbenone (0.05%), *p*-cymen-8-ol (0.06%), isopulegol (0.06%), terpinen-1-ol (0.05%), β-terpineol* (0.07%), γ-terpineol (0.11%), (*Z*,*E*)-farnesol (0.06%), spathulenol (0.06%).
8. Selvaray et al. (2002). India; one sample hydrodistilled of Seedless lime oil; GC/FID on capillary columns coated with Carbowax 20M (25 m × 0.2 mm × 0.2 μm) and HP-101 (50 m × 0.32 mm); LRI on HP-101 are reported; relative percentage of peak areas.
9. Atti-Santos et al. (2005). Brazil; one sample hydrodistilled; GC/FID on capillary column (30 m × 0.32 mm × 0.5 μm) coated with HP-Innowax; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-Innowax; LRI on HP-Innowax are reported; wt%.

1.4.2.5 Other Lime Oils

Chraske et al. (2005) reported the composition of an Australian native lime (*Microcitrus australe*) which appears very similar to the composition of an Australian Key lime oil analyzed by the same authors and reported in Table 1.6. The major differences among these oils are the higher content of limonene (35.1%) and lower content of γ-terpinene (11.2%) and of neryl acetate (0.1%) in the Australian native lime than those determined for these three components in Key lime (30.5%, 19.2%, and 2.2% respectively).

Shaw et al. (2000) studied the volatile components in the peel oil and in the juice of an Australian wild lime (*Microcitrus inodora*) since they considered that the knowledge of these components in *M. inodora* could help to plant breeders in screening derived hybrids for fruit quality traits. These authors identified 20 components in the peel oil but did not report the quantitative data.

1.5 MANDARIN (*CITRUS DELICIOSA* TEN.), TANGERINE (*CITRUS TANGERINA* HORT. EX TAN.), AND CLEMENTINE (*CITRUS CLEMENTINA* HORT. EX TAN.) OILS

The description of mandarin oil is difficult compared to other citrus oils. From the botanical point of view, the mandarin group is a very complex one. It consists of the Mediterranean mandarin (*Citrus deliciosa* Ten.); the tangerines (*C. tangerina* Hort. ex Tan.); the clementines (*C. clementina* Hort. ex Tan.); and the *C. temple* Hort. ex Tan.; the *C. nobilis* Lour.; the *C. unshiu* (Mak.) Marc; as well as the *C. reticulata* Blanco. Often, when describing the composition of mandarin oil, the authors refer to oils obtained from different mandarin species; seldom the botanical origin is not specified, and when it is, it is sometimes not univocal. Mandarins are also commonly described using botanical classifications that differ from the one mentioned above, generating confusion.

In this section, only the composition of mandarin (*C. deliciosa* Ten.), tangerine (*C. tangerina* Hort. ex Tan.), and clementine (*C. clementina* Hort ex Tan.) oils of ensured origin will be described, along with the composition of commercial oils that appear to have the same botanical origin.

Most of the literature published before 1979 on the composition of mandarin and tangerine oils has been reviewed by Shaw (1979) and later by Dugo et al. (2002). Literature published from 1979 to 1999 on the composition of these two oils was reviewed by Dugo et al. (2002), and it is summarized in Table 1.8.

TABLE 1.8
Percentage Composition of the Volatile Fraction of Mandarin and Tangerine Oils (1979–1999)

	Mandarin Oils			Tangerine Oils
	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils	Industrial Cold-Pressed and Commercial Oils
		Hydrocarbons		
Monoterpene				
Camphene	tr–0.02	tr–0.05	0.01–0.02	tr
δ-3-Carene	tr	0–0.06	tr	–
p-Cymene	0.12–1.28	1.20–4.25	0.23–0.39	0.04–1.28[–]
Limonene	65.30–77.14	65.51–77.02	72.71–73.58	87.40–91.65
p-Mentha-1,3,8 triene	tr[t]	tr	–	–
Myrcene	1.57–2.27	1.62–2.27	1.71–1.73	1.87–3.16
(*E*)-β-Ocimene	tr–0.03	tr–0.06	0.02	0.12–0.23
(*Z*)-β-Ocimene	tr–0.01	0.01	tr	–
α-Phellandrene	0.03–0.49	0.04–0.48	0.06–0.07	tr–0.05
β-Phellandrene	tr	0.21	0.52	tr
α-Pinene	1.75–5.24	2.22–2.87	1.78–1.79	0.84–2.01
β-Pinene	1.15–2.44	1.13–2.48	1.36–1.41	0.06–0.58
Sabinene	0.10–0.59	0.23–0.45	0.17–0.22	0.18–1.44
α-Terpinene	tr–0.52	tr–0.30	0.24–0.40	tr–0.02
γ-Terpinene	12.97–22.75	11.94–20.89	16.68–17.17	0.02–4.48
Terpinolene	0.54–1.04	0.49–1.38	0.78–0.82	0.07–0.24
α-Thujene	tr–1.06	0.34–0.91	0.64–0.66	0.12–0.22
Sesquiterpene				
trans-α-Bergamotene	tr	–	–	–
β-Bisabolene	tr	–	–	–
γ-Cadinene	–	tr–0.05	–	–
δ-Cadinene	tr	0–0.02	–	tr–0.02
β-Caryophyllene	0.03–0.14	0.06–0.11	0.06	0.02–0.13
α-Copaene	tr	0–tr	-	tr–0.03
(*E*,*E*)-α-Farnesene	0.06–0.26	0.13–0.30	0.04–0.05	tr–0.03
Germacrene D	tr	–	–	–
α-Humulene	tr–0.02	tr	0.02–0.03	–
α-Selinene	tr–0.06	–	–	–
Valencene	+	–	–	0.02–0.05
		Aldehydes		
Aliphatic				
Decanal	0.05–0.12	0.05–0.13	0.11	0.10–0.23
(*E*,*E*)-2,4-Decadienal	tr	–	–	0.04
(*E*)-2-Decenal	tr–0.03	–	–	–
Dodecanal	tr–0.05	0–tr	–	0.03–0.05
(*E*)-2-Dodecenal	tr–0.03	–	–	–

continued

TABLE 1.8 (continued)
Percentage Composition of the Volatile Fraction of Mandarin and Tangerine Oils (1979–1999)

	Mandarin Oils			Tangerine Oils
	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils	Industrial Cold-Pressed and Commercial Oils
Hexadecanal	tr	–	–	–
Nonanal	tr–0.07	tr–0.06	0.01–0.02	tr–0.06
Octanal	0.03–0.22	–	0.06–0.08	0.19
Tetradecanal	tr–0.01	–	–	–
Undecanal	tr–0.05	0–tr	–	0.02–0.05
Monoterpene				
Citronellal	tr–0.07[a]	tr–0.02	0.02–0.04	0.02–0.06
Geranial	0–0.12[b]	tr	0.07–0.08	0–0.05
Neral	tr–0.03	–	tr	tr–0.07
Perilla aldehyde	tr–0.12[b]	tr–0.08	–	0.04
Sesquiterpene				
α-Sinensal	0.12–0.53	0.15–0.31	0.14–0.16	0.09–0.26
		Ketones		
Aliphatic				
6-Methyl-5-hepten-2-one	tr	–	–	–
Monoterpene				
Carvone	tr–0.02	tr	–	–
Piperitone	tr	–	–	–
		Alcohols		
Aliphatic				
Nonanol	tr	–	tr	tr–0.20
Octanol	tr–0.14	–	0.02–0.03	tr–0.02
Monoterpene				
cis-Carveol	tr	tr–0.05	–	–
trans-Carveol	tr	tr	–	0–0.02
Citronellol	tr–0.04	tr–0.05	–	0–0.23
p-Cymen-8-ol	tr	–	–	–
Geraniol	tr–0.01	–	0.03	0–0.02
Linalol	0.04–0.31	0.13–0.25	0.16–0.19	0.34–1.22
Nerol	tr–0.03	–	0.04–0.07	tr
Perilla alcohol	tr–0.02	–	–	–
cis-Sabinene hydrate	0.01–0.07	tr	–	–
trans-Sabinene hydrate	tr–0.11	–	–	–
Terpinen-4-ol	0.01–0.08	tr–0.10	0.20–0.27	tr–0.05
α-Terpineol	0.04–0.46	0.05–0.24	0.37–0.38	0.04–0.09
Thymol	0.01–0.18	tr–0.08	0.02–0.11	0–0.07
		Esters		
Aliphatic				
Octyl acetate	tr–0.07[a]	–	–	0.02–0.04[a]

TABLE 1.8 (continued)
Percentage Composition of the Volatile Fraction of Mandarin and Tangerine Oils (1979–1999)

	Mandarin Oils			Tangerine Oils
	Cold-Pressed Oils	Commercial Oils	Laboratory-Extracted Oils	Industrial Cold-Pressed and Commercial Oils
Monoterpene				
Citronellyl acetate	tr–0.01	–	–	0–0.03
Geranyl acetate	tr–0.01	tr–0.09	–	tr–0.02
Linalyl acetate	tr–0.04	tr–0.49	–	tr–0.07
Neryl acetate	tr–0.01	tr–0.03	–	0–0.02
Terpinyl acetate*	tr	–	–	–
		Ethers and oxides		
Monoterpene				
1,8-Cineole	tr	–	–	–
Limonene dioxide	–	tr–0.14	–	tr–0.07
Limonene oxide*	–	tr–0.11	–	tr–0.07
cis-Limonene oxide	tr	–	–	–
trans-Limonene oxide	tr	–	–	–
Thymol methyl ether	+	–	–	0.10–0.13
		Others		
Methyl *N*-methyl anthranilate	0.15–0.66	0.50–0.72	0.46–0.48	0–0.07

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined; [a] citronellal + octyl acetate; [b] geranial + perilla aldehyde.

Appendix to Table 1.8

- The results reported in Table 1.8 and in this appendix, for mandarin and tangerine oils, are taken from the following original papers:
  - Industrial cold-pressed mandarin oils: Wilson and Shaw (1981); Koketsu et al. (1983); Mazza (1987a); Calvarano et al. (1989b); Boelens and Jimenez (1989a); Dugo et al. (1984a,1990,1999); Dugo (1994); Cotroneo et al. (1994); Verzera et al. (1997a). The qualitative results reported by Berger et al. (1985); Micali et al. (1990); Lanuzza et al. (1991); Mondello et al. (1995b); Mazza (1987b) were also included.
  - Commercial mandarin oils: Formàček and Kubeczka (1982); Inoma et al. (1989); Della Porta et al. (1997).
  - Laboratory-extracted mandarin oils: Ruberto et al. (1997b); Caccioni et al. (1998).
  - Industrial cold-pressed tangerine oils: Moshonas and Shaw (1979); Wilson and Shaw (1981,1984); Koketsu et al. (1983); Lancas et al. (1988); Inoma et al. (1989).
- Coeluitions indicated by one or more authors in chromatographic separations of mandarin and tangerine oils:
  - Mandarin: *p*-cymene + α-terpinene; limonene + β-phellandrene + 1,8-cineole; terpinolene + octanal; α-selinene + valencene; citronellal + octyl acetate; geranial + perilla aldheyde; citronellol + nerol.
  - Ranges reported in Table 1.8 for mandarin cold-pressed oils, relatively to some minor components (β-phellandrene, octanal) coeluted in chromatographic separation in some cases with other components, were determined considering the results where coelutions did not occur.
  - Tangerine: terpinolene + octanal; citronellal + octyl acetate.

continued

TABLE 1.8 (continued)
Percentage Composition of the Volatile Fraction of Mandarin and Tangerine Oils (1979–1999)

- In addition to those listed in Table 1.8 in mandarin oils was also determined the presence of the components listed below:
  - Cold-pressed mandarin oil: nonyl acetate (tr–0.01%), and trace amounts of *p*-cimenene, bicyclogermacrene, β-elemene, (*E*,*Z*)-2,4-decadienal, heptadecanal, (*E*)-2-hexadecenal, (*E*)-2-hexenal, (*E*)-2-nonenal, pentadecanal, (*E*)-2-tetradecenal, tridecanal, (*E*)-2-tridecenal, (*E*)-2-undecenal, cumin aldehyde, camphor, methyl acetophenone, nootkatone, heptanol, carvacrol, γ-isogeraniol[t], *cis*- and *trans*-isopiperitenol, limonen-4-ol, *p*-mentha-1,8(10)-dien-9-ol, *p*-menth-1-en-9-ol, *cis*-pinene hydrate[t], (*Z*,*E*)-farnesol, (*E*)-nerolidol, spathulenol, decyl acetate, *p*-mentha-1,8(10)-dien-9-yl acetate, *p*-menth-1-en-4,5-oxide, *p*-menth-4-en-1,2-oxide, methyl anthralinate, acetic acid, octanoic acid, and also not quantitatively determined linear chained hydrocarbons C_{21}–C_{33}, and correspondent "iso" isomers C_{23}–$C_{31,}$ 1,3(*E*),5(*Z*)-undecatriene, β-cubebene, hexanal, *p*-menth-1-en-9-al, *cis*- and *trans*-dihydrocarvone[t], isopiperitenone, β-pinone[t], α-thujone[t], cumin alcohol, *p*-mentha-1,8-dien-4-ol, *cis*- and *trans*-*p*-mentha-1(7),8-dien-2-ol, *cis*- and *trans*-*p*-menth-2,8-dien-1-ol, myrtenol, *trans*-pinocarveol, terpinen-1-ol, 3,7-dimethyl-1,5-octadien-3,7-diol, 3,7-dimethyl-1,7-octadien-3,6-diol, citronellyl formate, *cis*- and *trans*-linalol oxide, perillene, caryophyllene oxide*, humulene oxide II, cresol, diethyl succinate. Many of these components, present at trace levels, identified by only one author probably need further confirmation.
  - Commercial mandarin oils: δ-2-carene (0.03%), decanol (0.03%), hexanol (0.02%), 3-octanol (0.12%), γ-terpineol (0.01%), (*E*)-2-hexenyl butyrate (0%–0.14%), terpinen-4-yl-acetate (0.01%), benzaldehyde (0.02%), and trace amounts of β-selinene, acetophenone, isopulegol, *cis*-*p*-menth-2-en-1-ol, *cis*-β-terpineol, *trans*-β-terpineol, 3-methyl benzaldehyde.
- Among the results relative to genuine cold-pressed mandarin oils are included those obtained by Dugo et al. (1984, 1990, 1994) and by Dugo (1999) relative to about 400 samples of Italian oils acquired during numerous productive seasons. These authors observed that the composition of Italian mandarin oil varied during the productive season. These variation were observed during different productive years. In particular, limonene content increased during the season of production, ranging from the 68% average content in October, to 74% in February; the monoterpene hydrocarbons, such as γ-terpinene, β-pinene, α-pinene, the sesquiterpene hydrocarbons and all the oxygenated compounds decreased during the season of production. Due to such variations it was noticed that, red mandarin, produced at the end of the season, contains only 60% of methyl *N*-methyl anthranilate of the amount present in green mandarin, produced at the beginning of the season, and a total content of alcohols equal to the 25% of the oil produced at the start of the season. If the variation of the components present in mandarin oil are correlated, a good method of evaluation can be set-up, to determine the oil purity. For example (Figure 1.7), if a mandarin oil contains 73% of limonene, 20% of γ-terpinene, and 0.15% of α-terpineol, all these values taken singularly can be considered acceptable for a genuine oil, but this oil cannot be considered genuine: in fact, the values of γ-terpinene and of α-terpineol are typical for a green mandarin oil, while the limonene content is characteristic of a red mandarin oil. It is possible that this oil was a green mandarin oil diluted with other product containing high percentages of limonene, such as sweet orange terpene.
- More papers than those cited in Table 1.8 are found in literature. These papers have not been included because the botanical origin of the fruits was not clear, or because the composition appeared to us incompatible with that normally obtained for *Citrus deliciosa* Ten, or for *C. Tangerina* Hort. ex Tan. oils. Among these were the papers by Blanco Tirado et al. (1995) on Columbian oils, by Cappello et al. (1981) on oils from Argentina, by Dellacassa et al. (1989, 1992), and by Calvarano et al. (1989a) on oils from Uruguay.

1.5.1 1998–2009

1.5.1.1 Mandarin Industrial Oils

Table 1.9 reports the results for industrial mandarin oils; these are relative to two commercial samples, one of which was surely adulterated and therefore was not considered. They were analyzed by Oberhofer et al. (1999) in an investigation on the alteration of some essential oils used in aroma lamp therapy. Two papers by Pino et al. (2006) and Pino and Quijano-Celís (2007) are relative to one sample, of not specified botanical origin, from Cuba; two papers by Mondello et al. (2003, 2004b) report the composition of one Sicilian sample within the comparison of conventional and fast gas chromatographic analyses; two papers report the results of a large number of samples of

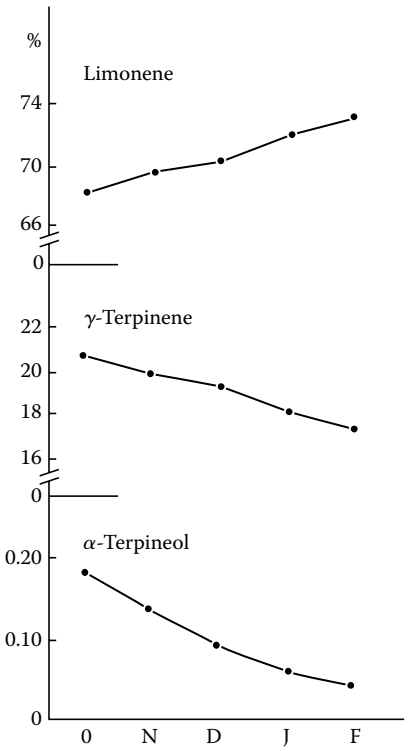

FIGURE 1.7 Average variation in the limonene, γ-terpinene, and α-terpineol content for mandarin oil for each month of the productive season. (This image originally appeared in Dugo, G., G. Lamonica, A. Cotroneo, et al. *Perfum. Flav.* 17(5):57–74, 1992. Printed with permission of Allured Business Media.)

TABLE 1.9
Percentage Composition of the Volatile Fraction of Industrial Extracted Mandarin Oils (1999–2009)

	1	2,3	4,5	6	7	8	9a	9b
				Hydrocarbons				
Monoterpene								
Camphene	–	0.02	–	0.01–0.02	0.01	–	0.01	0.01
δ-3-Carene	tr	tr	–	–	0–tr	–	0–tr	0–tr
p-Cymene	1.00	b	0.1	0.24–0.36	0.22–0.86	2.0	0.23–0.68	0.26–0.55
Limonene	77.44	73.84[b]	78.3	68.64–72.87[d]	68.51–77.59	74.7	71.38–77.82	71.56–77.70
Myrcene	1.72	1.79	5.3	1.65–1.78[d]	1.29–1.55	1.4	1.37–1.66	1.40–1.49
(*E*)-β-Ocimene	–	0.02	–	0.01–0.02	0.01–0.02	–	0.01	0.01–0.02
(*Z*)-β-Ocimene	–	tr	0.1	d	–	–	–	–
α-Phellandrene	–	0.13[a]	–	0.06–0.08	0.03–0.05	tr	0.04–0.06	0.04
α-Pinene	1.68	2.21	2.7	2.26–2.75	1.55–2.14	2.0	1.64–2.29	1.64–1.95

continued

TABLE 1.9 (continued)
Percentage Composition of the Volatile Fraction of Industrial Extracted Mandarin Oils (1999–2009)

	1	2,3	4,5	6	7	8	9a	9b
β-Pinene	1.41	1.47	1.0	1.57–1.82	1.06–1.47	1.3	1.07–1.41	1.07–1.84
Sabinene	tr	0.24	tr	0.24–0.27	0.16–0.20	0.2	0.16–0.19	0.17–0.19
α-Terpinene	0.27	0.32	tr	0.29–0.44	0.18–0.34	0.2	0.21–0.36	0.21–0.29
γ-Terpinene	14.65	17.06	7.2	18.20–20.72	13.13–20.70	15.7	13.13–18.83	15.19–18.85
Terpinolene	0.72	0.73	0.5	0.67–0.86	0.47–0.72	0.5	0.50–0.79	0.50–0.65
α-Thujene	0.57	0.74	0.3	0.83–1.01	0.48–0.72	0.7	0.51–0.76	0.51–0.64
Sesquiterpene								
β-Caryophyllene	0.06	0.07	tr	0.01–0.08	0.04–0.08	tr	0.04–0.08	0.02–0.07
δ–Cadinene	–	–	0.1	0–0.01	tr–0.01	–	–	–
α-Copaene	–	–	0.1	0–0.01	tr–0.02	–	–	–
β-Cubebene	–	–	tr	–	0–0.01	–	–	.
(*E*,*E*)-α-Farnesene	–	0.15[c]	0.1	0.02–0.10	0.06–0.16	tr	0.06–0.14	0.06–0.14
Germacrene D	–	–	0.3	0–tr	0–0.01	–	–	–
α-Humulene	–	0.01	tr	tr–0.01	tr–0.01	–	tr–0.01	tr–0.01
α-Selinene	–	0.03	–	0.01–0.03	tr–0.04	–	0.01–0.03	0.02–0.03
Aldehydes								
Aliphatic								
Decanal	0.21	0.08	0.2	0.04–0.08	0.01–0.18	tr	0.06–0.08	0.06–0.08
(*E*,*E*)-2,4-Decadienal	–	–	0.1	0–tr	0–0.01	–	–	–
(*E*)-2-Decenal	–	–	tr	–	0–0.01	–	–	–
(*E*,*Z*)-2,6-Dodecadienal	–	–	–	0–0.01	tr–0.01	–	–	–
Dodecanal	–	tr	0.1	0.01–0.02	–	–	tr–0.01	0.01–0.02
(*E*)-2-Dodecenal	–	–	tr	0–0.02	0.01–0.03	–	tr–0.02	tr–0.02
Nonanal	–	0.03	0.1	0.02–0.03	0–0.03	tr	0–0.03	0.01–0.03
Octanal	–	0.13[a]	tr	0.11–0.15	0.05–0.13	tr	0.05–0.14	0.05–0.13
Tetradecanal	–	–	tr	0–0.01	tr–0.01	–	tr–0.01	tr–0.01
Undecanal	–	0.01	tr	tr–0.03	tr–0.05	–	tr–0.01	tr–0.01
Monoterpene								
Citronellal	0.02	0.02	0.1	0.02–0.03	tr–0.03	–	0–0.03	0.02
Geranial	–	0.04	tr	tr–0.02	tr–0.02	–	0–0.02	0.01–0.03
Neral	0.09	tr	tr	0–0.04	tr–0.05	–	tr–0.04	tr–0.02
Perilla aldehyde	–	–	0.1	0.02–0.03	0.02–0.04	–	–	–
Sesquiterpene								
α-Sinensal	–	0.29	0.5	0.12–0.44	0.18–0.31	–	0.18–0.29	0.18–0.29
Ketones								
Monoterpene								
Camphor	–	–	–	tr–0.01	tr–0.02	–	–	–
Carvone	–	–	tr	0–0.01	0–tr	–	–	–
Piperitone	–	–	tr	–	0–tr	–	–	–

TABLE 1.9 (continued)
Percentage Composition of the Volatile Fraction of Industrial Extracted Mandarin Oils (1999–2009)

	1	2,3	4,5	6	7	8	9a	9b
				Alcohols				
Aliphatic								
Octanol	–	0.01	tr	0.02–0.05	–	–	–	–
Monoterpene								
cis-Carveol	–	–	tr	0–0.01	0–0.08	–	–	
Citronellol	–	–	tr	0–0.01	0–0.02	–	0–0.02[f]	0.01–0.02[f]
p-Cymen-8-ol	–	–	tr	–	tr–0.03	–	–	–
Geraniol	–	–	tr	0–tr	–	–	tr–0.01	tr–0.01
Linalol	0.02	0.09	1.0	0.10–0.21[e]	0.06–0.16	0.1	0.06–0.16	0.06–0.15
Nerol	–	0.01	–	–	–	–	0–0.02[f]	0.01–0.02[f]
cis-Sabinene hydrate	–	0.01	tr	–	0.01–0.04	–	0.01–0.04	0.01–0.03
trans-Sabinene hydrate	–	–	–	e	0–0.08	–	0.02–0.03	0.02–0.04
Terpinen-4-ol	–	0.02	0.1	0.02–0.05	tr–0.04	–	0.01–0.03	0.01–0.03
α-Terpineol	–	0.08	0.2	0.07–0.21	tr–0.20	0.1	0–0.18	0.07–0.18
Thymol	–	0.04	0.2	0.02–0.06	tr–0.06	–	0–0.06	0.01–0.04
Sesquiterpene								
(*Z*,*E*)-Farnesol	–	tr	–	0–tr	–	–	–	–
				Esters				
Monoterpene								
Citronellyl acetate	–	tr	tr	0–0.01	–	–	tr–0.01	tr–0.01
Geranyl acetate	0.31	tr	tr	0–0.04	0–0.01	tr	tr–0.01	tr–0.01
Neryl acetate	–	0.01	–	tr–0.01	tr–0.01	–	tr–0.01	tr–0.01
α-Terpinyl acetate	–	0.03	–	0–tr	–	–	–	
				Ethers and oxides				
Monoterpene								
cis-Limonene oxide	–	–	–	tr	0–0.02	–	–	–
trans-Limonene oxide	–	–	–	0–tr	tr–0.02	tr	0–0.01	0–0.01
(*E*)-Miroxide	–	0.02	–	tr–0.01	–	–	–	–
Thymol methyl ether	–	–	0.3	0.01–0.02	0–0.01	–	–	–
				Others				
Methyl *N*-methyl anthranilate	–	0.33	tr	0.22–0.50	0.17–0.54	–	0.02–0.45	0.20–0.40

Notes: tr, traces; [a] α-phellandrene + octanal; [b] *p*-cymene + limonene; [c] (*E*,*E*)-α-farnesene + unknown sesquiterpene hydrocarbon; [d] limonene + (*Z*)-β-ocimene; [e] linalol + *trans*-sabinene hydrate; [f] citronellol + nerol.

continued

TABLE 1.9 (continued)
Percentage Composition of the Volatile Fraction of Industrial Extracted Mandarin Oils (1999–2009)

Appendix to Table 1.9

1. Oberhofer et al. (1999). One sample of commercial oil acquired in Italy; GC/FID on capillary columns coated with HP-5 (25 m × 0.32 mm × 0.52 μm), OV-1 (25 m × 0.25 mm × 0.3 μm), Carbowax (25 m × 0.25 mm × 0.3 μm); GC/sniffing technique on capillary column (25 m × 0.53 mm × 0.3 μm) coated with FSOT-RSL-150; GC/IR/MS on capillary columns coated with RSL-200 (30 m × 0.52 mm × 0.25 μm) or with Stabilwax (60 m × 0.32 mm × 0.25 μm); NBS and Wiley MS libraries; EPA-REVA and Robertet IR libraries; LRI on OV-1 are reported; relative percentage of peak areas. Oberhofer et al. also found β-bisabolene (0.11%).

2 and 3. Mondello et al. (2003, 2004b). Sicily, Italy, one sample; conventional GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX–5MS; fast GC/FID on capillary column (10 m × 0.1 mm × 0.1 μm) coated with RTX–5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. In the table are reported the results obtained with conventional method; the results obtained with fast method were very similar. More information is reported in Chapter 11. Mondello et al. also found nootkatone (0.01%).

4 and 5. Pino et al. (2006), Pino and Quijano-Celís (2007). Cuba; 5 samples produced with FMC-in line extractor; GC/FID and GC/MS on capillary column (25 m × 0.25 mm × 0.25 μm) coated with HP-5MS; LRI on HP-5MS are reported; relative percentage of peak areas. These authors also found δ-elemene (0.1%), germacrene B (0.3%), and trace amounts of 1,3,8-*p*-menthatriene, bicyclogermacrene, α-cubebene, β-elemene, γ-elemene, α-guaiene, heptanal, (*E*)-2-nonenal, (*E*)-2-tetradecenal, 6-methyl-5-hepten-2-one, cryptone, *p*-methyl acetofenone, umbellulone, nonanol, *trans*-carveol, *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol, 4-isopropenyl-1-methyl-1,2-cyclohexan-diol, cubebol, elemol, β-eudesmol, (*E*)-nerolidol, spathulenol, octyl acetate, octyl formate, (*E*)-anethole, 1,8-cineole, *cis*- and *trans*-linalol oxide, decanoic acid, (*E*)-β-ionone.

6. Bonaccorsi et al. (2009). Sicily, Italy; range of the composition of 27 samples of green, yellow, and red mandarin oils produced by Sfumatrice and FMC-in line extractor during the 2007/2008 productive season (October 2007–January 2008); GC/FID on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; LRI on SLB-5 MS are reported; relative percentage of peak areas. Bonaccorsi et al. also found (*E*)-2-trideceanal (0–tr).

7. Dugo, P. et al. (2010). Sicily, Italy; range of the composition of 124 samples of green, yellow, and red mandarin oils produced by Torchi and Brown extractor during the 2008/2009 productive season (October 2008–March 2009); GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found viridiflorene (0%–0.01%), (*E*)-2-tetradecenal (tr–0.02%) tridecanol (0%–0.01%) and, trace amounts of decane, *p*-cymenene, (*Z*)-8-undecenol.

8. Viuda-Martos et al. (2009). Spain; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5MS; LRI on HP-5MS are reported; relative percentage of peak areas; Viuda-Martos et al. also found trace amounts of α-bergamotene*.

9. Schipilliti et al. (2010). (a) 53 genuine cold-pressed mandarin oils from Sicily, Italy; (b) 7 commercial mandarin oils from Italy and 2 from Japan; GC/FID on capillary column (25 m × 0.25 mm × 0.25 μm) coated with SLB-5; relative percentage of peak areas.

Sicilian mandarin oils produced in the seasons 2006/2007 (Bonaccorsi et al., 2009) and 2008/2009 (Dugo, P. et al., 2010). The results of these last investigations basically confirm what was previously observed for Italian mandarin oils (Dugo et al., 1984a, 1990, 1999) for the seasonal variation (from October to January) of the composition in function of the fruit ripening stages: increase of limonene content and decrease of all the other principal components. A slight decrement at the beginning of the season (from September to October) was observed for limonene, while some other components slightly increase (Figure 1.8). Some components at the end of the season (from February to March and in some cases from January) show the tendency to reach their initial values. The same can be observed for some classes of components (alcohols, aldehydes, sesquiterpene hydrocarbons).

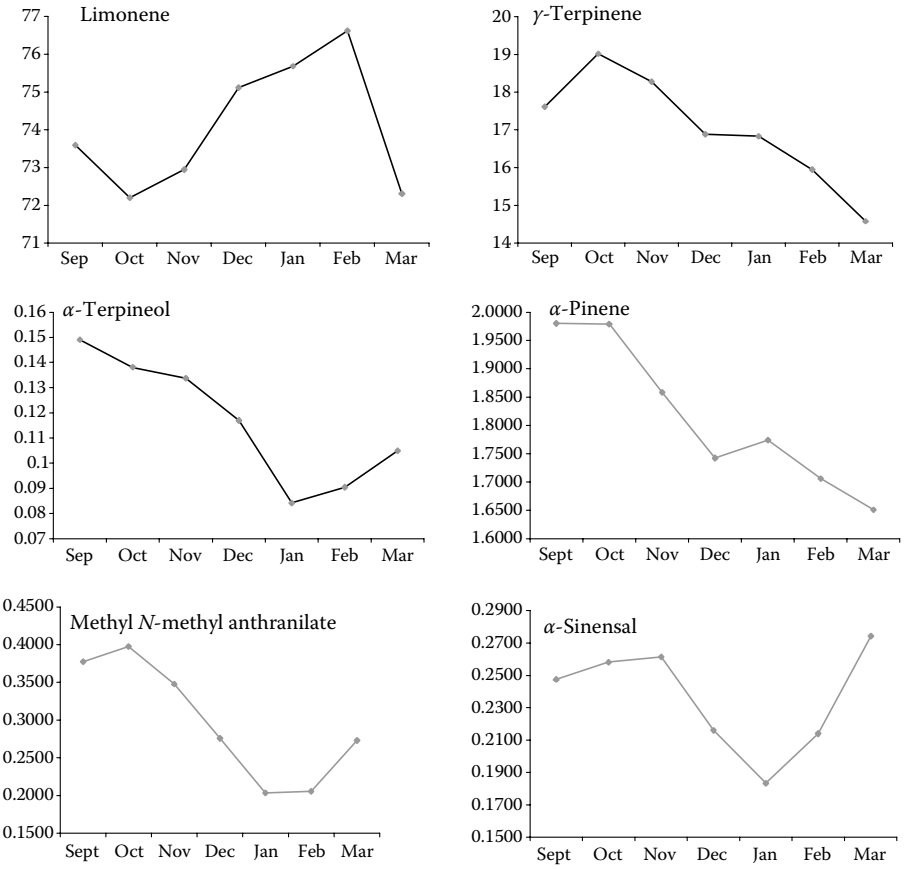

FIGURE 1.8 Seasonal variations of limonene, γ-terpinene, α-terpineol, α-pinene, methyl *N*-methyl anthranilate, and α-sinensal in cold-pressed mandarin oil produced during 2008–2009. (From Dugo, G., I. Bonaccorsi, C. Ragonese, et al., *Flavour Fragr. J.* 2010. In press.)

Monoterpene hydrocarbons maintain a constant value from September to February—in fact, limonene is compensated by other components of this class—then drastically decrease in March, when each monoterpene hydrocarbon presents the same behavior. The graphs in Figures 1.8 and 1.9 show the seasonal variation of some components and classes of components. It should be recalled that previous papers by Dugo et al. (1984a, 1990, 1999) on four productive seasons never included samples produced in September and March, and reported only two samples produced at the beginning of February during the 1983/84 productive season. Therefore the behavior of mandarin oil composition in September, February, and March has never before been investigated. Dugo, P. et al. (2010) also observed that the content of alcohols and aldehydes in oils produced by Torchi were lower than those produced in the same period by the Brown machine. Another paper (Schipilliti et al., 2010) reports the results relative to 53 samples of genuine Sicilian oils and 9 commercial samples. On these samples in addition to the volatile fraction were determined the oxygen heterocyclic compounds, the enantiomeric distribution, and the isotopic ratio (by GC-C-IRMS) of some volatile components. Based on the analyses carried out all nine commercial samples resulted genuine. The same article also reports the results relative to five adulterated commercial samples.

The high contents of limonene and of myrcene and the low content of γ-terpinene reported by Pino et al. (2006, 2007) should be noted.

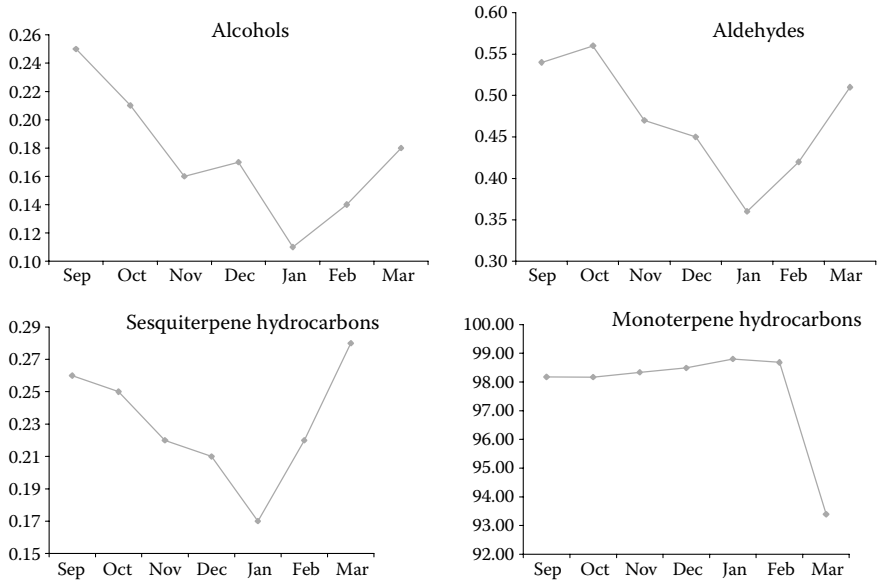

FIGURE 1.9 Seasonal variations of classes of compounds (aldehydes, alcohols, monoterpenes, and sesquiterpene hydrocarbons) in cold-pressed mandarin oil produced during 2008–2009. (From Dugo, G., I. Bonaccorsi, C. Ragonese, et al., *Flavour Fragr. J.* 2010. In press.)

In addition to those reported in Table 1.9, other papers on mandarin oil can be found in the literature. Feger et al. (2001a), in their investigation on germacrenes content in some citrus essential oils, determined in Italian, Greek, Cypriote, and Argentinean mandarin oils the following contents expressed as relative peak areas: bicyclogermacrene (tr–0.01%); germacrene A (tr–0.02%); germacrene B (0–tr); germacrene C (0%–0.1%); and germacrene D + valencene (tr–0.04%).

Steuer et al. (2001) and Schulz et al. (2002), during a study for the qualitative classification of citrus oil by ATR/FT-IR and NIR-FT Raman spectroscopy, reported the content of some monoterpene hydrocarbons in mandarin oil determined by GC: limonene (77.3%); myrcene (1.7%); α-pinene (1.8%); β-pinene (1.1%); γ-terpinene (14.2%); terpinolene (0.6%). Veriotti and Sacks (2002), in a study of citrus oils performed by High-Speed GC and GC/TOF/MS, reported the percentage of some components of mandarin oil. Among those that should be mentioned are the very unlikely of *p*-cymene (16.06%) and of γ-terpinene (5.85%). Reeve and Arthur (2002) in a paper entitled "When is mandarin a tangerine?" reports the following percentages for major components for mandarin oil: limonene (73%); γ-terpinene (17%); farnesene* (0.1%); α-sinensal (0.3%); methyl *N*-methyl anthranilate (0.3%). In a study on the evaluation of validity of conventional enantio GC for the analysis of volatile chiral compound of mandarin oil, Sciarrone et al. (2010) reported the composition of the volatile fraction of a mandarin oil also included among the samples analyzed by Dugo, P. et al. (2010c).

1.5.1.2 Mandarin Laboratory Oils

Table 1.10 summarizes the information found in literature on the composition of mandarin oils extracted in laboratory by manual pressure on the peels (Sawamura, 2000; Lota et al., 2001b; Catalfamo et al., 2004; Frizzo et al., 2004), by solvent extraction (Naef and Velluz, 2001), and by distillation techniques (Ruberto et al. 1999; Frizzo et al., 2004; Karioti et al., 2007).

Naef and Velluz (2001) in their brilliant study on mandarin and tangerine oils not only identified the main components, among which limonene in mandarin oil represents only 58.9%, but also

TABLE 1.10
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Mandarin Oils (1998–2009)

	Cold Extracted									Distilled			
	1	**2**	**3a**	**3b**	**3c**	**4a**	**4b**	**5a**	**5b**	**5c**	**5d**	**6**	**7**
							Hydrocarbons						
Monoterpene													
Camphene	0.01	–	–	–	–	tr–0.01	tr–0.01	0–0.01	0–0.01	0.01	0.01	tr	–
p-Cymene	1.46	0.3	0.2–0.5	–	05–6.9[a]	0.34–0.55	0.43–0.47	–	–	–	–	–	–
Limonene	69.27	58.9	89.6–91.4	92.6	65.3–75.3	69.42–71.87	72.17–74.75	69.29–75.04	70.35–73.64	70.54–76.41	67.14–75.80	73.6	67.0
Myrcene	tr	1.7	1.5–1.6	1.8	1.5–1.7	1.63–1.74	1.51–1.97	1.33–1.78	1.42–1.72	1.53–1.71	1.47–1.70	1.7	1.4
α-Phellandrene	1.49	–	–	–	–	0.06–0.07	0.06–0.08	–	–	–	–	0.1	–
β-Phellandrene	tr	–	0.2–0.3	0.3	0.2–0.3	–	–	–	–	–	–	0.4	–
(*E*)-β-Ocimene	–	–	tr–0.2	0.3	0–tr	0.02–0.11	0.03–0.11	0.02–0.03	0.01–0.02	0.01–0.02	0.01–0.02	tr	0.1
(*Z*)-β-Ocimene	0.07	–	–	–	–	–	–	–	–	–	–	tr	0.2
α-Pinene	1.77	1.5	0.4–0.7	0.6	0.7–1.9	1.29–1.86	1.35–1.92	0.26–1.53	0.35–1.49	1.04–1.44	1.21–1.36	1.8	0.6
β-Pinene	1.57	1.6	0.2–0.3	1.5	1.3	1.06–1.41	0.99–1.37	0.50–1.17	0.55–1.24	1.01–1.11	1.02–1.12	1.4	0.8
Sabinene	0.24	0.3	0.1–0.3	0.3	0.2–2.1	0.19–0.22	0.19–0.24	0.11–0.17	0.12–0.19	0.17–0.19	0.17–0.19	0.2	–
α-Terpinene	0.20	0.6	tr–0.1	–	0.1–0.4	0.29–0.39	0.27–0.38	0.23–0.32	0.22–0.31	0.32–0.38	0.32–0.42	0.4	0.4
γ-Terpinene	19.56	21.6	3.4–4.7	–	13.7–17.3	17.54–18.89	14.74–17.27	15.41–19.52	17.20–19.76	13.76–15.80	13.67–16.80	16.7	10.8
Terpinolene	0.99	1.3	0.1–0.2	–	0.5–0.8	0.74–0.89	0.75–0.97	0.73–1.11	0.71–0.98	0.66–0.83	0.67–0.86	0.8	0.7
α-Thujene	–	1.5	0.1	–	0.5–0.6	0.45–0.71	0.37–0.66	0.10–0.53	0.13–0.54	0.38–0.50	0.44–0.48	0.7	0.2
Sesquiterpene													
δ-Cadinene	–	–	–	–	–	–	–	0.01–0.05	0.01–0.10	0.01	0.01	–	0.2
β-Caryophyllene	0.21	0.2	–	–	–	0.07–0.12	0.05–0.09	0.11–0.27	0.10–0.25	0.05–0.10	0.04–0.09	0.1	0.3
α-Copaene	0.01	–	–	–	–	–	–	tr–0.05	tr–0.08	0–0.02	0.01	–	0.1
β-Cubebene	tr	–	–	–	–	–	–	0–0.01	0–0.02	0–0.01	0–tr	–	–

continued

TABLE 1.10 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Mandarin Oils (1998–2009)

	Cold Extracted									Distilled			
	1	**2**	**3a**	**3b**	**3c**	**4a**	**4b**	**5a**	**5b**	**5c**	**5d**	**6**	**7**
(E,E)-α-Farnesene	0.14	0.2	–	–	–	0.10–0.37	0.11–0.31	0.31–0.84	0.30–0.71	0.14–0.22	0.15–0.25	–	0.2
Germacrene D	–	–	–	–	–	–	–	tr–0.03	tr–0.07	0–0.01	0–0.01	–	0.1
α-Humulene	–	–	–	–	–	–	–	0.01–0.06	0.02–0.05	0.01	0.01	tr	–
α-Selinene	–	–	–	–	–	tr–0.06	0.01–0.06	tr–0.18	0.07–0.30	0.03–0.05	0.04–0.07	–	0.1
Aldehydes													
Aliphatic													
Decanal	0.12	0.1	–	–	–	0.25–0.32	0.10–0.16	0.12–0.30	0.09–0.24	0.11–0.15	0.08–0.10	0.1	0.4
(*E*,*E*)-2,4-Decadienal	–	+	–	–	–	–	–	0–0.01	0–tr	tr–0.01	0–0.01	–	–
(*E*)-2-Decenal	0.02	+	–	–	–	–	–	–	–	–	–	–	–
Dodecanal	0.04	0.1	–	–	–	tr–0.02	0.01–0.02	0.03–0.16	0.02–0.15	0.02–0.04	0.02–0.03	–	–
(*E*)-2-Dodecenal	–	0.1	–	–	–	0.01–0.04	0.01–0.04	–	–	–	–	–	–
Nonanal	0.04	tr	–	–	–	0.03–0.05	0.02–0.04	0.04–0.08	0.03–0.06	0.04–0.06	0.03–0.04	tr	0.5
Octanal	tr	0.2	0–0.2	0.1	tr–0.1	0.09–0.15	0.09–0.15	0.06–0.19	0.07–0.12	0.25–0.38	0.14–0.29	0.1	1.6
Pentadecanal	0.01	+	–	–	–	–	–	–	–	–	–	–	–
Tetradecanal	0.01	+	–	–	–	–	–	tr –0.03	0–0.01	0–0.01	0–tr	–	–
Undecanal	tr	+	–	–	–	tr–0.06	0.01–0.08	0.01–0.04	0.02–0.03	0.01	0.01	–	–
Monoterpene													
Citronellal	0.03	–	0–tr	tr	tr	tr–0.03	0.01–0.03	0.04–0.10	0.02–0.11	0.04–0.07	0.03–0.04	tr	0.3
Geranial	–	–	–	–	–	0.01–0.04	0.02–0.04	0–tr	0–tr	0.01–0.04	0.01–0.03	0.1	0.1
Neral	0.01	–	–	–	–	0.01–0.05	0.02–0.05	0–0.02	0.01–0.03	0.03–0.06	0.03–0.06	tr	0.1
Perilla aldehyde	0.05	0.1	–	–	–	–	–	0.08–0.16	0.11–0.23	0.16–0.27	0.19–0.32	–	–
Sesquiterpene													
α-Sinensal	0.32	0.5	tr–0.3	–	0.2–0.3	0.31–0.51	0.12–0.34	0.55–1.05	0.26–0.82	0.05–0.28	0.19–0.45	0.1	0.5

Ketones													
Aliphatic													
Camphor	–	–	–	–	–	tr–0.01	tr–0.01	0–tr	0–tr	0.01–0.03	0.01–0.02	–	–
Alcohols													
Aliphatic													
Octanol	0.01	0.1	–	–	–	tr–0.02	0.02–0.24	0–0.01	tr–0.02	0.06–0.21	0.02–0.16	tr	0.4
Monoterpene													
trans-Carveol	0.01	–	–	–	–	–	–	0.01–0.09	0.01–0.03	0.02–0.03	0.02	–	0.1
Citronellol	0.04	0.1	0–tr	–	0–tr	–	–	0.03–0.12	0.07–0.15	0.10–0.20	0.12–0.21	–	–
p-Cymen-8-ol	0.02	–	–	–	–	–	–	tr–0.05	–	–	–	–	–
Geraniol	–	–	–	–	–	tr–0.03	0.01–0.05	0–tr	0–tr	0.02–0.04	0.01–0.03	tr	–
Linalol	0.33	0.7	0.4–0.6	0.1	0.2–0.6	0.07–0.16	0.05–0.19	0.12–0.26	0.18–0.25	0.34–0.69	0.38–0.73	0.2	4.7
Nerol	0.05	–	–	–	–	tr–0.03	tr–0.05	–	–	–	–	tr	0.9
trans-p-Mentha-2,8-dien-1-ol	0.01	–	–	–	–	–	–	–	–	–	–	–	0.2
cis-Sabinene hydrate	–	0.3	–	–	–	–	–	0.05–0.09	0.06–0.11	0.03–0.06	0.04–0.07	–	–
trans-Sabinene hydrate	0.05	0.6	–	–	–	0.02–0.09	0.04–0.25	0.12–0.19	0.14–0.22	0.07–0.14	0.07–0.17	–	–
Terpinen-4-ol	0.02	0.2	–	–	–	0.03–0.07	0.03–0.08	0.06–0.13	0.07–0.14	0.32–0.75	0.32–0.88	0.2	1.6
α-Terpineol	0.29	1.3	tr–0.3	tr	0.2–0.4	0.11–0.16	0.07–0.20	0.29–0.53	0.33–0.59	0.61–1.46	0.68–1.69	0.4	4.8
Thymol	0.05	0.4	–	–	–	–	–	0.16–0.32	0.09–0.24	0.20–0.67	0.13–0.47	0.1	–
Esters													
Aliphatic													
Decyl acetate	tr	–	–	–	–	tr–0.01	tr–0.01	–	–	–	–	–	–
Octyl acetate	–	–	–	–	–	tr	tr	0–tr	0–tr	0.02–0.04	0.02–0.05	–	–

continued

TABLE 1.10 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Mandarin Oils (1998–2009)

	Cold Extracted									Distilled			
	1	**2**	**3a**	**3b**	**3c**	**4a**	**4b**	**5a**	**5b**	**5c**	**5d**	**6**	**7**
Monoterpene													
Citronellyl acetate	0.02	–	–	–	–	tr–0.01	tr–0.01	0–tr	0–0.01	0–0.01	0–0.01	–	–
Geranyl acetate	0.02	–	0–tr	0.2	0–tr	tr–0.02	tr–0.05	0.01–0.04	0.01–0.09	0.01	0.01	–	–
Neryl acetate	–	–	–	–	0–tr	tr–0.01	0.01–0.05	0–tr	0–0.01	0–0.01	0–0.01	–	–
Ethers and oxides													
Monoterpene													
trans-Limonene oxide	0.01	–	tr	–	0–0.1	–	–	–	–	–	–	–	–
Others													
Methyl *N*-methyl anthranilate	–	1.4	–	–	0–1.3[b]	0.42–0.53	0.19–0.65	0.41–1.05	0.67–1.27	0.59–1.45	0.93–3.36	0.5	–.

Notes: tr, traces; *, correct isomer not characterized; +, identified but not quantitatively determined; [a] *p*-cymene is present at the following levels in the four cvs.: 0.5% (de Chios and Avana Apireno), 2.8% (Tardivo di Ciaculli), 6.9% (Willow leaf); [b] methyl *N*-methyl anthranilate is absent in the cv. Tardivo di Ciaculli and is present at the following levels in the other cvs.: 0.3% (Avana Apireno), 0.4% (de Chios), 1.3% (Willow leaf).

Appendix to Table 1.10

1. Sawamura (2000). Japan; cv. Tardivo di Ciaculli; one sample hand pressed; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T; Sawamura also found 3,3-dimethyl-1-octene (0.02%), β-selinene (0.03%), 2,4-decadienal* (0.01%), 2-dodecenal* (0.08%), carvone (0.06%), hexadecanol (0.02%), *p*-mentha-1,8-dien-9-ol (0.02%), (*E*)-nerolidol (0.03%), geranyl isobutyrate (0.03%), *cis*-limonene oxide (0.01%), octanoic acid (0.05%), nonanoic acid (0.04%), linalyl anthranilate (0.06%), and trace amounts of 1,3,8-*p*-menthatriene, *p*-mentha-1-en-9-ol, 1,8-cineole.
2. Naef and Velluz (2001). One sample extracted with CH_2Cl_2 separated into 14 fractions by flash chromatography on silica gel with a gradient pentane-ether; GC/FID and GC/MS conditions are not reported; LRI of numerous aliphatic aldehydes on apolar and polar columns are reported; relative percentage of peak areas. Naef and Velluz also found not quantitatively determined (*E*,*E*,*Z*)-1,3,5-undecatriene, (*Z*,*Z*,*E*,*Z*)-1,3,5,8-undecatetraene, (*E*,*E*,*Z*,*Z*)-1,3,5,8-undecatetraene, hexanal, (*E*,*Z*)-2,4-decadienal, (*E*,*Z*,*Z*)-2,4,7-decatrienal, (*E*,*E*,*Z*)-2,4,7-decatrienal, (*Z*)-4-decenal, (*E*)-2-undecenal, (*E*,*Z*)-2,6-dodecadienal, (*E*,*Z*)-2,4-dodecadienal, (*Z*)-6-dodecenal, (*Z*)-4-dodecenal, (*E*)-4-dodecenal, (*Z*)-2-dodecenal,

(*E*)-2-tridecenal, (*E*)-2-tetradecenal, hexadecanal, (*E*,*E*)-2,4-decadienol, (*E*,*Z*,*Z*)-2,4,7-decatrienol, (*E*)-2-dodecenol, (*Z*)-4-dodecenol, indole, 2-(*N*-methylamino)-benzaldehyde, 3-butyl-pyridine, 2,3-dimethyl-pyrazine, 2,6-dimethyl-pyrazine, 2-methyl-5-ethyl-pyrazine, 2-methyl-6-ethyl-pyrazine, diethyl disulfide.

3. Lota et al. (2001b). Corsica, France; samples manually cold-pressed; (a) range of the composition of oils extracted from the cvs.: Late Emperor, Empress, Emperor, Peau rugueuse, Peau lisse, (b) oil extracted from the cv. Commune, (c) range of the composition of oils extracted from the cvs.: Willow leaf, de Chios, Avana Apireno, Tardivo di Ciaculli; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/MS analysis was also performed with capillary column (50 m × 0.22 × 0.25 μm) coated with BP-20; Wiley and NIST MS libraries; LRI on BP-20 and BP-1 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found, β-elemene (0–tr), linalyl acetate (0–tr), in samples (a), trace amounts of β-bisabolene, (*E*)-β-farnesene in sample (b), β-elemene (0–tr), and linalyl acetate (0–tr) in samples (c).
4. Catalfamo et al. (2004). Calabria, Italy; samples manually cold-pressed; (a) range of the composition of 9 oils extracted from the cv. Avana; (b) range of the composition of 7 oils extracted from the cv. Tardivo di Ciaculli; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; MS library: NIST 1.7; relative percentage of peak areas. Catalfamo et al. also found (*Z*,*E*)-farnesol (0.01%–0.03%), and trace amounts of δ-3-carene, tricyclene, *cis*-sabinene hydrate acetate.
5. Frizzo et al. (2004). Brazil; range of the composition of samples cold extracted from the cv. Cai (a) and from the cv. Montenegrina (b) from fruits collected from March to April, range of the composition of samples hydrodistilled from the cv. Cai (c) and from the cv. Montenegrina (d) from fruits collected in the same period; GC/FID on capillary columns coated with SE-52 (25 m × 0.32 mm × 0.40–0.45 μm) and Carbowax 20M (25 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (25 m × 0.25 mm × 0.25 μm) coated with SE-52 and BP-20; Adams MS library; LRI on SE-52 and Carbowax 20M are reported; relative percentage of peak areas. Frizzo et al. also found in samples (a), (b), (c), and (d) respectively 3-dodecen-1-al* (0.02%–0.12%, 0.02%–0.12%, 0.01%–0.02%, 0.01%–0.02%), piperitone (0–tr, 0–tr, 0.01%–0.02%, 0.01%–0.02%), borneol (0–tr, 0%–0.01%, 0.01%–0.02%, 0%–0.02%), carvacrol (tr–0.05%, 0.02%–0.03%, 0%–0.01%, 0%–0.01%), *p*-cymen-7-ol (tr–0.05%, 0.03%–0.04%, 0.03%–0.06%, 0.04%–0.07%), *cis*-pinene hydrate (0%–0.01%, 0–tr, 0.03%–0.05%, 0.03%–0.06%), *p*-menth-2-en-1-ol (0.01%–0.04% only in samples c and d), thymol methyl ether (0.01%–0.03% only in samples c and d).
6. Ruberto et al. (1999). Sicily, Italy; Avana mandarin; one sample laboratory extracted by steam distillation; GC/FID and GC/MS on capillary column (25 m × 0.2 mm × 0.33 μm) coated with HP-1; MS libraries: Wiley and NBS; relative percentage of peak areas. Ruberto et al. also found trace amounts of farnesene*, nonanol.
7. Karioti et al. (2007). Nigeria; one sample hydrodistilled; GC/FID on capillary column (30 m × 0.32 mm × 0.25 μm) coated with Supelcowax-10; GC/MS on capillary columns coated with HP-5MS (30 m × 0.25 mm × 0.25 μm) and HP-Innowax (30 m × 0.25 mm × 0.5 μm); LRI on HP-5MS and HP-Innowax are reported; relative percentage of peak areas. Karioti et al. also found *cis*-carveol (0.1%).

numerous minor components, many of which are newly identified and very interesting for their odor properties. These components are listed in the appendix to Table 1.10.

Lota et al. (2001b) studied the composition of 58 cvs. of mandarin belonging to 15 different species, and based on cluster analysis and discriminant analysis they classified them in different chemotypes. Ten of the cvs. analyzed belong to the *C. deliciosa* Ten. specie, and were grouped into three different chemotypes. In Table 1.10 for these groups the percentage composition is reported separately as indicated in the appendix to the table. Two of these groups are characterized by very high contents of limonene (ca. 90%–93%) and by the absence of or very low values of γ-terpinene. These values were never reported for any of the samples considered in this review.

Catalfamo et al. (2004) analyzed samples of the cvs. Avana and Tardivo di Ciaculli. The average composition of the two types of oil is quite similar; in the cv. Avana, a modestly higher content of oxygenated compounds in comparison to Tardivo di Ciaculli (1.84% vs. 1.66%) is noticed.

Frizzo et al. (2004) studied the seasonal variation during the period March–June of the composition of two cvs. of mandarin cultivated in Brazil, both hydrodistilled and cold-pressed. The composition of the two oils is quite similar. The authors suggested, however, that the ratio methyl *N*-methyl anthranilate/α-sinensal could be used to differentiate the two oils. The oils extracted by hydrodistillation for both cvs., as is obvious, present higher amounts of monoterpene alcohols and lower content of α-sinensal. Some of the results of this research are indicative of the fact that the compositions of oils extracted by distillation techniques are sometimes not representative of the natural composition of the oils. As a consequence, it is our opinion that the analysis of oils obtained by distillation is not adequate for the comparison of oils of different cvs., or for the evaluation of seasonal variation of the composition of the oils during ripening. It is sufficient to examine the graphs reported in Figure 1.10 relative to the curves of α-sinensal obtained for the two cvs. studied by Frizzo et al. for both the extraction techniques. In fact, for both the cvs. cold-pressed α-sinensal, after a moderate decrease between March and April, increases from April to June; the behavior of α-sinensal in the hydrodistilled oils is not the same for the two cvs. In one case, it decreases from March to April, increases in the other, and then remains constant. It is absolutely clear that the extraction technique affects the composition of the oil more than the ripening stage of the fruits.

The mandarin oil analyzed by Karioti et al. (2007) could belong, although differently indicated by the authors, to the *C. deliciosa* Ten. specie, mostly for the composition of the monoterpene and sesquiterpene hydrocarbons and for the α-sinensal content; the amount of aliphatic aldehydes and of monoterpene alcohols is, however, higher when compared to Mediterranean mandarin oil.

More information on volatile components in mandarin peel oil is reported by Alonzo et al. (2003). These authors sampled and analyzed the head space of the peel of mandarin of the Ciaculli

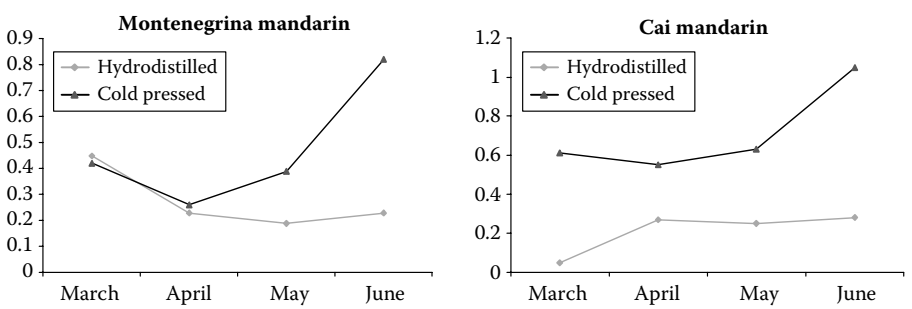

FIGURE 1.10 Variation of the content of α-sinensal in mandarin oils extracted by cold pressing and hydrodistillation from cvs. Montenegrina and Cai. (From the results reported by Frizzo, C.D., et al., *J. Agric. Food Chem.* 52, 3036–3041, 2004.)

late variety using a polydimethylsiloxane (PDMS) fiber. They identified only monoterpene hydrocarbons, among which the most abundant were limonene (79.5%) and γ-terpinene (12.0%). The absence of oxygenated compounds was probably due to the low affinity of the fiber toward polar compounds.

1.5.1.3 Tangerine Industrial Oils

Papers on the composition of industrially produced tangerine oils that were published in the period considered are limited to those about one sample of Murcott tangerine from Brazil (Feger et al., 2003) and six Mexican samples of Dancy tangerine of a different geographic origin (Dugo et al., 2005). The results are summarized in Table 1.11. Murcott is probably an hybrid obtained in Florida that shows great similarity to tangerine (Kryger, 2002). It is characterized by quite a high content of limonene (94.60%) and by the absence of γ-terpinene. The composition of the six samples analyzed by Dugo et al. (2005) does not seem to be affected by the different geographic origin, and only for

TABLE 1.11
Percentage Composition of the Volatile Fraction of Tangerine Oils (1998–2009)

	Industrial Oils		Laboratory Oils					
			Cold-Pressed					Solvent
	1	2	3	4	5	6	7	8
			Hydrocarbons					
Aliphatic								
Tetradecane	tr	–	–	–	–	–	0.1	–
Tridecane	tr	–	–	–	–	–	tr	–
Monoterpene								
Camphene	tr	tr–0.01	–	tr	tr	–	tr	–
p-Cymene	0.01	tr–1.12	–	3.32	–	0.1–0.4	–	tr
Limonene	94.60	89.58–90.94	91.72	89.15	95.67	87.1–90.9	95.1	95.2
Myrcene	1.86	1.76–2.34	–	1.94	1.74	1.5–1.7	2.0	1.6
(*E*)-β-Ocimene	0.04	0.02–0.06	–	0.07	0.08	tr–0.2	tr	–
(*Z*)-β-Ocimene	–	–	–	0.27	tr	–	tr	–
α-Phellandrene	0.03	0.03–0.04	–	0.40	tr	–	tr	–
β-Phellandrene	0.27	–	–	0.11	tr	0–0.3	0.3	–
α-Pinene	0.53	0.83–1.02	0.62	0.89	0.53	0.5–0.9	0.5	0.6
β-Pinene	0.03	0.29–0.34	1.18	0.30	0.03	0.3–0.4	0.4	tr
Sabinene	0.29	0.14–0.18	0.22	0.15	0.44	0.1–0.2	0.2	0.1
α-Terpinene	–	0.02–0.06	0.06	0.08	–	0.1	–	–
γ-Terpinene	–	2.15–3.46	–	0.03	tr	3.9–5.2	–	–
Terpinolene	0.01	0.12–0.18	2.82	1.32	0.01	0.2	tr	–
α-Thujene	tr	0.14–0.16	–	0.15	–	0.1–0.2	–	–
Sesquiterpene								
δ-Cadinene	0.04	0.01–0.02	–	tr	–	–	–	–
β-Caryophyllene	0.01	tr–0.01	0.01	0.06	tr	–	–	tr
α-Copaene	0.03	0.01	tr	0.03	0.02	–	–	–

continued

TABLE 1.11 (continued)
Percentage Composition of the Volatile Fraction of Tangerine Oils (1998–2009)

	Industrial Oils		Laboratory Oils					
			Cold-Pressed					Solvent
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
β-Cubebene	0.04[c]	0.02–0.04[c]	–	–	0.01	–	–	–
β-Elemene	0.04[c]	0.02–0.04[c]	–	0.04	0.01	0–0.1	tr	–
γ-Elemene	–	tr–0.01	–	tr	0.01	–	–	–
(*E*,*E*)-α-Farnesene	0.07	0.04–0.05	–	0.04	0.06	–	–	–
(*E*)-β-Farnesene	0.05	–	–	–	0.03	–	–	–
Germacrene D	0.02	–	–	–	0.01	–	tr	–
α-Humulene	0.01	0.01	–	0.02	–	–	tr	–
γ-Muurolene	tr	0.02–0.08	–	0.07	–	–	–	–
Valencene	0.01	–	tr	–	–	–	–	–
			Aldehydes					
Aliphatic								
Decanal	0.36	0.10–0.14	–	0.12	0.13	–	0.1	0.2
(*E*,*E*)-2,4-Decadienal	0.01	0.02	–	–	–	–	–	+
(*E*)-2-Decenal	0.01	0.01–0.02	–	–	–	–	tr	+
Dodecanal	0.07	0.02–0.03	–	0.03	0.02	–	tr	0.1
(*E*)-2-Dodecenal	–	tr	–	–	–	–	tr	tr
Hexadecanal	tr	–	–	–	–	–	–	+
Nonanal	0.10	0.03–0.06	–	–	0.03	–	–	0.1
Octanal	0.36	0.08–0.12	–	–	0.33	0.1–0.2	0.2	0.2
Tetradecanal	0.01	tr	–	–	–	–	–	+
Undecanal	0.03	0.01–0.02	–	tr	0.01	–	tr	+
Monoterpene								
Citronellal	0.12	0.03–0.06	0.05	0.07	0.07	0–tr	tr	–
Geranial	0.03	0.01–0.02	–	0.02	–	–	tr	–
Neral	0.08	tr–0.01	0.01	tr	tr	–	tr	–
Perilla aldehyde	0–04[b]	0.03–0.05	0.02	0.03	0.02	–	tr	tr
Sesquiterpene								
α-Sinensal	tr	0.11–0.20	0.26	0.12	–	tr–0.2	–	tr
			Ketones					
Monoterpene								
Carvone	0.01	0.01–0.03	–	–	0.02	–	tr	–
			Alcohols					
Aliphatic								
Octanol	0.04	0.02–0.06[d]	–	tr	0.01	–	tr	0.1
Monoterpene								
cis-Carveol	tr	tr–0.02	–	–	–	–	tr	–
trans-Carveol	0.01	–	–	–	tr	–	–	–
Citronellol	0.06	0.03–0.05	0.05	0.08	0.04	0–tr	tr	0.1
Geraniol	tr	tr	–	tr	–	–	–	–
Linalol	0.37	0.54–0.74	1.14	0.03	0.52	0.4–2.2[e]	0.2	0.3

TABLE 1.11 (continued)
Percentage Composition of the Volatile Fraction of Tangerine Oils (1998–2009)

	Industrial Oils		Laboratory Oils					
			Cold-Pressed					Solvent
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Nerol	0.01	–	–	tr	0.01	–	0.1	–
cis-Sabinene hydrate	–	0.02–0.06[d]	–	0.18	–	–	–	–
trans-Sabinene hydrate	tr	–	–	0.02	0.01	–	–	–
Terpinen-4-ol	tr	0.01–0.02	0.03	tr	–	–	–	tr
α-Terpineol	0.04	0.07–0.24	0.08	0.10	0.04	tr–0.1	0.1	0.1
Thymol	–	0.05–0.07	–	0.09	–	–	–	–
Sesquiterpene								
Elemol	0.01	tr–0.01	–	–	tr	–	–	–
Esters								
Monoterpene								
Citronellyl acetate	0.02	tr	0.01	0.04	0.01	–	tr	–
Geranyl acetate	0.01	tr–0.01	–	tr	tr	0–tr	tr	–
Neryl acetate	0.04	0.01–0.03	–	0.02	–	0–tr	tr	–
α-Terpinyl acetate	tr	0.01–0.04	–	–	–	–	–	–
Ethers and oxides								
Monoterpene								
cis-Limonene oxide	0.01[a]	0.03–0.21	–	–		–	tr	–
trans-Limonene oxide	0.01	0.03–0.14	–	–	tr	0–tr	tr	–
Thymol methyleter	–	0.03–0.05	0.05	0.11	–	–	–	–

Notes: tr, traces; t, tentative identification; +, identified but not quantitatively determined; *, correct isomer not characterized; [a] *cis*-limonene oxide + *cis*-*p*-mentha-2,8-dien-1-ol; [b] perilla aldehyde + decanol; [c] β-cubebene + β-elemene; [d] octanol + *cis*-sabinene hydrate, [e] linalol is present in the cvs. Dancy and Redskin at level of 2.2% and 1.7% respectively, in the other cvs. ranges from 0.4% to 0.7%.

Appendix to Table 1.11

1. Feger et al. (2003). Brazil; one sample industrial cold-pressed of Murcott tangerine oil; GC/FID and GC/MS on capillary columns (66 m × 0.25 mm × 0.5 μm) coated with DB-1 and (30 m × 0.25 mm × 0.5 μm) coated with OV-1701; relative percentage of peak areas. Feger et al. also found δ-3-carene (0.02%), bicyclogermacrene (0.01%), β-copaene + 2-hexylcyclopropane acetic acid (0.01%), germacrene A (0.01%), γ-geraniol[t] (0.01%), *p*-mentha-1(2),8-dien-10-ol (0.02%), *trans*-*p*-mentha-2,8-dien-1-ol (0.01%), octyl acetate (0.01%), *p*-mentha-1(2),8-dien-10-yl-acetate (0.01%), and trace amounts of undecane, α-bulnesene, α-cubebene, α-guaiene, α-muurolene, 7-epi-α-selinene, β-sesquiphellandrene, tridecanal, β-sinensal, isopiperitone, nootkatone, dodecanol, nonanol, *cis*-*p*-mentha-1(7),8-dien-2-ol, cubebol, (*E*)-nerolidol, caryophyllene oxide*, decanoic acid, octanoic acid.
2. Dugo P. et al. (2005). Mexico; six samples of cold-pressed Dancy tangerine oil of different geographical origins; GC/FID and GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with MDN-5S; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on MDN-5S column are reported; relative percentage of peak areas. Dugo P. et al. (2005) also found nonane + heptanal (tr–0.01%), 1-tetradecene (tr–0.01%), germacrene B (0.03%–0.09%), tetradecanol (tr–0.01%), germacrene D-4-ol (tr–0.01%), spathulenol (0.03%–0.04%), miroxide* (0.01%–0.02%), and trace amounts of tridecanol, octyl formate.

continued

TABLE 1.11 (continued)
Percentage Composition of the Volatile Fraction of Tangerine Oils (1998–2009)

3. Li and Qi (1997). China; one sample; Li and Qi also found β-ocimene* (0.07%), α-bisabolene* (0.01%), α-cubebene (0.03%), farnesene* (0.01%), β-selinene (0.04%), neryl propanate (0.01%), and trace amounts of β-cadinene, elemene*.
4. Huang and Wu (1998). Japan; one sample hand pressed; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-17 or OV-101; relative percentage of peak areas. Huang and Wu also found δ-elemene (0.07%), α-farnesene* (0.04%), β-farnesene* (0.02%), (*Z*)-3-hexenol (0.02%), and trace amounts of hexanol.
5. Sawamura (2000). Japan; one sample hand pressed of Murcott tangerine oil; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T. Sawamura also found *p*-mentha-1,8-dien-9-ol (0.02%), decyl acetate (0.01%), geranyl isobutyrate (0.02%).
6. Lota et al. (2001b). Corsica, France; one sample hand pressed of each of the following cvs.: Vohangisahy, Beauty of Glen Retreat, Brickaville, Dancy, Redskin, Swatow; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; NIST and Wiley MS libraries; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found β-bisabolene (0%–0.1%), linalyl acetate (0–tr).
7. Minh Tu et al. (2002). Vietnam; one sample hand pressed; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; LRI on DB-Wax are reported; relative percentage of peak areas. Minh Tu et al. also found trace amounts of octadecane, *trans-p*-mentha-2,8-dien-9-ol, perilla alcohol, *cis*-linalol oxide, *trans*-linalol oxide.
8. Naef and Velluz (2001). One sample extracted with CH_2Cl_2 separated into 14 fractions by flash chromatography by gradient elution with penthane and diethyl ether; GC condition not reported; relative percentage of peak areas. Naef and Velluz also found (*E*,*Z*)-2,4-decadienal; (*E*,*Z*,*Z*)-2,4,7-decatrienal; (*E*,*E*,*Z*)-2,4,7-decatrienal; (*Z*)-4-decenal, (*E*,*Z*)-2,6-dodecadienal, (*Z*)-2-dodecenal, (*E*)-2-hexenal, (*E*,*E*)-2,4-heptadienal, (*E*)-2-nonenal, (*E*)-2-octenal, pentadecanal, (*E*)-2-tridecenal.

one of the samples cultivated in the mountain region did the authors observe a lower content of some monoterpene hydrocarbons (α-thujene, β-pinene, α-, and γ-terpinene) and an higher content of decanal and nonanal compared to the other oils analyzed.

Besides what is reported in Table 1.11, there is only a little information available on industrially processed tangerine oils. Feger et al. (2001a), in their investigation on germacrenes content in some citrus essential oils, determined in Murcott tangerine oils from Brazil and Florida the following contents expressed as a relative percentage of peak areas: bicyclogermacrene (0.01%) germacrene A (0.01%) and germacrene D + valencene (0.02%–0.03%), whereas in samples of tangerine from China, the content of germacrenes was: bicyclogermacrene (0.01%–0.02%); germacrene A (0.03%); germacrene B (0.08%–0.10%); germacrene C (0.05%–0.07%); and germacrene D + valencene (0.06%–0.08%). Reeve and Arthur (2002) reported a chromatogram of tangerine oil where it appeared identified and quantitatively determined the following components: limonene (90%); γ-terpinene (4%); thymol (0.07%); and α-sinensal (0.1%). Kryger (2002) reported the chromatograms of a tangerine oil and of some tangerine-like oil from Florida. Authors identified only the main components. Veriotti and Sacks (2002) analyzed a commercial tangerine oil by high-speed GC with TOF MS detection. The oil was characterized by a high content of *p*-cymene (2.75%).

1.5.1.4 Tangerine Laboratory Oils

Table 1.11 also reports the results of studies carried out on tangerine and tangerine-like oils extracted in laboratory. Some of these were published before 1999, but were not revised in the previous review by Dugo et al. (2002). Most of the research looked at samples from the East (China, Vietnam), and all of them are characterized by absence or trace amounts of γ-terpinene. Only in the papers by Sawamura (2000) and by Lota et al. (2001b) is the botanical origin of the fruit reported. The main differences in composition of these oils relate to the content of *p*-cimene, γ-terpinene, and linalol. Naef and Velluz (2001) reported in addition to the main components of an oil analyzed numerous

minor components, some identified for the first time and very interesting for their odor properties. These components are listed in the appendix to Table 1.11.

1.5.1.5 Industrial and Laboratory-Extracted Clementine Oils (1974–2009)

The composition of clementine oil was not included in the previous review by Dugo et al. (2002).

In 1974 Calvarano et al. asserted that due to the appreciation of the fresh fruit in the market the industrial transformation of clementine was not extensive. Considering the very scant data available on industrially produced clementine oil, the situation is probably not substantially changed. It is difficult to determine the quantity of the fruits produced and sent to the industrial transformation, since in international statistics clementines are accounted with mandarins and tangerines. In Spain, one of the major producers of clementine, probably 300,000 tons per year are sent to the industrial transformation. The clementine oil is however present on the international market at a price of about 7 €/kg.

The composition of industrially produced oil is studied in limited research by Calvarano et al. (1974), Dugo et al. (1999), Mondello et al. (1995b)—the latter limited to the hydrocarbon fraction—and by Feger et al. (2001a) on a commercial oil, focused on the determination of germacrenes. Numerous are the articles published on the laboratory-extracted oil. The results on the composition of clementine oil were revised and shortly reported by Lawrence (2002) and later reported by the same author in a more detailed review (Lawrence, 2007).

Calvarano et al. (1974) identified and quantitatively determined the principal components of clementine essential oil: camphene (0.02%); limonene (83.03%); myrcene (7.58%), α-pinene (1.99%); β-pinene + sabinene (2.04%); γ-terpinene (2.05%); terpinolene (0.22%); decanal (0.46%); nonanal (0.03%); citronellal (0.21%); geranial (0.23%); neral + α-terpineol (0.13%); linalol (0.96%); terpinen-4-ol (0.06%); and methyl *N*-methyl anthranilate (tr). The low value of limonene and the high value of myrcene may be due to a chromatographic separation that is not sufficient for the correct quantitative determination. Mondello et al. (1995b) isolated and analyzed by automated on-line HPLC-HRGC/FID and MS the monoterpene and sesquiterpene hydrocarbons of clementine oil industrially produced. Among the monoterpene hydrocarbons only limonene (96.03%) and myrcene (1.90%) exceeded 1% of the analyzed fraction. The other monoterpene hydrocarbons identified, listed in decreasing amount were: γ-terpinene, α-pinene, sabinene, β-pinene, terpinolene, (*E*)-β-ocimene, α-phellandrene, α-terpinene, α-thujene, (*Z*)-β-ocimene, and camphene. In the sesquiterpene hydrocarbon fraction (qualitatively very complex), β-bisabolene, *trans*-α-bergamotene, and β-caryophyllene were the principal components, then, listed in decreasing amount order were germacrene B, δ-elemene, germacrene D, *cis*-α-bergamotene, β-elemene, (*Z*)-β-farnesene, α-humulene, α-selinene, β-santalene, (*Z*)-γ-bisabolene, (*E*)-γ-bisabolene, *epi*-β-santalene. In Italian commercial oils Feger et al. (2001a) identified the following germacrenes: biciclogermacrene (tr–0.01%), germacrene A (0.01%), germacrene D (coeluted with valencene) (0.03%).

The results relative to the composition of the whole volatile fraction of clementine essential oil are reported in Table 1.12.

The values reported in Table 1.12 refer to oils of different geographic origin; they appear quite homogeneous and well represent the composition of the volatile fraction of clementine essential oil. However, the following comments should be noted: low values of limonene obtained by Lota et al. (2001b) in the oils extracted from the cvs. Ragheb and Guillermina cultivated in Corsica (90.1% and 89.1% respectively), along with high values of sabinene (4.0% and 2.8% respectively); the values of sabinene reported by Merle et al. (2004) are also high in the cvs. Arrufatina, Clemenpons, and Marisol (2.22%, 1.85%, and 1.74% respectively); values of γ-terpinene, usually present at trace levels are too high in the samples analyzed by Dugo et al. (1999) and by Gazea et al. (1998) extracted from fruits cultivated in Calabria (0.68% and 0.81% respectively); in the same samples linalol presented low values (0.21% and 0.16% respectively), and in the Chinese oil analyzed by Huang et al. (1998) linalol was present only at trace levels.

TABLE 1.12
Percentage Composition of the Volatile Fraction of Clementine Oils

	Industrial		Laboratory								Distilled	
			Cold-Pressed								SDE	Hydrodistillation
	1	2	3	4	5	6	7	8	9	10	11–15	16
						Hydrocarbons						
Monoterpene												
Camphene	–	tr	tr	tr	tr	tr	0.01	–	–	–	tr	tr
δ-3-Carene	–	0.04	0.02	0.03–0.07	0.03–0.06	0.08	0.03	–	0–0.1	0–0.13	tr–0.05	–
p-Cymene	–	a	d	–	–	tr	–	0.02	tr	0–0.03	–	–
Limonene	94.95	94.62[a]	95.01[d]	91.50–94.78	92.94–93.78	92.85	93.86	93.32	89.1–95.5	91.79–95.13	94.68–95.46	94.0
Myrcene	1.50	1.86	1.63	1.61–2.03	1.99–2.10	2.00	1.66	1.23	1.4–2.0	1.84–1.93	1.8–1.83	1.7
(*E*)-β-Ocimene	–	0.04	0.04	0.02–0.16	0.04–0.10	tr	0.06	0.05	tr–0.2	0.03–0.16	0–0.03	tr
(*Z*)-β-Ocimene	–	0.01	0.01	tr–0.02	tr–0.01	0.28	0.01	–	–	–	tr	tr
α-Phellandrene	–	0.09	0.27[c]	0.02–0.04	0.03	0.02	0.11	–	tr	0.04–0.05	tr–0.03	tr
β-Phellandrene	–	a	–	–	–	0.03	–	tr	0.2–0.3	–	0–0.01	–
α-Pinene	0.10	0.57	0.32	0.30–0.51	0.55–0.58	0.53	0.56	0.48	0.3–0.6	0.49–0.56	0.33–0.5	0.3
β-Pinene	–	0.09	0.02	0.03–0.22	0.21–0.26	0.04	0.15	0.01	tr–0.2	0.03–0.11	tr–0.03	tr
Sabinene	0.10	0.51	0.44	0.28–1.26	0.94–1.09	0.66	0.65	0.09	0.3–4.0	0.52–2.22	0.15–0.4	0.1
α-Terpinene	–	0.02	–	tr–0.01	tr	–	0.02	–	–	–	0–0.06	tr
γ-Terpinene	0.01	0.68	tr	tr–0.02	tr	0.05	0.81	–	0–0.1	0–0.02	tr–0.02	tr
Terpinolene	–	0.05	0.01	0.01–0.02	0.01–0.02	0.67	0.02	–	tr	0–0.23	tr–0.02	tr
α-Thujene	–	0.03	tr	tr–0.01	0.01	tr	0.04	–	–	0–0.02	tr	–
Sesquiterpene												
trans-α-Bergamotene	–	–	–	tr	tr	–	0.01	–	0–0.1	–	–	–
Bicyclogermacrene	–	tr	–	tr–0.02	tr–0.01	–	–	–	–	–	–	tr
β-Bisabolene	–	–	–	tr–0.01	tr	–	–	–	–	–	0–0.01	–
β-Caryophyllene	–	0.01	0.01	tr–0.02	0.01	0.02	0.01	tr	–	0–0.01	tr–0.02	–

δ-Cadinene	0.04	0.03	–	0.03–0.06	0.03–0.05	0.07	–	–	0–0.1	0.01–0.05	tr	tr
α-Copaene	–	0.03	0.04	0.02–0.04	0.03–0.04	0.07	–	0.02	tr–0.1	0.01–0.04	tr–0.03	tr
β-Cubebene	0.04	0.03	0.03	0.02–0.05	0.03–0.04	–	–	tr	–	0.01–0.05	0–0.02	tr
β-Elemene	–	0.01	0.03	0.01–0.02	0.01	0.07	–	–	–	–	–	tr
β-Farnesene*	–	–	–	–	–	0.03	–	–	–	–	0–0.02	–
(*E*,*E*)-α-Farnesene	–	0.03	0.04	0.03–0.15	0.03–0.04	0.06	0.05[f]	0.01	–	–	0–0.01	tr
(*E*)-β-Farnesene	–	–	–	0.01–0.11	0.01–0.02	–	–	0.06	0–0.1	–	–	tr
(*Z*)-β-Farnesene	–	0.02	–	0.01–0.03	0.01	–	–	tr	–	–	–	–
Germacrene D	0.06	0.02	–	0.02–0.04	0.02–0.04	–	–	–	0–0.1	0–0.04	–	tr
β-Gurjunene	0.02	–	–	0.01–0.04	0.01–0.02	–	–	–	–	–	–	tr
α-Humulene	–	0.01	0.03	tr–0.01	0.01	0.02	0.04	–	–	0–0.08	tr–0.01	tr
γ-Muurolene	–	–	–	tr–0.01	tr–0.02	0.05	–	–	–	–	–	–
Valencene	–	tr	–	0.01–0.04	0.01	–	0.50	–	–	0.01–0.09	–	tr
Aldehydes												
Aliphatic												
Decanal	0.40	0.19	0.31	0.21–0.47	0.36–0.40	0.18	0.18	0.01	0.1–0.5	–	0.27–0.34	0.5
(*E*,*E*)-2,4-Decadienal	0.02	tr	–	tr–0.02	0.01	–	–	0.01	–	–	–	tr
(*E*)-2-Decenal	–	0.01	–	tr–0.02	tr–0.01	–	–	0.01	–	–	–	tr
Dodecanal	–	0.05	–	0.04–0.11	0.06–0.08	0.05	–	0.01	–	–	0.05–0.1	0.1
(*E*)-2-Dodecenal	–	0.01	–	0.01–0.04	0.01	–	–	–	–	–	tr–0.02	–
Nonanal	0.02	0.01	0.02	0.01–0.03	0.01–0.02	–	0.09	–	tr	–	0–tr	tr
Octanal	0.19	0.09	0.27[c]	0.17–0.53	0.18–0.58	–	–	0.01	0–0.3	0.30–0.50	0.13–0.4	0.6
Tetradecanal	–	tr	–	tr–0.01	tr–0.01	–	–	–	–	–	–	–
Tridecanal	–	–	–	tr–0.01	tr	–	–	–	–	–	–	–
Undecanal	–	0.01	0.02	tr–0.01	tr–0.01	tr	0.01	–	–	–	–	tr
Monoterpene												
Citronellal	0.09	0.05	0.13	0.06–0.10	0.04–0.07	0.09	0.04	0.05	0–0.1	0.03–0.06	0–0.1	0.1
Geranial	0.03	0.27[b]	0.04	0.02–0.10	0.02–0.03	0.02	0.03	–	0–0.1	–	0–0.1	tr
Neral	–	0.01	0.01	0.01–0.06	0.01–0.04	tr	0.01[f]	0.03	0–0.1	–	0–0.03	tr
Perilla aldehyde	0.01	0.27[b]	–	0.02–0.05	0.02–0.04	0.02	–	0.04	–	–	0–0.07	0.1

continued

TABLE 1.12 (continued)
Percentage Composition of the Volatile Fraction of Clementine Oils

	Industrial		Laboratory								Distilled	
			Cold-Pressed								SDE	Hydrodistillation
	1	2	3	4	5	6	7	8	9	10	11–15	16
Sesquiterpene												
α-Sinensal	0.22	0.19	0.30	0.24–0.68	0.21–0.28	0.35	0.21	–	0–0.7	0.08–0.27	0.07–0.21	tr
β-Sinensal	–	0.04	0.05	0.02–0.19	0.04–0.07	0.10	0.04	0.04	0–0.3	–	tr–0.03	tr
Ketones												
Monoterpene												
Carvone	–	tr	–	tr	tr	–	–	–	–	0–0.25	0–0.05	tr
Sesquiterpene												
Nootkatone	–	–	–	0.01–0.02	tr–0.03	–	–	–	–	–	tr–0.01	–
Alcohols												
Aliphatic												
Octanol	–	0.01	0.01	tr–0.02	tr–0.11	0.02	–	–	–	–	tr–0.06	0.1
Tetradecanol	–	–	–	tr–0.01	tr	–	–	–	–	–	–	–
Monoterpene												
cis-Carveol	0.03	tr	–	tr–0.06	0.01	–	–	–	–	–	–	tr
Citronellol	–	–	0.03[e]	0.01–0.04[e]	–	0.04	0.09[f]	0.11	tr	–	–	tr
Geraniol	–	–	–	tr	tr	tr	tr	–	–	–	0–0.04	tr
Linalol	0.89	0.21	0.69	0.64–1.52	0.46–0.67	tr	0.16	0.93	0.6–1.9	0.49–1.18	0.53–1.15	0.9
trans-p-Mentha-2,8-dien-1-ol	–	–	–	–	–	–	–	0.19	–	0–0.17	–	–
Nerol	–		0.03[e]	0.01–0.04[e]	0.01–0.02	tr	0.02	–	–	–	tr–0.09	–
Perilla alcohol	0.03	tr	–	0.01–0.04	0.01	–	0.02[f]	–	–	–	–	–
cis-Sabinene hydrate	–	tr	0.01	tr–0.07	0.03	tr	–	–	–	–	–	–

trans-Sabinene hydrate	–	tr	–	tr–0.01	tr	tr	–	–	0–0.2	–	–	–
Terpinen-4-ol	–	0.01	tr	tr–0.02	tr–0.01	tr	0.02	–	–	–	tr–0.05	0.1
α-Terpineol	0.09	0.02	0.06	0.05–0.13	0.05–0.07	0.09	0.02	0.04	0–0.2	0.31–0.56	0.07–0.12	0.1
Thymol	–	tr	–	–	–	tr	–	–	–	–	–	–
Sesquiterpene												
Elemol	–	0.01[t]	–	–	–	–	–	–	–	–	–	tr
(*E*)-Nerolidol	–	tr	–	0.01	tr–0.01	–	–	–	–	–	–	tr
Esters												
Aliphatic												
Heptyl acetate	–	tr	–	tr–0.02	tr–0.01	–	–	–	–	–	–	–
Octyl acetate	–	tr	–	tr–0.01	tr–0.01	–	tr	–	–	–	–	tr
Monoterpene												
Citronellyl acetate	–	–	0.01	–	–	–	0.03	–	–	–	–	–
Geranyl acetate	0.04	tr	0.01	tr–0.01	tr	tr	tr	–	–	–	0–tr	tr
Linalyl acetate	–	tr	–	–	–	–	–	–	–	–	–	–
Neryl acetate	–	tr	0.01	tr	tr	tr	0.01	–	–	–	0–tr	tr
α-Terpinyl acetate	–	tr	–	tr–0.01	tr	–	0.06	–	–	–	0–0.03	tr
Ethers and oxides												
Monoterpene												
cis-Limonene oxide	–	0.01	–	0.01–0.03	tr–0.01	–	–	0.43	tr	0–0.18	–	tr
trans-Limonene oxide	–	0.01	–	0.01	tr–0.01	–	–	0.20	tr	–	–	tr
Others												
Methyl *N*-methyl anthranilate	–	0.02	0.07	0–0.01	tr	–	–	–	–	–	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; [a] limonene + *p*-cymene + β-phellandrene; [b] geranial + perilla aldehyde; [c] α-phellandrene + octanal; [d] limonene + *p*-cymene; [e] citronellol + nerol; [f] identified and quantified only by GC/MS.

continued

TABLE 1.12 (continued)
Percentage Composition of the Volatile Fraction of Clementine Oils

Appendix to Table 1.12

1. Baaliouamer et al. (1992). Algeria; semi industrial extractor; one sample; preparative GC on packed columns of FFAP; GC/FID and GC/MS on capillary column (50 m × 0.2 mm) coated with FFAP; LRI on FFAP are reported; relative percentage of peak areas. Baaliouamer et al. also found isopiperitone (0.04%), citronellyl propanate (0.07%).
2. Dugo et al. (1999). Italy; one sample; GC/FID and GC/MS on capillary column (25 m × 0.25 mm × 0.25 μm) coated with SE-52; LRI on SE-52 are reported; Adams, Nist and Wiley MS libraries; relative percentage of peak areas. Dugo et al. also found α-cadinene[t] (0.01%), *trans*-carveol (0.01%), and trace amounts of decyl acetate.
3. Dugo et al. (1988). Italy; average values of three samples laboratory cold-pressed; GC/FID on capillary column (25 m × 0.32 mm) coated with SE-52; GC/MS on capillary column (25 m × 0.35 mm) coated with DB-1; relative percentage of peak areas.
4. Verzera et al. (1997b). Calabria, Italy; three samples, each laboratory cold-pressed from the cvs. Oroval, Monreal and Comune; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary column (60 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; relative percentage of peak areas.
5. Verzera et al. (1998b). Uruguay; two samples, each laboratory cold-pressed from the cvs. Nules and Comune; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary column (30 m × 0.25 mm × 0.40–0.45 μm) coated with SE-52; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas.
6. Huang and Wu (1998). China; one sample laboratory cold-pressed; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-17 and OV-101; relative percentage of peak areas. Huang and Wu also found (*Z*)-3-hexenol (0.03%) and trace amounts of γ-elemene, hexanol.
7. Gazea et al. (1998). Calabria, Italy; one sample laboratory cold-pressed; GC/FID on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; GC/MS on capillary column (15 m × 0.25 mm × 0.25 μm) coated with SE-54; relative percentage of peak areas. Gazea et al. also found γ-gurjunene[f] (0.06%), β-selinene[f] (0.08%), (*Z*,*Z*)-farnesol[f] (0.04%), nonyl acetate (0.01%), terpinen-4-yl acetate (0.05%), thujyl acetate[f] (0.01%).
8. Sawamura (2000). Japan; one sample laboratory cold-pressed; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T. Sawamura also found bicyclopentyl-2-one (0.05%), *p*-mentha-1-en-9-ol (0.01%), *p*-mentha-1,8-dien-9-ol (0.03%), spathulenol (0.04%), *cis*-carvyl acetate (0.01%), *trans*-linalol oxide (0.01%).
9. Lota et al. (2001b). Corsica, France; range of the composition of the oils cold-pressed from the cvs. MA2, MA3, Nules, Hernandina, Tardia Villareal, Reina, Caffin, Mac Bean, Oroval, Monreal, Bruno, Tomatera, Comune, Marisol, Ragheb, Guillerma; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/MS analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; Wiley and Nist MS libraries; LRI on BP-20 and BP-1 are reported; ^{13}C-NMR; relative percentage of peak areas.
10. Merle et al. (2004). Spain; range of the composition of the oils cold-pressed from the cvs. Hernandina, Clemenules, Clemenpons, Arrufatina, Loretina, Marisol, Oronules; GC/FID and GC/MS on capillary column (25 m × 0.25 mm × 0.25 μm) coated with DB-5; LRI on DB-5 are reported; Adams MS library. Merle et al. also found β-copaene (0.01%–0.05%), *cis*-*p*-menth-2,8-dien-1-ol (0%–0.04%).

11–15. Ruberto et al. (1993, 1994, 1997a,b, 1999). Sicily, Italy; SDE; range of the composition of the oils analyzed in the five papers; GC/FID and GC/MS on capillary column (25 m × 0.2 mm) coated with HP-1; Adams, NBS and Wiley MS libraries; relative percentage of peak areas. Ruberto et al. also found γ-cadinene (0%–0.04%), α-cubebene (0%–0.02%), β-farnesene* (0%–0.02%), decanol (0–tr), 1-hexen-3-ol (0–tr), nonanol (0%–0.02%), carveol* (tr–0.02%), isopulegol (0%–0.07%), decyl acetate (0–tr).

16. Ruberto and Rapisarda (2002). Sicily, Italy; one sample; hydrodistillation; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; Adams and NBS MS libraries; relative percentage of peak areas. Ruberto and Rapisarda also found trace amounts of α-muurolene, β-selinene, β-sesquiphellandrene, *cis*- and *trans*-dihydrocarvone, 6-dodecenal*, 3-dodecenal*, decanol, *trans*-carveol, *p*-mentha-1,8(10)-en-9-ol, *cis*-*p*-menth-2-en-1-ol, *cis*- and *trans*-carvyl acetate.

Two articles (Buettner et al. 2003; Chisholm et al., 2003a,b) also reported the odor active and the potent odorant volatiles present in clementine oil; many of these components are discussed in Chapter 10.

1.6 SWEET ORANGE OIL (*CITRUS SINENSIS* [L.] OSBECK)

1.6.1 1979–1999

Many of the papers on the quantitative composition of sweet orange oils published before 1979 have been reviewed by Shaw (1979). A summary of these papers was reported by Dugo et al. (2002). Between 1960 and 1979 more papers than those reviewed by Shaw (1979) report quantitative or qualitative information on the composition of sweet orange oil. Shaw (1977) reported some of these in his earlier review. These papers have been reviewed by Dugo et al. (2002). Most of the papers published from 1979 to 1999 on the composition of sweet orange oils (cold-pressed surely genuine and commercial of not specified origin, industrially processed, and laboratory-extracted oils), have been reviewed by Dugo et al. (2002).

Table 1.13 summarizes the results, relative to these last papers.

TABLE 1.13
Percentage Composition of the Volatile Fraction of Sweet Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial and Unusual Oils	Laboratory-Extracted Oils
		Hydrocarbons	
Monoterpene			
Camphene	tr–0.01	tr	tr
δ-3-Carene	0.04–0.21	0–0.17	0–0.22
p-Cymene	tr–0.06	tr–0.2	tr
Limonene	91.15–96.08	85.16–94.51	86.18–96.80
Myrcene	1.71–2.04	1.37–2.3	0.93–2.05
(*E*)-β-Ocimene	tr–0.10	tr–0.21	0.01–0.06
(*Z*)-β-Ocimene	tr–0.03	tr	tr–0.01
α-Phellandrene	tr–0.07	0.05	0.02–0.09
β-Phellandrene	0–0.2	1.5	0–0.20
α-Pinene	0.36–1.4	0.51–0.9	0.28–0.55
β-Pinene	tr–0.11	0–1.25	tr–0.06
Sabinene	0.24–0.80	0.13–2.45	0.13–0.93
α-Terpinene	0–0.02	0.09	tr–0.30
γ-Terpinene	0–0.33	tr–4.66	tr–0.18
Terpinolene	tr–0.07	tr–0.24	tr–0.08
α-Thujene	tr–0.01	0.15–1.72	tr–0.01
Sesquiterpene			
cis-α- Bergamotene	–	0–tr	tr
Bicyclogermacrene	tr	–	0.01
β-Bisabolene	0.01	0–1.50	0.01–0.02
γ-Cadinene	tr–0.11	tr–0.10	tr–0.01
δ-Cadinene	tr–0.05	tr–0.07	0.02–0.03

continued

TABLE 1.13 (continued)
Percentage Composition of the Volatile Fraction of Sweet Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial and Unusual Oils	Laboratory-Extracted Oils
β-Caryophyllene	tr–0.04	tr–0.11	tr–0.04
α-Copaene	tr–0.04	0–0.08	0.02
β-Copaene	tr–0.03	–	–
α-Cubebene	tr–0.02	0–0.14[a]	–
β-Cubebene	0.02	0–0.27	0.01–0.03
β-Elemene	0.02	0–0.14[a]	0.02–0.03
δ-Elemene	tr	0.06	–
(*E*,*E*)-α-Farnesene	0–0.25	0.05–0.69	0.01–0.03
β-Farnesene*	tr–0.02	0.15	–
(*E*)-β-Farnesene	0.01	tr–0.02	tr–0.03
Germacrene D	0.01–0.02	0–tr	0.01–0.02
β-Gurjunene	–	0–tr	0.01
α-Humulene	tr–0.04	0.04–0.07	tr–0.01
α-Muurolene	+	–	tr
γ-Muurolene	0.02	–	tr–0.02
Valencene	tr–0.38	tr–0.05	tr–0.31
Aldehydes			
Aliphatic			
Decanal	0.12–0.43	0.08–0.28	0.14–0.43
(*E*,*E*)-2,4-Decadienal	tr–0.01	0–tr	–
(*E*,*Z*)-2,4-Decadienal	tr–0.02	–	–
(*E*)-2-Decenal	tr	–	tr
Dodecanal	tr–0.11	0–0.11	0.02–0.06
Heptadecanal	+	0–tr	tr
Heptanal	+	–	tr
Hexadecanal	tr	0–tr	–
Hexanal	+	0–0.02	tr
Nonanal	0.02–0.11	0.02–0.1	tr–0.10
Octanal	0.04–0.34	0.10–0.35	0.09–0.68
Tetradecanal	tr–0.01	–	tr
Tridecanal	tr–0.01	–	tr
Undecanal	tr–0.06	0.01–0.07	0.01–0.02
Monoterpene			
Citronellal	tr–0.11	0–0.17	0.02–0.06
Geranial	0.04–0.22	0.13–0.33	tr–0.23
Neral	0.02–0.15	tr–0.34	tr–0.18
Perilla aldehyde	tr–0.04	0–0.05	0–0.01
Sesquiterpene			
α-Sinensal	0–0.04	0–0.15	0–0.06
β-Sinensal	tr–0.1	0.04–0.18	0.01–0.05
Ketones			
Aliphatic			
6-Methyl-5-hepten-2-one	0–0.04	0–0.05	tr

TABLE 1.13 (continued)
Percentage Composition of the Volatile Fraction of Sweet Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial and Unusual Oils	Laboratory-Extracted Oils
Monoterpene			
Carvone	tr–0.09	0.04–0.07	tr
Sesquiterpene			
Nootkatone	tr–0.03	–	0.01–0.04
Alcohols			
Aliphatic			
Decanol	tr	–	tr
Nonanol	0.04–0.34	0–0.81	tr
Octanol	tr–0.16	tr–0.25	tr–0.24
Monoterpene			
cis-Carveol	tr	0–tr	tr–0.01
trans-Carveol	0–0.12	0–0.06	tr
Citronellol	tr–0.02	tr–0.18	tr–0.02
Geraniol	0–0.02	0–0.08	tr–0.09
Linalol	0.17–0.8	0.06–1.23	0.31–2.56
Nerol	tr–0.05	0–0.10	0–0.02
Perilla alcohol	0.01	0.02	tr
cis-Sabinene hydrate	tr–0.01	tr	0.01–0.05
trans-Sabinene hydrate	–	tr	tr
Terpinen-4-ol	tr–0.01	0.01	tr–0.31
α-Terpineol	0.02–0.12	0.02–0.34	tr–0.25
Sesquiterpene			
Elemol	tr	0–tr	tr
Farnesol*	tr	tr	–
(*E*)-Nerolidol	tr	0–tr	tr–0.01
Esters			
Aliphatic			
Heptyl acetate	–	–	tr–0.01
Decyl acetate	0.01–0.03	0–tr	tr–0.03
Nonyl acetate	tr	–	tr–0.01
Octyl acetate	tr–0.03	+	tr–0.01
Monoterpene			
Bornyl acetate	0.01[t]	–	0.01–0.04
Citronellyl acetate	tr–0.07	0–0.09	tr–0.01
Geranyl acetate	0–0.03	tr–0.24	tr–0.02
Linalyl acetate	tr–0.06	0–0.16	tr
Neryl acetate	tr–0.06	0.01–0.09	tr–0.01
α-Terpinyl acetate	tr	0–tr	tr–0.01

continued

TABLE 1.13 (continued)
Percentage Composition of the Volatile Fraction of Sweet Orange Oils (1979–1999)

	Cold-Pressed Oils	Commercial and Unusual Oils	Laboratory-Extracted Oils
		Ethers and oxides	
Monoterpene			
Limonene dioxide	tr–0.20	0.05	–
Limonene oxide*	tr–0.38	0.09	–
cis-Limonene oxide	0.01	tr	0.01–0.02
trans-Limonene oxide	0.02	–	0.01–0.02
cis-Linalol oxide	–	–	tr
trans-Linalol oxide	–	tr	tr
Sesquiterpene			
Caryophyllene oxide*	–	tr	tr
		Acid	
Aliphatic			
Octanoic acid	–	0–tr	–
		Others	
Methyl *N*-methyl anthranilate	–	0.01	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined;
[a] α-cubebene + β-elemene.

Appendix to Table 1.13

- The results reported in Table 1.13 and in this appendix, for the different types of oils of sweet orange, derive from the following original papers:
  - Industrial cold-pressed oils: Moshonas and Shaw (1979, 1980); Cappello et al. (1981); Koketsu et al. (1983); Vora et al. (1983); Owusu-Yaw et al. (1986); Ferrer and Matthews (1987); Dugo et al. (1988, 1994, 1999); Inoma et al. (1989); Boelens and Jimenez (1989a); Boelens (1991); Micali et al. (1990); Lanuzza et al. (1991); Dellacassa et al. (1992); Pino et al. (1992); Thomas and Bassols (1992); Dugo (1994); Mondello et al. (1994a, 1995b); Chamblee et al. (1997).
  - Commercial and unusual oils: Koketsu et al. (1983); Barukadze (1985); Dugo et al. (1988); Haubruge et al. (1989); Inoma et al. (1989); Baaliouamer et al. (1992).
  - Laboratory-extracted oils: Uchida et al. (1984); Arras et al. (1985); Sugisawa et al. (1987, 1989); Lin and Hua (1988); Verzera et al. (1996c); Caccioni et al. (1998); Trozzi et al. (1999); Sawamura et al. (1999a). Not included in the table are the results by El-Samahy et al. (1982); MacLeod (1988); Usai et al. (1992); Blanco Tirado et al. (1995), who reported unusual composition if compared to other sweet orange oils. El-Samahy et al. determined in an Egyptian sample 3.86% of camphene, 28.89% of limonene, 22.40% of myrcene, 14.83% of α-pinene. MacLeod in a sample from Libia found 52% of limonene, 15.8% of linalol, 4.5% of β-copaene, 3.5% of geranial and 2.5% of carvone. The oils from Sardinia of the cv. Thompson Navel, analyzed by Usai et al. (1992), were characterized high values of α-phellandrene (0.27%), germacrene D (0.02%–0.15%), aliphatic aldehydes (4.13% total), α-terpineol (1.41%), while the Columbian oils analyzed by Blanco Tirado et al. (1995) were characterized by high values of camphene (0.27%–0.52%), β-pinene (0.63%–1.05%), γ-terpinene (0.02%–1.09%), terpinolene (0.58%–2.61%), and by the presence of trace amounts of myrcene; in both the last two groups of oils valencene, α- and β-sinensal, and nootkatone were absent.
- Coelution indicated by one or more authors in chromatographic separations of sweet orange oils:
  - Limonene + *p*-cymene; limonene + β-phellandrene; myrcene + octanal; α-phellandrene + octanal; β-pinene + sabinene; terpinolene + octanal; myrcene + β-pinene; β-cubebene + β-elemene; dodecanal + unknown; (*E*,*E*)-2,4-decadienal + nonyl acetate; nonanal + linalol; undecanal + unknown; citronellal + octyl acetate; citronellol + nerol; neral + carvone;

TABLE 1.13 (continued)
Percentage Composition of the Volatile Fraction of Sweet Orange Oils (1979–1999)

neral + unknown; *cis*-limonene oxide + *trans*-limonene oxide. Ranges reported in Table 1.13, relative to industrial cold-pressed oils, when coelution occurred in the chromatographic separation of some papers for a number of components (*p*-cymene, α-phellandrene, β-phellandrene, β-pinene, terpinolene, nonanal, octanal, carvone, nerol, octyl acetate, *cis*-and *trans*-limonene oxide), are relative to the results reported on papers where coelution did not occur. Were also not included in the table values unusually high of myrcene (2.95%–3.31%) reported by Koketsu et al. (1983) for Brazilian oils; the range of the values of myrcene (2.49%–3.17%) reported by Inoma et al. (1989) as well as the value of neryl acetate (0.24%) reported by the same authors for a Spanish oil; of myrcene + octanal (4.3%) reported by Pino et al. (1992) for a Cuban oil. The limited number of data, the different geographical and botanical origin of the fruits determine large variability ranges, therefore for the results relative to commercial and unusual oils it was not possible to determine the contribute of single components coeluted, and the data are reported in the table as ranges of variability even for the raw data generated by chromatographic coelutions. Must be highlighted the high content of sabinene (2.45%), α-thujene (1.72%) and octanal (0.82%) found in a sample cold extracted from Navel oranges cultivated in Georgia, ex URSS, (Barukadze, 1985); the high content of γ-terpinene (4.66%), and of (*E*,*E*)-α-farnesene (0.69%) in a sample extracted from Wenzhou honey oranges cultivated in China (Dugo et al. 1988); the high content of β-bisabolene (1.07%–1.50%) and of nonanol (0.53%–0.81%) found in oils extracted from Piralima oranges cultivated in Brazil (Koketsu et al., 1983). Variability ranges in Table 1.13 relative to laboratory-extracted oils do not include the values of α-phellandrene + octanal (0.24%–0.49%) reported by Arras et al. (1985) for Sardinia Valencia oils, and those of β-pinene + sabinene (1.93%) reported by Trozzi et al. (1999) for the oils extracted from Maltese oranges cultivated in Calabria, Italy.

- In addition to those listed in Table 1.13, in sweet orange oils were found the components listed below:
  - Cold-pressed oils: α-cadinene[t] (0.01%–0.02%), myrtenal (0.03%), nerolidol* (0.01%), *p*-mentha-1,8-dien-10-yl-acetate (tr–0.01%), peryllene (0.01%), and trace amounts of linear chained hydrocarbons C_{21}–C_{33} and correspondent "iso" isomers, C_{23}–C_{31}, ar-curcumene, germacrene B, 7-epi-α-santalene, germacrene D-4-ol, methyl anthranilate, alkyl, acetyl and phenyl pyridines, *N*-methylaniline, *N*,*N*-dimethylaniline, 2-(methylamino) benzyl alcohol.
  - Commercial and unusual oils: eucarvone[t] (0.10%), neryl propanate (0.12%), linalol oxide* (0.08%), methyl nopinone* (0.66%), and trace amounts of *trans*-α-bergamotene, γ-elemene, β-guaiene, α-himachalene, octadecanal, (*Z*)-9-octadecenal, isopiperitone, δ-cadinol, spathulenol, geranyl formate, linear chained acids C_9–C_{12}.
  - Laboratory-extracted oils: allo-aromadendrene (0.01%). Usai et al. (1992) and Blanco Tirado et al. (1995) (their quantitative data are not reported in Table 1.12) in addition to compounds listed in Table 1.13, identified by other authors, found formaldehyde, pulegone, 3,7-dimethyl-2,6-octadien-1-ol, pentanol, *p*-mentha-4(8)-en-9-ol, isopulegol, 1,4-cineole, acetic acid, formic acid; Uchida et al. (1984) and Sugisawa et al. (1987, 1989) also found, in addition to those listed above, in Japanese sweet orange oils, within the fraction of the oxygenated compounds, separated by chromatography on silica gel, the following components: heptanal, (*E*)-2-hexenal, benzaldehyde, phenyl acetaldehyde, 2-heptanone, dihydrocarvone, camphor, butanol, dodecanol, hexanol, heptanol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, 2-methyl-butanol, 3-methyl-butanol, 2-methyl-3-buten-2-ol, 3-methyl-2-buten-1-ol, fenchol*, *p*-mentha-1,8(10)-dien-9-ol, β-terpineol*, eugenol, methyl eugenol, methyl isoeugenol, 2,6-di tertbutyl-*p*-cresol, patchouli alcohol, *n*-butyl butyrate, *n*-butyl hexanoate, heptyl acetate, (*Z*)-3-hexenyl hexanoate, ethyl octanoate, (*Z*)-3-hexenyl butyrate, hexyl acetate, hexyl butyrate, methyl octanoate, octyl butyrate, octyl hexanoate, *p*-mentha-1,8(10)-dien 9-yl acetate, 1,8-cineole, caryophyllene oxide*, geranyl acetone, β-ionone. The presence of many of these components in sweet orange oils need further confirmation.
- Although the composition of sweet orange can be subject to variation in function of the botanical origin and the harvest period of the fruits (Lifshitz et al., 1970; Shaw and Coleman, 1974; Braddok and Kesterton, 1976; Dugo et al., 1994; Verzera et al., 1996c), the data reported in the first column of the table vary in a limited range and appear to be representative of the composition of industrially cold-pressed sweet orange oils.

1.6.2 1998–2009

1.6.2.1 Industrial Oils

Table 1.14 reports the results published after the review by Dugo et al. (2002) relative to the composition of the volatile fraction of industrial cold-pressed sweet orange oils.

TABLE 1.14
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Sweet Orange Oils (1998–2009)

	1	2	3	4	5	6,7	8a	8b	9
Hydrocarbons									
Monoterpene									
Camphene	–	tr	–	–	–	0.01	0.01	0.01	–
δ-3-Carene	tr–0.16	0.04	0.07	0.09	0.10	0.16	0.31	0.31	tr
p-Cymene	0–0.20	–	0.01	tr	–	c	tr	tr	–
Limonene	95.40–96.10	95.21	94.39	94.74	95.40	93.33[c]	93.67	94.69	94.9
Myrcene	1.20–2.02	1.76	1.84	1.66	1.90	2.03	2.09	2.49	1.2
(*E*)-β-Ocimene	–	–	0.03	0.03	–	0.06[h]	0.05	0.03	–
(*Z*)-β-Ocimene	–	–	–	–	–	0.01	0.01	tr	–
α-Phellandrene	–	–	–	0.03	–	0.18[b]	0.07	0.03	–
α-Pinene	0.51– 0.55	0.47	0.53	0.46	0.50	0.64	0.65	0.71	0.5
β-Pinene	tr–0.07	0.69[a]	0.03	0.03	0.20[a]	0.09[h]	1.00[a]	0.42[a]	tr
Sabinene	0.31–0.32	0.69[a]	0.47	0.46	0.20[a]	0.52	1.00[a]	0.42[a]	0.3
α-Terpinene	–	–	–	tr	–	0.02	0.01	0.01	–
γ-Terpinene	tr–0.21	0.02	–	tr	–	0.06[h]	0.26	0.04	–
Terpinolene	tr	0.02	0.01	0.02	0.01	0.05	0.06	0.05	tr
α-Thujene	–	tr	–	tr	–	0.03	0.02	0.01	–
Sesquiterpene									
trans-α-Bergamotene	–	–	–	–	0.03	0.03	–	–	–
δ-Cadinene	–	–	0.01	0.02	0.03	0.02	0.02	0.03	–
β-Caryophyllene	–	0.02	0.02	0.01	0.01	0.03	0.02	0.01	–
α-Copaene	–	–	0.02	0.01	–	0.02	0.02	0.02	–
β-Cubebene	–	–	0.02	0.01	0.02	0.03[d]	0.02[d]	0.02[d]	–
β-Elemene	–	–	–	–	–	0.03[d]	0.02[d]	0.02[d]	–
(*E*,*E*)-α-Farnesene	–	–	–	0.02	–	0.17[e]	0.03	0.11	–
(*E*)-β-Farnesene	–	–	–	–	–	0.02	0.01[f]	tr[f]	–
Germacrene D	–	–	–	0.01	–	0.02	0.02	0.02	–
α-Humulene	–	–	–	–	0.01	tr	0.01	0.02	–
Valencene	–	0.16	0.06	0.03	0.03	–	0.05	tr	tr
Aldehydes									
Aliphatic									
Decanal	tr–0.18	0.24	0.29	0.38	0.30	0.17	0.27	0.08	0.1
Dodecanal	–	0.04	0.05	0.11	0.10	0.03	0.04	0.01	–
Nonanal	–	0.04	0.05	0.04	0.10	0.04	0.06	0.02	tr
Octanal	–	0.18	0.30	0.22	0.20	0.18[b]	0.41	0.10	0.1

TABLE 1.14 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Sweet Orange Oils (1998–2009)

	1	2	3	4	5	6,7	8a	8b	9
Tetradecanal	–		–	–	–	0.01	tr	tr	–
Undecanal	–	0.01	–	–	–	0.01	0.01	0.01	–
Monoterpene									
Citronellal	tr–0.05	0.04	0.04	0.05	0.05	0.04	0.04	0.04	–
Geranial	tr	0.06	0.09	0.03	0.10	0.13	0.13	0.05	–
Neral	tr–0.20	0.03	0.06	0.02	0.03	0.06	0.05	0.02	–
Perilla aldehyde	–	–	–	0.06	0.03	tr	0.01	0.01	–
Sesquiterpene									
α-Sinensal	–	–	0.02	0.04	0.02	0.01	0.01	tr	–
β-Sinensal	–	–	0.04	0.03	0.02	0.02	0.02	0.02	–
Ketones									
Monoterpene									
Carvone	–	–	0.08	0.08	0.02	–	–	–	0.2
Sesquiterpene									
Nootkatone	–	–	0.02	–	0.01	0.02	0.01	0.01	–
Alcohols									
Aliphatic									
Octanol	–	0.02	0.10	0.12	0.04	–	0.03	0.01	0.1
Monoterpene									
cis-Carveol	–	–	–	0.05	–	–	0.01	0.01	0.3
trans-Carveol	–	–	–	0.05	0.01	–	–	–	0.3
Citronellol	–	–	0.02	–	–	–	0.01[g]	0.01[g]	–
Geraniol	0–tr	0.01	0.02	–	tr	0.01	0.01	tr	–
Linalol	0.24–0.34	0.33	0.40	0.40	0.40	0.30	0.31	0.32	0.4
Nerol	–	0.01	0.02	–	0.02	0.02	0.01[g]	0.01[g]	–
cis-Sabinene hydrate	–	–	–	–	–	0.04	0.01	0.01	–
trans-Sabinene hydrate	–	tr	–	–	–	–	tr	tr	–
Terpinen-4-ol	–	–	0.01	–	tr	tr	0.01	tr	–
α-Terpineol	–	0.04	0.15	0.06	0.10	0.06	0.05	0.05	0.1
Esters									
Aliphatic									
Decyl acetate	–	0.01	–	–	–	0.01	tr	tr	–
Nonyl acetate	–	0.01	–	–	–	–	tr	tr	–
Octyl acetate	–	tr	–	–	0.03	tr	tr	tr	0.1
Monoterpene									
Bornyl acetate	–	–	–	–	–	0.01	0.01	0.01	–
Citronellyl acetate	–	tr	–	–	–	0.01	tr	0.01	–
Geranyl acetate	0–tr	0.03	0.01	0.01	0.01	0.03	0.02	0.01	–
Neryl acetate	–	0.01	–	0.01	tr	0.04	0.02	0.01	–

continued

TABLE 1.14 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Sweet Orange Oils (1998–2009)

	1	2	3	4	5	6,7	8a	8b	9
Ethers and oxides									
Monoterpene									
cis-Limonene oxide	–	–	0.07	0.08	–	0.01	tr	0.01	0.5
trans-Limonene oxide	–	–	0.04	0.05	–	0.02	0.01	0.02	0.5

Notes: tr, traces; *, correct isomer not characterized; [a] β-pinene + sabinene; [b] α-phellandrene + octanal; [c] limonene + *p*-cymene; [d] β-cubebene + β-elemene; [e] (*E*,*E*)-α-farnesene + unknown sesquiterpene hydrocarbon; [f] (*E*)-β-farnesene + β-santalene; [g] citronellol + nerol; [h] in the original paper, due to a material mistake, were reported for (*E*)-β-ocimene, β-pinene, and γ-terpinene, respectively the values 0.62%, 0.91%, and 0.59%.

Appendix to Table 1.14

1. Oberhofer et al. (1999). Two samples of blond and two samples of blood orange oils acquired in Austria and in Italy; GC/FID on capillary columns coated with HP-5 (25 m × 0.32 mm × 0.52 μm), OV-1 (25 m × 0.25 mm × 0.3 μm), Carbowax (25 m × 0.25 mm × 0.3 μm); GC/sniffing technique on capillary column (25 m × 0.53 mm × 0.3 μm) coated with FSOT-RSL-150; GC/IR/MS on capillary columns coated with RSL-200 (30 m × 0.52 mm × 0,25 μm) or with Stabilwax (60 m × 0.32 mm × 0.25 μm); NBS and Wiley MS libraries; EPA-REVA and Robertet IR libraries; LRI on OV-1 are reported; relative percentage of peak areas. Oberhofer et al. also found trace amounts of linalyl acetate and in one of the four oils 3-methyl butyl acetate (0.13%).
2. Di Giacomo et al. (1999). Italy; one sample extracted with Pelatrice Speciale; GC/FID on capillary column (25 m × 0.32 mm) coated with SE-52; relative percentage of peak areas. Di Giacomo et al. also found β-ocimene* (0.05%) and trace amounts of β-bisabolene.
3. Kubeczka and Formàček (2002). One commercial sample; GC/FID on capillary column (50 m) coated with VG-11; ^{13}C-NMR; relative percentage of peak areas. Kubeczka and Formàček also found β-phellandrene (0.24%).
4. Shen et al. (2002). Australia; one sample of Navel orange oil; GC/FID on capillary column (30 m × 0.32 mm × 0.25 μm) coated with DB-5; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; Varian terpene MS library. Shen et al. also founded β-farnesene* (0.02%) and iso dihydrocarveol (0.01%).
5. Lopes et al. (2003). Brazil; one sample extracted by FMC in-line; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; Wiley MS library. Relative percentage of peak areas. Lopes et al. also found allo aromadendrene (0.02%), α-muurolene (0.02%), α-ylangene (probably a misidentification, 0.02%), *p*-mentha-2,8-dien-1-ol (0.02%), perilla alcohol (0.01%), perillyl acetate (0.02%), limonene oxide* (0.03%), methyl *N*-methyl anthranilate (0.01%), and trace amounts of hexadecanoic acid.

6 and 7. Mondello et al. (2003, 2004b). Sicily Italy; one sample; conventional GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX-5 MS; Fast GC/FID on capillary column (10 m × 0.1 mm × 0.1 μm) coated with RTX-5 MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. In the table are reported the results obtained with conventional method. The results obtained with fast method were very similar. More information is reported in Chapter 11. Mondello et al. also found β-copaene (0.02%), 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol (0.01%).

8. Verzera et al. (2004). (a) Average values of ten samples of oils extracted by FMC-in line from fruits grown under biological techniques, (b) average values of ten samples of oils extracted by FMC in-line from fruits grown with traditional methods. GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with Mega-5 MS or Megawax; MS Adams library; relative percentage of peaks areas. Verzera et al. also found γ-cadinene (0.02%), γ-muurolene (tr–0.01%), (*Z*)-nerolidol (tr–0.01%), and trace amounts of tricyclene, tridecanal, 6-methyl-5-hepten-2-one, camphor, piperitone, borneol, α-terpinyl acetate.
9. Viuda-Martos et al. (2009). Spain; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5MS; LRI on HP-5MS are reported; relative percentage of peak areas; Viuda-Martos et al. also found verbenol (0.2%) and trace amounts of myrcenol, linalyl acetate.

Among the papers reported in the table only few were oriented to the study of the composition of sweet orange essential oil (Kubeczka and Formàček, 2002; Verzera et al., 2004; Viuda-Martos et al., 2009). Most were mainly dedicated to the study of alteration of the oil used in an aroma lamp (Oberhofer et al., 1999), the composition of concentrated oil by vacuum distillation (Di Giacomo et al., 1999; Lopes et al., 2003) or by adsorption on silica gel and desorption with supercritical CO_2 (Shen et al., 2002), and to the development of advanced analytical methods (Mondello et al., 2003, 2004b). Many were on a limited number of samples, often only one.

Oberhofer et al. (1999) only considered the olfactory impact components and evaluated all the odor differences between the head space of the oil prior and after heating at 65°C, in a tube at the top of a commercial aroma lamp, before and after the reduction of 50% of the oil volume. In heated oils, before the evaporation, a moderate increment of decanal, neral, geranial, geraniol, and linalyl acetate was observed, the latter present only at trace levels in the original oil. After the reduction of 50% of the volume a drastic decrease of limonene and a sensible increase of decanal, neral, geranial and linalyl acetate; geraniol almost disappears in three of the four samples, while it increases unreasonably in the forth oil. Verzera et al. (2004) observed that the content of aliphatic and monoterpene aldehydes and of valencene was higher in biological oils than in traditional oils. Among the results determined by Viuda-Martos et al. (2009) the high content of *cis*- and *trans*-limonene oxide (0.5% for both components) should be noted.

Although limited to a scant number of samples, the results reported in Table 1.14 appear, however, to be representative of the composition of sweet orange essential oil and are in good agreement with the data previously revised by Dugo et al. (2002) and summarized in Table 1.13.

In addition to those reported in Table 1.14, other papers were published on sweet orange oil. Feger et al. (2001a), using GC/MS and a column coated with OV-1701, determined the presence in a sample of sweet orange oil of bicyclogermacrene (tr–0.01%), germacrene A (tr–0.02%), germacrene D + valencene (0.07%–0.38%). Veriotti and Sacks (2002) used fast gas chromatography and TOF MS for the identification and quantitative analysis of sweet orange oil main components (*p*-cymene 0.06%; limonene 96.10%; myrcene 1.77%; α-pinene 0.69%; β-pinene 0.52%; terpinolene 0.01%; β-caryophyllene 0.05%; octanal 0.06%; geranial 0.05%; neral 0.05%; and linalol 0.18%). Steuer et al. (2001) and Schultz et al. (2002) in two papers aimed at the characterization of some citrus oils by ATR/FT-IR and NIR-FT Raman spectroscopy reported the following average content of some components for several samples of sweet orange oil analyzed by GC: limonene (95.1%); myrcene (1.8%); α-pinene (0.6%). Elston et al. (2005) using multidimensional GC-O/GC-MS analysis and as internal standard 2-heptadecanone, noticed that valencene varied between 54.2 and 68.4 μg/g. The same paper reported that the aroma descriptors of numerous components of sweet orange oil identified based on their LRI on a column coated with DB-Wax (limonene, myrcene, (*Z*)-β-ocimene, α-pinene, decanal, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,4-decadienal, (*E*)-2-decenal, (*Z*)-2-decenal, dodecanal, (*E*)-2-nonenal, citronellal, geranial, neral, β-sinensal, 1-octen-3-ol, citronellol, geraniol, linalol, eugenol, ethyl eptanoate, *trans*-4,5-epoxide-(*E*)-2-decenal, linalol oxide*, wine lactone). Ogawa et al. (2002), using capillary liquid chromatography and micellar electrokinetic chromatography, identified limonene, myrcene, α-pinene, citral, carvone, and linalol in sweet orange oil and determined the detection limits of these components.

1.6.2.2 Laboratory-Extracted Oils

Table 1.15 summarizes the results relative to laboratory-extracted sweet orange oil that is cold-pressed and distilled. These oils are obtained from numerous varieties of sweet orange fruits some of which are internationally common and are industrially important, other are typical of the geographic region of production. In addition to those reported in Table 1.15, other papers report information on the composition of sweet orange laboratory-extracted oils. Marongiu et al. (2003) compared the composition of the oil hydrodistilled from Tarocco oranges cultivated in Sardinia, Italy, with the oils obtained from the same matrix by supercritical CO_2 under different experimental conditions. For the hydrodistilled oil they obtained the following composition: limonene (93.85%);

TABLE 1.15
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Sweet Orange Oils (1999–2009)

	Cold Extracted									Distilled					
	1a	**1b**	**2**	**3**	**4**	**5**	**6a**	**6b**	**7**	**8**	**9**	**10a**	**10b**	**11a**	**11b**
						Hydrocarbons									
Aliphatic															
Octadecane	–	–	tr	–	–	0–tr	–	–	–	–	–	–	–	–	–
Tetradecane	–	–	tr	–	–	0–tr	0.01	0.01	–	–	–	–	–	–	–
Monoterpene															
Camphene	tr	tr	tr	tr	–	tr	tr	0.01	tr	tr	tr	0.66	0.25	–	–
δ-3-Carene	0.03	0.01	0.1	0.07–0.28	–	0.1	–	–	0.4	tr	tr	0.15	–	0.2	0.1
p-Cymene	tr	tr	–	–	0–tr	0–tr	0.03	0.11	–	–	–	–	–	–	–
Limonene	96.19	96.57	94.7	92.34–95.50	90.5–94.6	93.6–94.4	95.59	95.27	95.6	95.6	91.3	83.62	78.60	76.7	78.5
Myrcene	1.79	1.77	2.0	0.10[a]–1.95	0–tr	2.0–2.1	1.11	2.26	2.0	1.9	1.7	3.12	2.24	4.3	5.3
(*E*)-β-Ocimene	0.02	tr	tr	0.01–0.06	–	tr–0.1	–	–	–	tr	tr	–	–	–	–
(*Z*)-β-Ocimene	tr	tr	tr	0.01–0.07	0–tr	tr	–	–	–	tr	tr	–	–	–	–
α-Phellandrene	–	–	tr	–	0–tr	tr	–	–	–	tr	tr	–	–	–	–
β-Phellandrene	–	–	0.2	–	0–tr	0.3	–	–	–	tr	–	–	–	–	–
α-Pinene	0.35	0.41	0.4	0.07–0.51	0.3–0.5	0.4–0.5	0.49	0.51	0.5	0.4	0.3	0.59	0.20	0.5	1.6
β-Pinene	0.01	tr	tr	0.01–0.07	tr	tr	0.10	0.24	tr	tr	tr	0.04	–	2.4	2.7
Sabinene	0.21	0.37	0.2	0.12–0.50	0.1–0.2	0.7–0.8	–	–	0.3	0.1	0.3	–	0.22	1.2	1.2
α-Terpinene	–	–	–	tr–0.03	1.5–1.7	–	–	–	–	–	tr	–	–	–	–
γ-Terpinene	tr	tr	–	0.02–0.03	0–tr	–	0.03	0.11	0.4	tr	0.1	0.07	0.19	0.3	0.2
Terpinolene	0.01	0.01	tr	0.01–0.03	tr	tr–0.3	0.01	0.01	tr	tr	tr	0.11	–	0.2	0.2
α-Thujene	tr	tr	–	tr–0.01	–	–	–	–	tr	tr					
Sesquiterpene															
Bicyclogermacrene	–	–	tr	–	–	–	–	–	–	–	tr	–	–	–	–
β-Bisabolene	–	–	–	–	0–tr	0–tr	–	–	–	–	–	–	–	–	–
δ-Cadinene	–	–	–	–	–	–	–	–	tr	–	tr	–	–	0.1	0.2

γ-Cadinene	–	–	–	0.01–0.03	–	–	–	–	–	tr	–	–	–	–	–
β-Caryophyllene	0.01	0.01	tr	0.01–0.06	tr	0–tr	–	–	tr	tr	–	–	–	0.2	0.2
α-Cedrene	–	–	–	–	tr	–	–	–	–	–	–	–	0.74	–	–
α-Copaene	0.02	0.02	–	–	–	–	–	–	tr	tr	tr	–	–	0.1	0.1
α-Cubebene	tr	tr	–	–	tr	–	–	–	–	tr	–	–	–	–	–
β-Cubebene	–	–	tr	–	–	0–tr	–	–	–	tr	tr	–	–	0.1	0.1
β-Elemene	0.02	0.02	–	–	–	0–tr	0.01	0.01	–	–	tr	–	–	0.1	0.1
(*E*,*E*)-α-Farnesene	–	–	tr	–	tr–0.1	tr–0.1	–	–	tr	–	tr	–	–	–	–
β-Farnesene*	–	–	tr	–	–	–	–	–	tr	–	–	–	–	–	–
(*E*)-β-Farnesene	0.01	tr	–	–	–	tr–0.1	–	–	–	–	tr	–	–	0.1	0.1
Germacrene D	tr	tr	tr	tr–0.01	0–tr	tr	–	–	tr	–	tr	–	–	0.1	0.1
β-Gurjunene	–	–	–	–	–	–	–	–	–	–	tr	–	–	0.1	0.1
α-Humulene	–	–	tr	0–0.01	–	0–tr	–	–	–	tr	tr	–	–	–	–
β-Sesquiphellandrene	–	–	–	–	–	0–tr	–	–	–	–	tr	–	–	–	–
Valencene	tr	tr	tr	0.01–0.12	–	0–tr	–	–	tr	0.3	0.1	0.18	0.13	0.2	0.4
Aldehydes															
Aliphatic															
Decanal	0.16	0.10	0.1	0.15–0.72	0.2	0.1–0.3	–	–	0.1	0.1	0.4	0.05	0.74	1.9	1.7
Dodecanal	0.02	0.01	–	tr–0.01	tr	tr–0.1	–	–	–	tr	tr	–	–	–	–
(*E*)-2-Dodecenal	–	–	–	–	–	0–tr	–	–	–	–	tr	–	–	–	–
Nonanal	0.04	0.03	–	0.01–0.07	tr	tr–0.1	–	–	0.1	tr	0.1	0.15	0.26	0.2	0.1
Octanal	0.23	0.23	0.1	0.10–0.59	0.2–0.3	tr–0.4	tr	0.03	–	tr	1.0	1.44	1.15	–	–
Undecanal	tr	tr	–	tr–0.02	tr–0.1	0–tr	–	–	–	–	tr	–	–	0.1	0.1
(*E*)-2-Undecenal	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
Monoterpene															
Citronellal	0.03	0.01	tr	0.03–0.13	tr	tr–0.1	0.15	0.02	tr	tr	0.1	0.14	0.28	0.4	0.3
Cumin aldehyde	–	–	–	–	tr–0.1	0–tr	–	–	–	–	–	–	–	–	–
Geranial	0.16	0.08	0.1	0.01–0.11	tr–0.1	0.2–0.3	0.02[b]	0.04[b]	tr	tr	0.2	–	–	0.5	0.5
Neral	0.09	0.04	0.1	0.01–0.05	tr	0.1–0.2	0.02[b]	0.04[b]	tr	tr	0.2	0.27	0.46	0.4	0.3
Perilla aldehyde	tr	tr	tr	tr–0.02	tr–0.1	tr	–	–	tr	tr	tr	0.09	0.24	–	–

continued

TABLE 1.15 (continued)
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Sweet Orange Oils (1999–2009)

	Cold Extracted									Distilled					
	1a	1b	2	3	4	5	6a	6b	7	8	9	10a	10b	11a	11b
Sesquiterpene															
α-Sinensal	0.02	tr	tr	0.01–0.09	0–tr	0–tr	–	–	–	tr	tr	–	–	0.7	0.1
β-Sinensal	0.03	tr	tr	–	0–tr	0–tr	–	–	–	tr	tr	–	–	0.2	0.2
Ketones															
Monoterpene															
Carvone	–	–	–	–	0.2–0.5	0–tr	–	–	tr	tr	tr	0.20	–	–	–
Sesquiterpene															
Nootkatone	–	–	–	0.01–0.03	tr–0.1	–	–	–	–	tr	tr	–	–	–	–
Alcohols															
Aliphatic															
Decanol	tr	tr	tr	–	–	–	–	–	–	–	0.1	–	–	0.3	–
Octanol	0.02	tr	tr	tr–0.01	tr	tr	tr	0.01	–	tr	0.4	0.60	–	1.1	0.5
Monoterpene															
cis-Carveol	0.02	0.01	–	–	0.1–0.5	0–tr	–	–	–	–	tr	–	–	–	–
trans-Carveol	0.02	0.01	–	–	0.1–0.3	–	–	–	–	–	tr	0.10	0.21	0.2	0.3
Citronellol	tr	tr	tr	–	–	tr	0.03	0.02	–	–	0.1	–	–	–	–
Geraniol	tr	tr	tr	tr–0.01	–	tr	0.01	tr	–	–	tr	–	0.28	0.3	0.1
Isoborneol	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
Linalol	0.35	0.12	0.4	0.25–1.06	0.4–0.5	0.4–0.7	0.12	0.19	0.2	0.4	2.6	2.80	5.87	3.1	2.0
Nerol	tr	tr	–	tr–0.01	–	tr	0.02	tr	–	–	0.1	0.09	0.24	0.6	0.3
Perilla alcohol	–	–	tr	–	tr–0.1	0–tr	–	–	–	–	tr	–	–	0.1	–
trans-Sabinene hydrate	–	–	–	0.01–0.06	tr	–	–	–	–	–	–	0.60	–	–	–
Terpinen-4-ol	0.01	tr	tr	tr–0.03	tr	0–tr	0.04	tr	–	tr	0.1	–	0.91	0.3	0.5
α-Terpineol	0.01	tr	0.1	0.01–0.15	0.1	0.1	–	–	tr	0.1	0.2	0.82	1.30	0.8	0.4

Sesquiterpene															
Cedrol	–	–	–	–	0–tr	0–tr	–	–	–	–	–	–	–	–	–
Elemol	–	–	tr	–	0–tr	0–tr	–	–	–	–	–	–	–	–	–
Eugenol	–	–	–	–	0–tr	0–tr	–	–	–	–	–	–	–	–	–
(*Z*,*E*)-Farnesol	–	–	–	–	tr	0–tr	–	–	–	–	–	–	–	–	–
(*Z*)-Nerolidol	–	–	–	tr	0–tr	0–tr	–	–	–	–	–	–	–	–	–
Esters															
Aliphatice															
Octyl acetate	–	–	–	0.01–0.03	–	–	–	–	–	–	tr	–	–	0.1	0.1
Monoterpene															
Citronellyl acetate	tr	tr	–	tr–0.01	–	–	0.05	0.01	–	–	tr	–	–	–	–
Geranyl acetate	tr	tr	–	tr–0.01	tr	tr	0.04	0.01	–	–	tr	–	–	–	–
Linalyl acetate	tr	tr	–	–	tr–0.1	–	0.03	0.01	–	–	–	–	–	–	–
Neryl acetate	tr	tr	tr	0–0.01	–	0–tr	–	–	–	–	tr	–	–	–	–
α-Terpinyl acetate	tr	tr	tr	tr–0.01	–	0–tr	–	–	–	–	tr	–	–	–	–
Ethers and oxides															
Monoterpene															
1,8-Cineole	–	–	–	–	tr	0–tr	–	–	–	–	–	–	–	–	–
Limonene diepoxide	–	–	–	–	0–tr	0–tr	–	–	–	–	–	–	–	–	–
cis-Limonene oxide	0.02	0.03	tr	tr–0.02	0–tr	0–tr	–	–	–	–	tr	–	–	–	–
trans-Limonene oxide	0.01	tr	–	0.01–0.04	0.1–0.4	0–tr	–	–	tr	–	tr	0.20	–	0.3	0.1
Sesquiterpene															
trans-Caryophyllene oxide	tr	tr	–	–	tr–0.2	–	–	–	–	–	–	–	–	–	–
Acid															
Undecanoic acid	–	–	–	–	tr	0–tr	–	–	–	–	–	–	–	–	–

continued

TABLE 1.15 (continued)
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Sweet Orange Oils (1999–2009)

Notes: tr, traces; *, correct isomer not characterized; [a] minimum value of 0.10% (maybe due to a printing error) is reported for the oil of Ovale calabrese, in Valencia late oil the minimum value of myrcene is 1.69%; [b] in the original paper these components were indicated as citral.

Appendix to Table 1.15

1. Mitiku et al. (2000). Ethiopia; (a) one sample from the cv. Valencia, (b) one sample from the cv. Hamlim; GC/FID on capillary columns coated with Thermon 600T (50 m × 0.22 mm × 0.25 μm) and DB-5 (30 m × 0.22 mm × 0.25 μm); GC/MS on capillary columns coated with Thermon 600T (50 m × 0.22 mm × 0.25 μm) and HP-5 (30 m × 0.32 mm × 0.25 μm); home-made MS library; LRI on DB-5 and Thermon 600T are reported; relative percentage of peak areas. Mitiku et al. also found tridecanal (0.01%) in sample (a) and trace amounts in sample (b) and trace amounts in both samples of α-caryophyllene, 2,4-decadienal*, (*E*)-2-undecenal, nerolidol*, decyl acetate.
2. Minh Tu et al. (2002). Vietnam; one sample hand pressed; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; LRI on DB-Wax are reported; relative percentage of peak areas. Minh Tu et al. also found trace amounts of cadinene*, menthone, 2-methyl butyrate.
3. Gionfriddo et al. (2003). Calabria, Italy; 9 samples from the cv. Valencia late, 10 samples from the cv. Ovale calabrese; GC and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; relative percentage of peak areas. Gionfriddo et al. also found γ-cadinene (0.01%–0.03%), nonyl acetate (tr–0.01%), bornyl acetate (0.01%–0.03%).
4. Njoroge et al. (2005). Kenya; one samples each from the cvs.: Salustiana, Valencia late, Washington navel; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; MS libraries: NIST 62 and 107; relative percentage of peak areas. Njoroge et al. also found tetradecanal (0%–0.1%), sabina ketone (0.1%–0.2%), nerolidyl acetate (tr–0.02%), *trans*-linalol oxide (0.1%–0.2%), nonanoic acid (0.01%) and in one or more of analyzed samples trace amounts of aromadendrene, δ-elemene, octadecanal, *p*-menth-1-en-9-al, ethanol, heptanol, dihydrocarveol, (*E*,*Z*)-2,6-nonadienol, *p*-menth-1-en-9-ol, *cis*-piperitol, globulol, perillyl acetate, carvone oxide, *cis*-linalol oxide, myrcene epoxide, α-pinene oxide, dodecanoic acid, nonanoic acid, 1,4,7,10-tetraoxaciclodecane, 1,4,7,10,13-pentaoxaciclopentadecane, 1,4,7,10,13,16-hexaoxacicloctadecane.
5. Sawamura et al. (2005). Japan; one sample each from the cvs.: Hongjiang, Anliu, Sihui, Washington navel; GC/FID and GC/MS on capillary columns (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax or DB-1; MS home made library; LRI on DB-Wax and DB-1 are reported; relative percentage of peak areas. Sawamura et al. also found 0–tr of isopiperitone, dehydrocarveol, farnesol*, (*E*)-nerolidol.
6. Choi (2006). Korea; one sample each from the cvs.: (a) Valencia late, (b) Washington navel; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; MS libraries: NIST and Wiley; LRI on DB-Wax are reported; relative percentage of peak areas. Choi also found, respectively in sample (a) and (b) tridecane (tr, 0.06%), undecane (0.07%, 0.17%), linalol oxide* (0.17%, 0.01%), octanoic acid (0.02%, 0.01%), and trace amounts of ethyl acetate.
7. Dharmawan et al. (2008). India; one sample hand pressed from the cv. Mosambi; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with BPX-5; NIST MS library; relative percentage of peak areas.
8. Ruberto et al. (1999). Sicily, Italy; one sample extracted by steam distillation from the cv. Tarocco; GC/FID and GC/MS on capillary column (25 m × 0.2 mm × 0.33 μm) coated with HP-1; MS libraries: Wiley and NBS; relative percentage of peak areas; Ruberto et al. also found trace amounts of carveol*. The same results were previously reported by Ruberto et al. (1997a).

9. Ruberto and Rapisarda (2002). Sicily, Italy; one sample hydrodistilled from the cv. Moro; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; Adams MS library; relative percentage of peak areas. Ruberto and Rapisarda also found *p*-mentha-1,8(10)-dien-9-ol (0.1%), and trace amounts of germacrene A, α-muurolene, 7-epi-α-selinene, β-selinene, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,4-decadienal, (*E*)-2-decenal, carvacrol, *cis*-*p*-menth-2-en-1-ol, (*E*,*E*)-farnesol, *trans*-carvyl acetate, limonene-10-yl-acetate.
10. Giamperi et al. (2002). Sicily, Italy; one sample each of (a) blond orange oil and (b) blood orange oil hydrodistilled; GC/FID and GC/MS on capillary column (25 m × 0.2 mm × 0.33 μm) coated with HP-1; NBS MS library; relative percentage of peak areas. Giamperi et al. also found in sample (a) *cis*-*p*-mentha-2,8-dien-9-ol (0.20%), *trans*-*p*-mentha-2,8-dien-9-ol (0.40%), linalyl formate (0.20%) and in sample (b) α-cedrene (0.74%), *cis*-*p*-mentha-2,8-dien-9-ol (1.98%), *trans*-*p*-mentha-2,8-dien-9-ol (0.39%).
11. Ferhat et al. (2006). Algeria; one sample each from the cv. Valencia late extracted by hydrodistillation (a) and microwave accelerated distillation (MAD) (b); GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5 MS; MS libraries: NIST and Wiley; LRI on HP-5MS are reported; relative percentage of peak areas. Ferhat et al. also found in sample (a) hexanol (0.2%). These authors assert that MAD technique offers, in comparison with traditional hydrodistillation, some important advantages: water and solvent free process, faster extraction time (30 min vs. 3 h), higher yields (0.42% vs. 0.39%), lower costs and better olfactive quality.

myrcene (2.97%); α-pinene (0.61%); sabinene (0.37%); decanal (0.75%); octanal (0.59%); and linalol (0.86%). Moussaid et al. (2000) studied the effect of radiation (1 kGy and 2 kGy) on sweet orange oil (Maroc late) extracted from waxed and unwaxed fruits. These authors observed that the content of essential oil was sensibly lower in samples radiated with 2 kGy compared to the control and that the radiation 2 kGy stimulated the synthesis of limonene and the degradation of linalol, citral, and methyl anthranilate. This last component found by the authors in all the samples analyzed with values of ca. 0.10 milligram in 100 grams of essential oil, is unusual for sweet orange oil. Thao et al. (2007) in a paper focused on the characterization of various Vietnamese citrus oils, manually cold-pressed, using the isotopic ratios of monoterpene hydrocarbons, in the oil extracted from a cv. locally named Xa Doai, determined by GC the following composition: limonene (92.26%); myrcene (0.31%); α-phellandrene (2.87%); β-phellandrene (0.60%); α-pinene (0.83%); β-pinene (0.05%); sabinene (1.01%); α-terpinene (0.10%); γ-terpinene (0.07%); terpinolene (0.11%).

1.7 BERGAMOT OIL (*CITRUS BERGAMIA*)

1.7.1 1979–1999

Table 1.16 summarizes the results relative to the papers published from 1979 to 1999 on the composition of bergamot essential oils already revised by Dugo et al. (2002). In the table are separately reported the data relative to industrially cold-pressed oils and oils that are laboratory extracted by cold extraction or by distillation techniques.

TABLE 1.16
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

	Cold-Pressed Oils	Laboratory-Extracted Oils	
		Cold Extracted	Distilled
	Hydrocarbons		
Aliphatic			
Dodecane	0–0.01	–	0.03
Monoterpene			
Camphene	tr–0.11	tr–0.05	0.01–0.21
δ-3-Carene	tr–0.01	0.14–0.9	2.04
p-Cymene	0.01–3.61	tr–0.57	0.10–1.56
Limonene	24.07–54.85	23.70–40.80	35.42–45.11
Myrcene	0.36–2.33	0.7–2.0	0.95–1.97
Allo-ocimene	tr	–	–
(*E*)-β-Ocimene	0.02–1.06	0.14	0–0.74
(*Z*)-β-Ocimene	0.01–0.43	tr–0.02	–
α-Phellandrene	0.01–0.18	–	tr–0.04
β-Phellandrene	0.02–0.04	0.15	–
α-Pinene	0.70–1.84	0.5–1.88	0.52–1.30
β-Pinene	4.11–10.60	3.0–8.9	2.90–5.79
Sabinene	0.72–1.69	0.5–0.55	0.58–1.00
α-Terpinene	0.08–0.28	0.07–0.2	–
γ-Terpinene	1.15–11.38	4.12–12.60	1.35–6.02
Terpinolene	tr–0.72	0.18–0.4	0.14–0.44

TABLE 1.16 (continued)
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

	Cold-Pressed Oils	Laboratory-Extracted Oils	
		Cold Extracted	Distilled
α-Thujene	0.15–0.49	0.2	0–0.18
Tricyclene	0–0.01	–	–
Sesquiterpene			
α-Bergamotene*	–	0.28–0.86	0.02–0.15
cis-α-Bergamotene	tr–0.05	tr	–
trans-α-Bergamotene	0.09–0.44	0.9	–
Bicyclogermacrene	0.01–0.08	–	–
α-Bisabolene*	tr	–	–
β-Bisabolene	0.16–0.73	1.2–1.28	0.02–0.95
(*Z*)-γ-Bisabolene	0–0.01	–	–
δ-Cadinene	tr	–	0.33
β-Caryophyllene	0.15–0.55	0.25–0.7	0.11–0.53
δ-Elemene	0–0.06	–	–
(*E*,*E*)-α-Farnesene	0–tr	–	–
(*E*)-β-Farnenese	tr	–	0.15–0.57
(*Z*)-β-Farnesene	0.03–0.09	0.07–0.1	–
Germacrene B	0–0.04	–	–
Germacrene D	tr–0.11	0.1	–
α-Humulene	tr–0.07	0.1	tr
γ-Muurolene	0.07	0.05	–
β-Santalene	tr–0.02	–	–
β-Selinene	tr–0.04	–	–
β-Sesquiphellandrene	tr	tr	–
Aldehydes			
Aliphatic			
Decanal	tr–0.16	tr–0.29	0.07–0.12
Dodecanal	tr–0.16	tr	–
Nonanal	tr–0.08	tr–0.09	0.03–0.04
Octanal	0.02–0.08	tr–0.03	0.02–0.11
Tetradecanal	0–0.01	–	–
Undecanal	0–0.02	–	0.07–0.23
Monoterpene			
Citronellal	tr–0.06	tr–0.02	0.02–0.07
Geranial	0.16–1.25	tr–0.3	0.06–0.48
Neral	0.05–0.72	0.16–0.62	0.04–0.36
Perilla aldehyde	tr–0.11	–	–
Ketones			
Aliphatic			
6-Methyl-5-hepten-2-one	tr–0.01	–	0.01–0.02
Monoterpene			
Camphor	tr–0.01	–	–

continued

TABLE 1.16 (continued)
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

	Cold-Pressed Oils	Laboratory-Extracted Oils	
		Cold Extracted	Distilled
Carvone	0–0.13	–	–
Sesquiterpene			
nootkatone	tr–0.10	tr–0.5	–
		Alcohols	
Aliphatic			
Decanol	tr	tr	–
Hexanol	tr	–	0.01
(*Z*)-3-Hexenol	0.01	–	tr
Nonanol	tr	–	0.02
Octanol	0–0.03	–	–
Monoterpene			
cis-Carveol	tr	–	–
trans-Carveol	tr	–	–
Citronellol	tr–0.29	tr–0.29	tr–0.06
Hotrienol	tr	–	–
Geraniol	0–0.07	tr–0.04	0.15–5.67
Linalol	1.58–24.22	4.2–18.2	7.56–17.89
Nerol	tr–0.26	tr–0.1	0.09–0.56
Perilla alcohol	tr	–	–
cis-Sabinene hydrate	tr–0.07	–	0.04
trans-Sabinene hydrate	tr	–	–
Terpinen-4-ol	tr–0.29	tr–0.1	0.03–0.16
α-Terpineol	0.03–0.27	0.1–0.80	0.07–3.11
Sesquiterpene			
α-Bisabolol	tr–0.03	0.1	–
β-Bisabolol	tr	–	–
Campherenol	0.01–0.03	–	–
(*E*)-Nerolidol	tr–0.04	0.1	tr
Norbornanol[a]	0.01–0.02	–	–
		Esters	
Aliphatic			
Decyl acetate	tr–0.05	0.1	0.02–0.12
Heptyl acetate	tr–0.02	–	–
Hexyl acetate	0–tr	0.2	0.01–0.16
Nonyl acetate	tr–0.05	tr	0.03–0.05
Octyl acetate	tr–0.22	0.02–0.2	0.11–0.19
Monoterpene			
Bornyl acetate	tr–0.04	tr	–
Citronellyl acetate	tr–0.06	0.05–0.1	0.09–0.15
Geranyl acetate	0.11–0.88	0.06–1.6	0.58–1.93
Linalyl acetate	15.09–41.36	23.0–39.0	11.37–31.66

TABLE 1.16 (continued)
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

	Cold-Pressed Oils	Laboratory-Extracted Oils	
		Cold Extracted	Distilled
Linalyl propanate	tr–0.07	–	–
Methyl geranate	tr–0.02	–	0.05
Neryl acetate	tr–0.67	0.02–1.6	0.48–1.28
trans-Sabinene hydrate acetate	0.05–0.13	–	–
α-Terpinyl acetate	0.07–0.27	0.18–0.49	0.03–0.31
Ethers and oxides			
Monoterpene			
1,8-Cineole	tr–0.02	–	0.16–0.28
cis-Limonene oxide	tr–0.02	–	0.02–0.07
trans-Limonene oxide	tr–0.01	–	–
Sesquiterpene			
Humulene oxide II	tr	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; [a] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol.

Appendix to Table 1.16

- The results reported in Table 1.16 and in this appendix, for the different categories of bergamot oils, are taken from the following original papers:
  - Cold-pressed industrial oils: Schenk and Lamparsky (1981); Huet (1981); Ricciardi et al. (1982); Koketsu et al. (1983); Mazza (1986); Inoma et al. (1989); Dellacassa et al. (1997); Dugo et al. (1987, 1991, 1999); Dugo (1994); Lamonica et al. (1990); Verzera et al. (1996b, 1998a); Chouchi et al. (1995). Were also included the qualitative studies carried out by Ehret and Maupetit (1982); Ohloff et al. (1986); Cartoni et al. (1987); Micali et al. (1990); Lanuzza et al. (1991); Mondello et al. (1994b, 1995a,b).
  - Laboratory cold extracted oils: Drescher et al. (1984); Calvarano et al. (1984); Baser et al. (1995); Sawamura et al. (1999a); Kirbaslar et al. (2000).
  - Laboratory-distilled oils: Huang et al. (1986, 1987).
- Coelutions indicated by one or more authors in chromatographic separations of bergamot oils:
  - β-Pinene + sabinene; limonene + β-phellandrene; terpinolene + octanal; citronellal + octyl acetate; geranial + perilla aldehyde; citronellol + nerol; α-humulene + α-terpineol; β-bisabolene + 2,7-dimethyl-2,6-octadien-1-ol[t]; α-terpineol + α-terpinyl acetate.
  - Ranges reported in Table 1.16 for some components in cold-pressed oils, coeluted in chromatographic separation reported by few authors (β-pinene, sabinene, β-phellandrene, citronellal, octyl acetate, perilla aldehyde, citronellol, nerol, α-humulene, β-bisabolene), are determined considering the results where coelutions did not occur.
- In addition to those listed in Table 1.16, in bergamot oil were found the components listed below:
  - Cold-pressed oils: tridecane (0.05%), δ-2-carene (0.11%), 1,3,8-*p*-menthatriene (0.17%), aromadendrene (0.36%), (*Z*)-α-bisabolene (0–tr), (*E*)-2-decenal[t]

continued

TABLE 1.16 (continued)
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

(0%–0.01%), isomenthone (0.01%), menthone (0%–0.06%), dodecanol (0%–0.01%), octen-3-ol* (0.08%), dihydrocitronellol (0.05%), isopulegol (tr–0.01%), menthol (0.16%), neomenthol (0.02%), (*E*,*E*)-α-farnesol (0.01%), (*Z*)-β-santalol (0.01%), isomenthyl acetate (0.10%), *cis*-sabinene hydrate acetate (0.07%), and trace amounts of linear chained hydrocarbons C_{21}–C_{33} and correspondent "iso" isomers, C_{23}–C_{31}, *p*-cymenene, γ-acoradiene, ar-curcumene, γ-elemene, α-muurolene; α-santalene, epi-β–santalene, α-selinene, lilial, piperitone, 2,6-dimethyl-6-acetoxy-octa-7-en-3-one, 2,6-dimethyl-6-acetoxy-octa-1,7-dien-3-one, 2,6-dimethyl-6-acetoxy-octa-1-en-7-one, (*E*)-2-hexenol, (*Z*)-3-hexenol, 1-pentanol, 2-pentanol, borneol, carvacrol, *p*-cymen-8-ol, cumin alcohol, *cis*- and *trans*-2,6-dimethyl-octa-1,5,7-trien-3-ol, 3,7-dimethyl-3-acetoxy-octa-1,5-dien-7-ol, 3,7-dimethyl-3-acetoxy-octa-1,7-dien-6-ol, limonen-4-ol, *cis*- and *trans*-*p*-menth-2,8-dien-1-ol, 3,7-dimethyl-octa-1,5-dien-3,7-diol, 3,7-dimethyl-octa-1,7-dien-3,6-diol, *p*-mentha-1,8-dien-9-ol, *p*-menth-1-en-9-ol, *cis*-and *trans*-*p*-menth-2-en-1-ol, *trans*-pinocarveol, thymol, (–)-(4S,8R)-8-epi-α-bisabolol, (–)-(4R,8S)-4-epi-β-bisabolol, α-cadinol, T-cadinol, caryophyllenol I and II, caryophyllene alcohol, β-eudesmol, farnesol*, spathulenol, *cis*-hex-3-en-1-yl acetate, undecyl acetate, carvyl acetate, hotrienyl acetate, geranyl propanate, linalyl propanate, *p*-mentha-1,3-dien-7-yl acetate, *p*-mentha-1,7-dien-4-yl acetate, *p*-mentha-1,7(10)-dien-2-yl acetate, *p*-mentha-1,8-dien-9-yl acetate, *p*-menth-1-en-9-yl acetate, neomenthyl acetate, neryl propanate, perillyl acetate, *trans*-pinocarvyl acetate, terpinen-4-yl acetate, 3-(3,4,5-trimethoxyphenyl)-propenyl acetate, *cis*- and *trans*-linalol oxide (furanoid and pyranoid form), *cis*- and *trans*-2,3-ocimene oxide, *p*-menth-1-en-4,5-oxide, *p*-menth-4-en-1,2-oxide, linalyl acetate oxide (two isomers), caryophyllene oxide*, humulene oxide I, isocaryophyllene oxide, 2,3-geranyl acetate oxide, 2,3 neryl acetate oxide, methyl *N*-methyl anthranilate, indole, acetic acid, octanoic acid. Many of these components, identified by a single author need further confirmation.

– Cold-pressed laboratory oils: *m*-cymene (0.3%), hexanal (0.02%), 1-hydroxylinalol (0.1%), phenyl ethyl alcohol (0.5%), octadienyl formate (0.1%), and trace amounts of (*E*)-2-hexenal, 6-methyl-3-heptanol, *p*-menth-1-en-8-yl acetate.
– Steam distilled laboratory oils: pentadecane (0.21%), dihydrolinalol (0.07%), endo-fenchol (0.01%), isopulegol (0.01%), lavandulol (0.02%), citronellyl formate (0.02%), geranyl formate (0.02%), γ-heptalactone (0.04%), and trace amounts of pulegone, (*Z*)-nerolidol, butyl acetate, 1,4-cineole.

- The values in Table 1.16 show that bergamot oil is subject to wide ranges of variation in its composition. These changes, as proved by many authors, could be due to the period of harvest of the fruits (Calvarano, 1968; Huet and Dupuis, 1969; Dugo et al., 1987, 1991; Dugo, 1994; Verzera et al., 1996b, 1998), to the cultivar of the fruits (Verzera et al., 1996b) and to the area of cultivation of the fruits (Huet and Dupuis, 1969), also if cultivated in very close fields (Figure 1.11), as noticed by Dugo et al. (1987, 1991, 1994) and by Verzera et al. (1998). The most evident changes are, however, noticed during the productive season, in particular for linalol and linalyl acetate. The former, as shown in Figure 1.12 (Dugo, 1994), varied, for a bergamot oil produced in Calabria, during the productive season, from an average content of 13% to about 6%; the latter, during the same period varied from an average content of 25% at the beginning of the season, to that of 31 % at the end. The ratio linalol/linalyl acetate varied from 0.5 to 0.2.

TABLE 1.16 (continued)
Percentage Composition of the Volatile Fraction of Bergamot Oils (1979–1999)

- In laboratory-extracted oils appear high values of δ-3-carene (0.14%, 0.9%, and 2.04%), respectively reported by Calvarano et al. (1984), Kirbaslar et al. (2000), and Huang et al. (1986), probably due to a not optimised chromatographic separation. This component in genuine samples of bergamot oil should never exceed 0.01% of the volatile fraction. High values and therefore not representative of the natural composition of bergamot oil of geraniol and α-terpineol are reported by Huang et al. (1986, 1987) in oils distilled in laboratory.

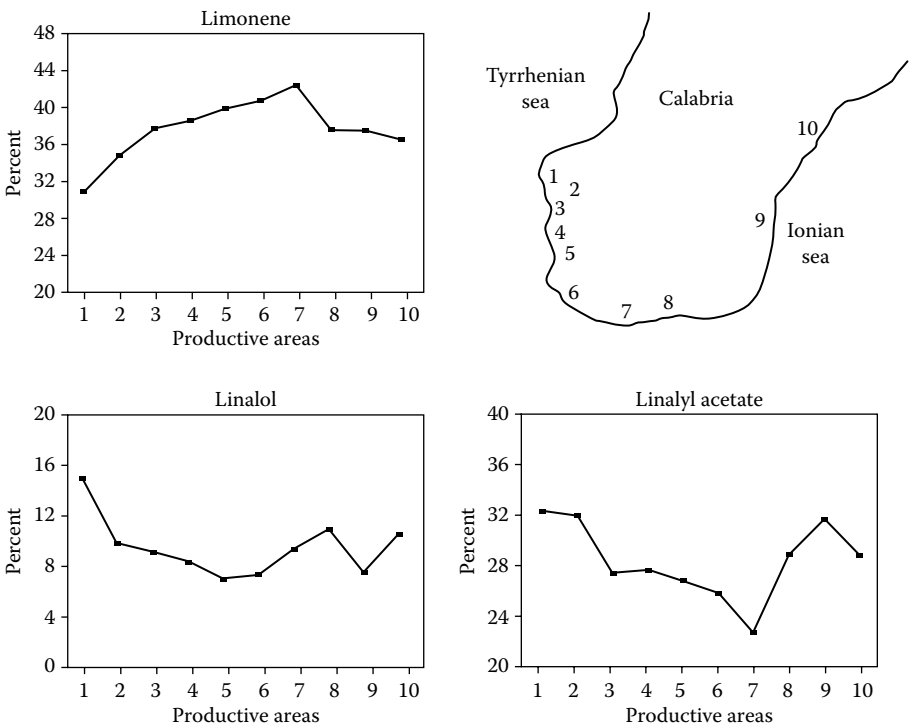

FIGURE 1.11 Variation in average content of limonene, linalol, and linalyl acetate for bergamot oil in relation to the production area. (From Dugo, G., et al., *J. Essent. Oil Res.* 6, 101–137, 1994.)

1.7.2 1998–2009

1.7.2.1 Industrial Oils

Table 1.17 reports the results published after the review by Dugo et al. (2002), relative to the composition of the volatile fraction of industrial cold-pressed bergamot oils.

Among the papers relative to the results reported in the table, some were focused on the study of the composition of bergamot oil (Sawamura et al., 1999b; Gionfriddo et al., 2000; Kubeczka and Formáčcek, 2002; Belsito et al., 2007; Costa et al., 2010; Sciarrone et al., 2009). Most of

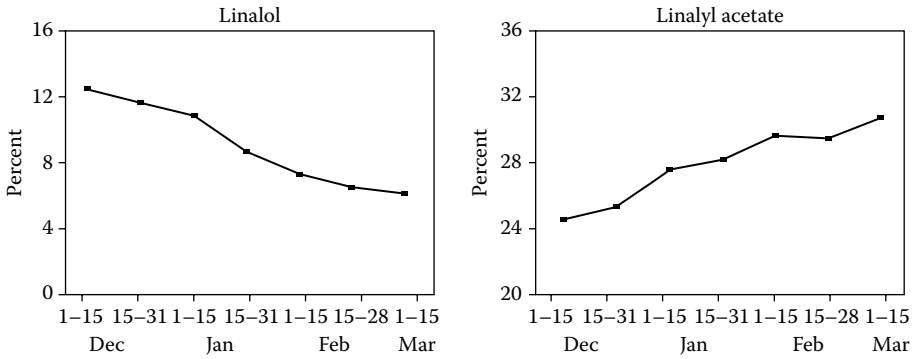

FIGURE 1.12 Variation in average content of linalol and linalyl acetate for bergamot oil in relation to the production season. (From Dugo, G., et al., *J. Essent. Oil Res*. 6, 101–137, 1994.)

these studies were limited to only one sample, with the exception of the results obtained by Sciarrone et al. (2010) relative to a large set of industrial samples. The other papers were focused on the antimicrobial activity (Ferrini et al., 1998); the alteration of the oil used in aroma lamp (Oberhofer et al., 1999); the use of CO_2 for the extraction and fractionation of the oils (Russo et al., 2001; Poiana et al., 2003; Franceschi et al., 2004); the identification of characteristic odor components (Sawamura et al., 2006); and the development of advanced analytical techniques (Mondello et al., 2003, 2004b; Tranchida et al., 2006). In these papers, too, the results were limited to one sample.

The main difference between the oils from Italy and from the Ivory Coast analyzed by Ferrini et al. (1998) is relative to the linalol content, which is higher in oils from Ivory Coast. It must be pointed out that a higher linalol content in the oil not always imply a better odor quality (Dugo et al., 1987). Ferrini et al. also analyzed a bergapten-free Italian essential oil (see the last column of the table for the reports of its composition) with a composition very similar to that of cold-pressed Italian bergamot oil and two reconstituted oils.

The results obtained by Oberhofer et al. (1999) are relative only to the olfactory impact components. They evaluated all the odor differences between the head space of the oil prior and after heating at 65°C, in a tube at the top of a commercial aroma lamp, before and after the reduction of 50% of the oil volume. In heated oils were observed a drastic decrease of limonene, an increment of linalol, and an increase before evaporation and a drastic decrease after evaporation of linalyl acetate.

Poiana et al. (2003) also analyzed two bergapten-free oils, one obtained by distillation, and the other by alkaline treatment. Both presented a composition very similar to the cold-pressed oil (these results are included in the last column of the table). The study carried out by Costa et al. (2010) is focused on the comparison between cold-pressed oils and some industrially treated oils to reduce the content of monoterpene hydrocarbons (terpeneless oils) by fractionated distillation, or to reduce the content of psoralens and coumarines (bergapten-free oils) by distillation or by alkali treatment. The article compares the analytical differences of the oils (composition of volatile fraction, of the coumarinic and psoralenic fraction, and the enantiomeric distribution of some volatiles).

Sciarrone (2009, personal communication) analyzed 13 oils produced in Calabria during the 2008/09 season and two oils from Ivory Coast. Compositional differences are not detected among the different oils. The same article reports the composition of three peratoner. Their composition is reported in Chapter 3.

Results reported in Table 1.17, although limited to few samples appear, however representative of the composition of bergamot oil and are in good agreement with those revised by Dugo et al. (2002) and summarized in Table 1.16. From these results it can also be noted, as has been reported

TABLE 1.17
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed							
	1a	**1b**	**2**	**3**	**4**	**5**	**6**	**7,8,9**
	Hydrocarbons							
Aliphatic								
Dodecane	–	–	–	–	–	–	–	–
Monoterpene								
Camphene	–	–	–	0.03	0.02–0.05	–	0.03	0.03–0.04
δ-3-Carene	–	–	–	–	tr–0.02	–	–	tr
p-Cymene	–	0.2–0.5	0.67	0.97	–	1.0	0.30	0.62
Limonene	29.8–30.4	23.5–24.5	24.50	35.62	37.61–50.48	41.3	33.10	42.07
Myrcene	1.2–1.4	1.0–1.1	1.78	0.83	0.74–1.38	5.1	0.89	0.88–0.98
β-Ocimene*	0.4–0.5	0.3	–	–	–	–	–	–
(*E*)-β-Ocimene	–	–	0.82	0.03	0.12–0.35[a]	2.7	0.27	0.21
(*Z*)-β-Ocimene	–	–	0.46	–	0.12–0.35[a]	1.5	0.04	0.02
α-Phellandrene	–	–	–	0.03	0.02–0.09	–	–	0.03
β-Phellandrene	–	–	–	0.19	–	–	0.21	–
α-Pinene	1.1–1.2	0.6–0.8	0.46	1.45	0.92–1.56	1.2	1.04	1.27–1.43
β-Pinene	6.7–7.5	3.6–4.5	2.97	8.93	4.11–9.55	7.3	5.87	7.04
Sabinene	1.2–1.3	0.7–0.9	tr	1.16	0.50–1.32	1.2	1.02	1.16
α-Terpinene	–	–	–	0.10	0.09–0.19	–	0.13	0.15
γ-Terpinene	7.2–7.9	3.5–4.1	2.54	6.53	5.73–10.23	7.9	6.54	6.82–7.84
Terpinolene	0.3–0.4	0.2	tr	0.23	0.03–0.40	0.6	0.28	0.24–0.32
α-Thujene	0.3	0.2	tr	–	0.21–0.40	0.3	0.27	0.32–0.33
Tricyclene	–	–	–	–	–	–	–	tr

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed							
	1a	**1b**	**2**	**3**	**4**	**5**	**6**	**7,8,9**
Sesquiterpene								
Bergamotene*	0.3	0.3	–	–	–	–	–	–
α-Bergamotene*	–	–	–	0.30	–	–	–	–
cis-α-Bergamotene	–	–	–	–	–	–	–	–
trans-α-Bergamotene	–	–	0.91	–	0.28–0.50	–	0.29	0.22–0.29
trans-β-Bergamotene	–	–	–	–	–	–	–	–
Bicyclogermacrene	–	–	–	–	–	–	–	0.03
β-Bisabolene	0.4–0.5	0.4	0.13	0.45	0.43–0.65	0.6	–	0.43
(E)-α-Bisabolene	–	–	–	–	–	–	–	–
(Z)-α-Bisabolene	–	–	–	–	–	–	–	-
(E)-γ-Bisabolene	–	–	–	–	–	–	–	–
(Z)-γ-Bisabolene	–	–	–	–	–	–	–	–
δ-Cadinene	–	–	–	–	–	–	–	–
β-Caryophyllene	0.3–0.4	0.4	0.11	0.21	0.26–0.48	–	0.33	0.33
β-Elemene	–	–	–	+	–	–	–	–
δ-Elemene	–	–	–	–	0.01–0.04	–	–	tr
(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	–
(*E*)-β-Farnesene	–	–	–	0.07	–	–	–	–
(*Z*)-β-Farnesene	–	–	–	–	–	–	–	0.03–0.07
Germacrene D	–	–	–	0.03	0.03–0.14	–	–	0.02–0.05
α-Humulene	–	–	–	+	–	–	–	–
β-Santalene	–	–	–	+	–	–	–	0.03
β-Sesquiphellandrene	–	–	–	–	–			0.01

Aldehydes								
Aliphatic								
Decanal	–	–	tr	–	0.04–0.08	–	0.04	0.06
(*E*)-2-Decenal	–	–	–	+	–	–	–	–
Dodecanal	–	–	–	+	–	–	–	0.01
Nonanal	–	–	–	–	–	–	0.02	0.03
Octanal	–	–	–	–	0.03–0.09	–	0.04	0.05
Tetradecanal	–	–	–	–	–	–	–	0.01
Undecanal	–	–	–	–	tr–0.04	–	0.05	0.01
Monoterpene								
Citronellal	–	–	–	–	0.01–0.03	–	–	0.01
Geranial	0.3–0.4	0.3	0.26	0.47	0.21–0.42	–	0.36	0.15–0.36
Perilla aldehyde	–	–	–	–	–	–	–	–
Neral	–	–	–	0.16	0.12–0.30	–	0.21	0.24
Ketones								
Aliphatic								
6-Methyl-5-hepten-2-one	–	–	–	–	tr–0.05	–	–	0.01
Monoterpene								
Camphor	–	–	–	–	0.01	–	–	–
Carvone	–	–	–	–	–	–	–	–
Sesquiterpene								
Nootkatone	–	–	–	0.07	0.01–0.10	–	–	0.05
Alcohols								
Aliphatic								
Octanol	–	–	–	–	–	–	–	tr

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed							
	1a	**1b**	**2**	**3**	**4**	**5**	**6**	**7,8,9**
Monoterpene								
Citronellol	–	–	–	+	–	–	–	–
Geraniol	–	–	–	0.11	–	–	0.07	c
Isopulegol	–	–	–	–	–	–	–	–
Linalol	10.1–13.5	26.4–29.4	22.49	8.67	2.23–9.96	6.9	14.63	6.79–7.53
Nerol	–	–	–	–	0.01–0.05	–	0.08	0.05
cis-Sabinene hydrate	–	–	–	0.04	–	–	0.08	0.04
trans-Sabinene hydrate	–	–	–	–	0.03–0.09	–	0.04	–
Terpinen-4-ol	–	–	–	0.03	0.02–0.04	–	0.03	0.03
α-Terpineol	–	–	–	0.16	0.02–0.10	–	0.09	0.09
Sesquiterpene								
α-Bisabolol	–	–	–	–	–	–	–	tr–0.03
Campherenol	–	–	–	–	–	–	–	0.01–0.02
Nerolidol*	–	–	–	–	–	–	–	–
(*E*)-Nerolidol	–	–	–	+	–	–	–	0.01–0.02
Norbornanol[e]	–	–	–	+	–	–	–	tr–0.01
Spathulenol	–	–	–	–	–	–	–	–
				Esters				
Aliphatic								
Decyl acetate	–	–	–	+	–	–	–	0.02–0.03
Heptyl acetate	–	–	–	+	–	–	–	–
Hexyl acetate	–	–	–	–	–	–	–	–
Nonyl acetate	–	–	–	+	tr–0.05	–	–	0.01–0.02
Octyl acetate	–	–	–	0.12	0.08–0.15	–	0.09	0.07–0.12

Monoterpene								
Bornyl acetate	–	–	–	–	tr–0.04	–	–	0.05
Citronellyl acetate	–	–	–	–	–	–	0.04	0.03
Geranyl acetate	0.4–0.5	0.2–0.3	0.38	0.47	0.25–0.87	2.4	0.20	0.36
Linalyl acetate	33.7–35.5	30.5–35.4	40.61	29.12	19.99–32.89	18.8	32.51	27.32–31.92[c]
Linalyl propanate	–	–	–	–	0.01–0.04	–	–	0.03
Methyl geranate	–	–	–	–	tr–0.05	–	–	0.01
Neryl acetate	0.4–0.5	0.4–0.8	0.21	–	0.25–0.53	1.2	0.68	0.18–0.39
α-Terpinyl acetate	–	–	–	–	0.14–0.29	–	0.14	0.17
Ethers and oxides								
Monoterpene								
cis-Limonene oxide	–	–	–	+	tr–0.02	–	0.02	0–tr
trans-Limonene oxide	–	–	–	+	tr–0.02	–	–	tr
cis-Linalol oxide	–	–	–	–	–	–	–	–
trans-Linalol oxide	–	–	–	–	–	–	–	–
Others								
(*E*)-Solanone[t]	–	–	–	0.10	–	–	–	–

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed								Bergapten Free[h]
	10	11	12	13	14	15a	15b		16,17,18,19,20
	Hydrocarbons								
Aliphatic									
Dodecane	–	–	–	–	–	–	–	–	tr
Monoterpene									
Camphene	tr	–	tr	0.02	0.03	0.01–0.03	0.01	0.02	tr–0.04
δ-3-Carene	–	–	–	tr	–	–	0.01	tr	tr
p-Cymene	0.7	–	1.4	b	0.37	0.07–0.49	0.14	0.15	0.09–0.6
Limonene	32.1	38.16	37.2	32.52[b]	42.80	37.40–49.10	40.90	45.89	32.4–46.29
Myrcene	1.0	0.94	0.8	1.10	0.91	0.68–0.99	0.71	0.83	0.86–1.47
β-Ocimene*	–	–	–	–	–	–	–	–	0.4
(*E*)-β-Ocimene	0.3	–	–	0.21	0.17	0.16–0.24	0.08	0.15	0.18–0.35
(*Z*)-β-Ocimene	tr	–	tr	0.05	0.04	0–0.03	0.02	0.02	tr–0.10
α-Phellandrene	tr	–	tr	0.10[d]	0.02	0.02	0.01	0.02	tr–0.06[d]
β-Phellandrene	–	–	0.2	–	–	–	–	–	tr
α-Pinene	1.5	0.64	1.3	1.01	1.04	0.51–1.15	0.57	1.09	0.99–1.4
β-Pinene	7.0	3.05	6.2	5.20	5.59	3.50–6.88	3.58	6.72	5.27–6.7
Sabinene	0.9	0.61	1.1	0.91	0.89	0.48–0.95	0.55	0.93	0.85–1.1
α-Terpinene	0.2	–	0.1	0.05	0.11	0.04–0.14	0.03	0.12	0.09–0.3
γ-Terpinene	8.5	4.12	6.8	5.49	6.19	6.36–8.10	3.78	8.03	5.20–8.7
Terpinolene	0.4	0.17	0.3	0.18	0.24	0.18–0.28	0.12	0.25	0.22–0.5
α-Thujene	0.4	0.15	–	0.27	0.27	0.12–0.27	0.11	0.25	0.14–0.4
Tricyclene	–	–	–	–	0.01	0–0.03	0.02	0.02	tr–0.01
Sesquiterpene									
Bergamotene*	–	–	–	–	–	–	–	–	0.3
α-Bergamotene*	–	–	tr	–	–	–	–	–	–

cis-α-Bergamotene	–	–	–	–	–	0.01–0.27	0.01	0.01	0.01–0.03
trans-α-Bergamotene	0.6	0.31	–	–	0.25	0.01–0.25	0.17	0.22	0.22–0.5
trans-β-Bergamotene	–	–	–	–	0.01	0–0.01	0.01	0.01	0.01
Bicyclogermacrene	–	–	–	–	0.01	0–0.01	tr	0.01	0.01–0.03
β-Bisabolene	0.8	0.44	0.8	0.47	0.36	0.10–0.39	0.26	0.34	0.25–0.7
(*E*)-α-Bisabolene	–	–	–	–	0.01	0–0.01	0.01	0.01	0.01
(*Z*)-α-Bisabolene	–	–	–	–	0.03	0.01–0.03	0.02	0.02	tr–0.03
(*E*)-γ-Bisabolene	–	–	–	–	tr	0–0.01	tr	tr	–
(*Z*)-γ-Bisabolene	–	–	–	–	tr	0–0.01	tr	0.01	tr
δ-Cadinene	–	–	–	–	tr	0–0.03	0.02	tr	–
β-Caryophyllene	0.6	0.45	0.3	0.55	0.25	0.11–0.27	0.24	0.22	0.24–0.6
β-Elemene	–	–	–	–	tr	0–0.02	0.04	tr	–
δ-Elemene	–	–	–	–	0.01	0–0.01	tr	0.01	tr–0.01
(*E*,*E*)-α-Farnesene	–	–	–	–	0.01	0–0.01	0.01	tr	tr
(*E*)-β-Farnesene	–	–	–	–	0.04	0–0.04	0.03	0.04	0.04–0.05
(*Z*)-β-Farnesene	–	–	–	0.45	0.01	0–0.04	0.01	0.01	0.01–0.12
Germacrene D	–	–	tr	–	0.03	0.01–0.04	0.02	0.04	0.03–0.06
α-Humulene	–	–	tr	–	0.02	0.01–0.02	0.02	0.01	0.02
β-Santalene	–	–	–	–	0.01	0–0.01	0.01	tr	0.01
β-Sesquiphellandrene	–	–	–	–	tr	–	–	tr	–
Aldehydes									
Aliphatic									
Decanal	0.1	–	tr	0.07	0.05	0.01–0.04	0.02	0.03	0.04–0.1
(*E*)-2-Decenal	–	–	–	–	–	–	–	–	tr
Dodecanal	–	–	+	–	–	0–0.03	0	tr	tr–0.03
Nonanal	–	–	tr	tr	0.03	0.01–0.02	0.01	0.01	tr–0.03
Octanal	tr	–	tr	0.10[d]	0.05	0.01–0.04	0.01	0.01	tr–0.06[d]
Tetradecanal	–	–	–	–	–	–	–	–	tr
Undecanal	–	–	–	–	0.01	0–0.02	0.01	tr	0.01

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed								Bergapten Free[h]
	10	11	12	13	14	15a	15b		16,17,18,19,20
Monoterpene									
Citronellal	–	–	–	–	0.01	0–0.01	tr	0.01	0.01
Geranial	0.3	0.27	0.3	0.69	0.24	0.14–0.30	0.24	0.17	0.17–0.31
Neral	0.2	0.18	0.2	0.14	0.16	0.12–0.21	0.18	0.11	0.10–0.21
Perilla aldehyde	–	–	–	–	tr	0–0.01	0.01	tr	tr
Ketones									
Aliphatic									
6-Methyl-5-hepten-2-one	–	–	tr	–	0.02	0–0.03	0.01	0.01	0.01–0.03
Monoterpene									
Camphor	–	–	–	–	–	–	0.01	tr	tr
Carvone	–	–	tr	–	0.01	0–0.01	0.02	0.01	tr–0.1
Sesquiterpene									
Nootkatone	0.1	–	0.1	–	0.08	0–0.07	tr	0.05	tr–0.1
Alcohols									
Aliphatic									
Octanol	tr	–	–	–	–	0.02–0.05[g]	0.03[g]	0.01[g]	tr
Monoterpene									
Citronellol	–	–	–	–	0.01	0–0.02	0.01	–	0.05–0.06[f]
Geraniol	–	–	0.1	–	–	–	–	–	tr
Isopulegol	–	–	–	–	–	0–0.01	tr	tr	–
Linalol	12.1	15.30	8.8	14.1	5.55	3.69–36.14	19.39	6.81	7.81–13.57
Nerol	0.1	–	0.1	0.17	0.02	0–0.08	0.10	–	tr–0.1
cis-Sabinene hydrate	0.1	–	–	0.06	0.02	0.02–0.05[g]	0.03[g]	0.01[g]	tr–0.08

trans-Sabinene hydrate	0.1	–	0.1	–	–	–	–	–	–
Terpinen-4-ol	tr	–	tr	0.05	0.02	0.02–0.16	0.01	0.03	tr–0.1
α-Terpineol	0.1	–	0.1	0.13	0.06	0.05–0.43	0.10	0.16	0.03–0.1
Sesquiterpene									
α-Bisabolol	–	–	tr	0.01	0.02	0–0.03	0.01	0.02	tr–0.02
Campherenol	–	–	–	–	0.01	0–0.01	0.01	tr	tr–0.02
Nerolidol*	–	–	tr	0.02	–	–	–	–	–
(*E*)-Nerolidol	–	–	–	–	0.02	0–0.02	0.01	0.02	tr–0.02
Norbornanol[e]	–	–	–	–	–	0–0.01	0.01	0.01	tr–0.01
Spathulenol	–	–	–	–	0.01	0–0.01	tr	–	–
Esters									
Aliphatic									
Decyl acetate	–	–	tr	0.03	0.04	0.01–0.03	0.01	0.02	0.01–0.03
Heptyl acetate	–	–	–	–	tr	–	tr	tr	0.01
Hexyl acetate	–	–	–	–	0.02	0–0.01	tr	0.01	0–0.01
Nonyl acetate	–	–	tr	0.10	0.01	0.01–0.02	tr	0.02	0.01–0.04
Octyl acetate	0.1	–	–	0.14	0.10	0.05–0.09	0.04	0.08	0.08–0.13
Monoterpene									
Bornyl acetate	–	–	–	0.12	–	0.01	0.01	0.01	0.02–0.05
Citronellyl acetate	–	–	0.1	–	–	0–0.02	0.01	0.02	0.01–0.02
Geranyl acetate	0.4	–	0.3	0.57	0.31	0.18–0.40	0.18	0.26	0.19–0.5
Linalyl acetate	29.7	34.71	30.1	31.01	27.14	11.80–30.00	27.53	26.18	25.07–33.6
Linalyl propanate	0.1	–	–	–	0.03	0–0.04	0.02	0.02	0.03–0.1
Methyl geranate	–	–	–	–	tr	0–0.01	0.01	tr	tr–0.01
Neryl acetate	0.5	0.50	+	0.28	0.30	0.24–0.36	0.38	0.27	0.14–0.4
α-Terpinyl acetate	0.3	–	0.2	–	0.14	0.05–0.16	0.05	0.12	0.11–0.3
Ethers and oxides									
Monoterpene									
cis-Limonene oxide	–	–	tr	–	–	0–0.01	tr	tr	tr–0.01

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

	Cold-Pressed								Bergapten Free[h]
	10	**11**	**12**	**13**	**14**	**15a**	**15b**		**16,17,18,19,20**
trans-Limonene oxide	–	–	tr	–	tr	0–0.01	tr	tr	tr–0.01
cis-Linalol oxide	–	–	tr	–	–	–	–	–	tr
trans-Linalol oxide	–	–	0.1	–	–	–	–	–	–
					Others				
(*E*)-solanone[t]	–	–	0.1	–	–	–	–	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, identified but not quantitatively determined; [a] (*E*)-β-ocimene + (*Z*)-β-ocimene; [b] limonene + *p*-cymene; [c] geraniol + linalyl acetate; [d] α-phellandrene + octanal; [e] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol; [f] citronellol + nerol (value reported by Verzera et al., 1998); [g] octanol + *cis*-sabinene hydrate; [h] ranges are relative to those oils where the components were identified.

Appendix to Table 1.17

1. Ferrini et al. (1998). (a) Reggio Calabria, Italy, two samples; (b) Ivory Coast, two samples; GC/FID and GC/MS on capillary column (60 m × 0.20 mm × 0.25 μm) coated with SPB-5; NIST/EPA/MSDC MS library; relative percentage of peak areas.
2. Oberhofer et al. (1999). Italy; one commercial sample; GC/FID on capillary columns coated with HP-5 (25 m × 0.32 mm × 0.52 μm), OV-1 (25 m × 0.25 mm × 0.3 μm), Carbowax (25 m × 0.25 mm × 0.3 μm); GC/sniffing technique on capillary column (25 m × 0.53 mm × 0.3 μm) coated with FSOT-RSL-150; GC/IR/MS on capillary columns coated with RSL-200 (30 m × 0.52 mm × 0.25 μm) or with Stabilwax (60 m × 0.32 mm × 0.25 μm); NBS and Wiley MS libraries; EPA-REVA and Robertet IR libraries; relative percentage of peak areas.
3. Sawamura et al. (1999b). Reggio Calabria, Italy; one sample; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with Thermon 600T; wt% using as internal standards *n*-heptanol and methyl myristate. Sawamura et al. also found not quantitatively determined, δ-muurolene, verbenol, (*Z*)-nerolidol, hydroxylinalol.
4. Gionfriddo et al. (2000). Reggio Calabria, Italy; 100 samples extracted with Pelatrice Speciale representative of Kg 7.802 of essential oil product from January to March; GC/FID on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; relative percentage of peak areas.
5. Russo et al. (2001). Reggio Calabria, Italy; one sample; GC/FID and GC/MS on capillary column (25 m × 0.25 mm × 0.25 μm) coated with SE-52; relative percentage of peak areas.

6. Kubeczka and Formàček (2002). Italy; one sample; GC/FID on capillary column (50 m) coated with VG-11; relative percentage of peak areas; Kubeczka and Formàček also found 1,8-cineole (0.04%).
7,8,9. Mondello et al. (2003, 2004b). Sicily, Italy; one sample; conventional GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX-5MS; Fast GC/FID on capillary column (10 m × 0.1 mm × 0.1 μm) coated with RTX-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. Tranchida et al. (2006). Sicily, Italy; one sample; conventional HS-SPME-conventional GC/FID: 30 μm PDMS fiber and a capillary column (25 m × 0.25 mm × 0.25 μm) coated with SE-52; HS-SPME-Fast GC/FID: 7 μm PDMS fiber and a capillary column (10 m × 0.1 mm × 0.1 μm) coated with SE-52; relative percentage of peak areas. These authors also found (*E*)-γ-bisabolol (0.01%–0.02%), *trans*-sesquisabinene hydrate (0.01%). In the table are reported the results obtained with conventional method. The results obtained with fast method were very similar. More information is reported in the Chapter 11.
10. Poiana et al. (2003). Calabria, Italy; one sample; GC/MS on capillary column (30 m × 0.2 mm × 0.25 μm) coated with HP-5MS; Wiley MS library; relative percentage of peak areas.
11. Franceschi et al. (2004). Brazil; one sample; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm); coated with HP-5MS; Wiley MS library; relative percentage of peak areas.
12. Sawamura et al. (2006). Calabria, Italy; one sample produced with a Pelatrice-type extractor; GC/FID, GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; for GC/MS was also used a DB-1 column; GC/O on capillary column (60 m × 0.53 mm × 1 μm) coated with DB-Wax; wt%. Sawamura et al. also found trace amounts of decanol, cedrol, peryllyl acetate, 1,8-cineole, caryophyllene oxide*, octanoic acid. In the work were reported the retention indices on DB-Wax and DB-1 columns of the identified components and their odor description at the GC sniffing port during GC/O. An example for a most probable aroma model of bergamot oil was also indicated. The same results were previously reported by Onishi et al. (2003).
13. Belsito et al. (2007). Calabria, Italy; one sample; GC/MS for qualitative and quantitative analyses on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5MS; wt% using tetradecane as internal standard. GC/FID (for calculation of LRI) on capillary column (30 m × 0.25 mm × 0.25 μm) coated with Equity-5MS.
14. Costa et al. (2010). Calabria, Italy; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; Adams 4th edn., Hochmuth; NIST-05; LRI on SLB-5MS are reported; relative percentage of peak areas. In this paper the composition of the oil is also reported as wt%. Costa et al. also found isobornyl acetate (0.01%) and trace amounts of epi-β-bisabolol.
15. Sciarrone (2009, personal communication). (a) 13 samples from Calabria, Italy; (b) two samples from Ivory Coast; for experimental conditions see point 14 of this appendix. Sciarrone et al. also found 4,8-dimethyl-1,3(*E*)-7-nonatriene (0%–0.01%), ascaridole (0%–0.01%) in samples (a), fenchol* (0%–0.01%), and trace amounts of 4,8-dimethyl-1,3(*Z*)-7-nonatriene γ-curcumene, *cis*- and *trans* sesquisabinene hydrate, ascaridole in samples (b).

continued

TABLE 1.17 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed and Bergapten Free Bergamot Oils (1998–2009)

16. Verzera et al. (1998a). Calabria, Italy; range of the composition of 7 distilled bergapten free oils, and 5 alkali treated bergapten free oils; GC on capillary column (30 m × 0.32 mm × 0.4–0.45 μm) coated with SE-52; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; MS libraries: Adams and FFNSC home made library; relative percentages of peak areas. Verzera et al. also found germacrene B (tr–0.01%), *trans*-sabinene hydrate acetate (0.04%–0.06%), and trace amounts of dodecanol, nonanol, indole.
17. Ferrini et al. (1998). Sicily, Italy; one sample of distilled bergapten free oil. For experimental conditions see point 1 of this appendix.
18. Poiana et al. (2003). Sicily, Italy; one sample distilled and one sample alkali treated of bergapten free oils. For experimental conditions see point 10 of this appendix. Poiana et al. also found *cis*-sabinene hydrate acetate (0.1%) and trace amounts of bergapten.
19. Belsito et al. (2007). Calabria, Italy; one sample of bergapten free oil obtained by vacuum distillation of the peel of bergamot fruits. For experimental conditions see point 13 of this appendix.
20. Costa et al. (2010). Calabria, Italy; one sample distilled and one sample alkali treated of bergapten free oils; for experimental conditions see point 14 of this appendix.

many times in literature (Calvarano, 1968a; Huet and Dupuis, 1969b; Dugo et al., 1987, 1991; Dugo, 1994; Verzera et al., 1996b, 1998a), that there is great variability in the composition of bergamot oil, mainly due to the geographic origin and the harvest period of the fruits. The high values reported by Russo et al. (2001) of (*E*)- and (*Z*)-β-ocimene must, however, be highlighted.

In addition to the papers reported in Table 1.17 more articles were published on bergamot oil. Feger et al. (2001a), using GC/MS and a column coated with OV-1701 determined the presence in Italian bergamot oil of bicyclogermacrene (0.01%); germacrene A (0.01%); germacrene B (0.01%); germacrene C (0.02%); germacrene D (0.05%–0.06%). Veriotti and Sacks (2002) used fast gas chromatography and TOF/MS for the identification and quantitative analysis of bergamot oil main components. The sample used (adulterated or at least damaged because of inadequate storage conditions considering the high content of *p*-cymene—7.24%) and the results reported by these authors, obtainable with less complex and less expensive technique, do not contribute to the knowledge of the composition of bergamot oil. Onishi et al. (2003) studied the characteristic odor components of bergamot oil. They identified 55 components, with their odor description obtained by GC-sniffing and FD-factors derived from aroma extraction dilution analysis (AEDA). This article did not include quantitative results.

The bergapten-free bergamot oils reported in the last column of Table 1.17, obtained by distillation or by alkali treatment, present a composition very similar to cold-pressed oils.

In addition to the papers on bergapten-free bergamot oils summarized in Table 1.17, two research must be mentioned by Figoli et al. (2000a,b) who studied the extraction of aromatic compounds of bergamot oil by a feed solution prepared with bergamot peels homogenized and mixed in different ratios with water/methanol using a polydimethylsiloxane membrane with encapsulated silicates. The permeate oils were all bergapten-free.

1.7.2.2 Laboratory Oils

Table 1.18 summarizes the results relative to papers on bergamot oils laboratory extracted using manual pressure on the peel, solvent extraction, and distillation. Most of the oils studied were from Italy (Sawamura, 2000; Verzera et al., 2000, 2003; Gionfriddo et al., 2003; Kiwanuka et al., 2000). The results on a Japanese oil (Sawamura, 2000) and a Turkish oil (Kirbaslar et al., 2001) are also included.

Sawamura (2000) reported results almost identical to those of a previous paper (Sawamura et al., 1999b) where the content of components was determined as wt% using two internal standards. In the more recent paper (Sawamura, 2000) this procedure is not mentioned. The Italian oil when compared to the Japanese oil presents higher content of limonene and a lower content of linalyl acetate and mainly of linalol. Since only two samples were analyzed, the differences determined cannot be considered generally representative of Italian and Japanese bergamot oils, particularly if we consider the great variability of the composition as widely reported in literature and already stated in this chapter.

From the results obtained, Verzera et al. (2000) concluded that Sicilian oils present a composition similar to those from Calabria previously studied (Verzera et al., 1996b, 1998a), although some differences were determined: the oils from the cv. Castagnaro presented in Sicilian oils higher content of limonene and other monoterpene hydrocarbons, and lower content of linalol and other monoterpene alcohols. The Sicilian oils of the cv. Fantastico compared to the Calabrese ones, also presents a lower content of linalol and the other monoterpene alcohols, while the Sicilian oils of the cv. Femminello presents a lower content of linalyl acetate than those from Calabria of the same botanical origin.

Kirbaslar et al. (2001) confirmed that during the productive season the composition of bergamot oil undergoes to consistent changes: for example, linalol varies from 18.7% to 7.9%, and linalyl acetate from 29.5% to 36.3%; β-pinene ranges from 2.7% to 3.9%, while limonene remains almost constant. In the oil obtained by distillation Kirbaslar et al. (2001) observed, as obvious, a higher content of alcohols and a lower content of esters than in cold-pressed oils.

TABLE 1.18
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Bergamot Oils (1998–2009)

	Cold Extracted							Distilled	
	1a	1b	2	3a	4	5	6a	6b	3b
				Hydrocarbons					
Aliphatic									
Monoterpene									
Camphene	0.03	0.03	0.02–0.03	tr	0.01–0.04	0–0.04	–	–	tr
δ-3-Carene	–	–	tr	–	0–0.01	–	–	–	–
p-Cymene	0.33	0.02	0.02–0.04	0.1	0.06–0.15	0.01–0.21	–	–	tr
Limonene	35.66	21.31	38.70–52.49[a]	36.4–37.2	30.49–47.14	16.95–60.00	40.20	41.65	33.2
Myrcene	0.87	0.66	0.91–1.24	1.2–1.3	0.52–1.53	0.37–1.89	1.53	2.13	2.3
(*E*)-β-Ocimene	0.03	0.02	0.13–0.16	0.3–0.4	0.15–0.29	0.01–0.19	–	–	0.3
(*Z*)-β-Ocimene	–	–	0.01	tr	0.02–0.06	0–0.04	–	–	–
α-Phellandrene	0.03	0.02	0.02–0.04	–	0.01–0.03	0–0.09	–	–	0.1
β-Phellandrene	0.23	0.19	a	–	–	–	0.31	0.35	-
α-Pinene	1.52	1.29	0.71–1.18	0.5–0.6	0.23–1.49	0.27–1.52	0.81	0.78	0.9
β-Pinene	10.09	7.47	3.70–7.57[b]	2.7–3.9	3.55–6.00	2.30–8.89[b]	4.94	4.96	3.8
Sabinene	1.47	1.25	3.70–7.57[b]	0.3–0.4	0.26–1.25	2.30–8.89[b]	1.04	0.98	0.5
α-Terpinene	0.16	0.13	0.10–0.17	0.2	0.07–0.19	0–0.20	0.13	0.17	0.7
γ-Terpinene	7.32	5.30	4.62–7.77	5.1–5.9	4.04–7.77	1.03–9.22	7.27	7.73	6.5
Terpinolene	0.28	0.21	0.21–0.33	0.3	0.20–0.37	0.07–0.38	0.29	0.43	–
α-Thujene	–	–	0.18–0.31	0.1–0.2	0.07–0.41	0.02–0.39	–	–	0.2
Tricyclene	–	–	tr	–	–	0–0.01	–	–	–
Sesquiterpene									
cis-α-Bergamotene	–	–	tr	tr	–	tr	–	–	0.4
trans-α-Bergamotene	–	–	0.28–0.29	0.4	0–0.01	0.09–0.42	–	–	tr
Bicyclogermacrene	–	–	0–0.03	–	–	0–0.03	–	–	–

β-Bisabolene	0.45	0.76	0.36–0.46	0.6	0.43–0.93	0.12–0.51	–	–	0.8
(Z)-γ-Bisabolene	–	–	tr	–	–	tr	–	–	–
β-Caryophyllene	0.15	0.22	0.22–0.31	0.3	0.30–0.61	0.11–0.80	–	–	0.3
δ-Elemene	tr	tr	tr	–	0.04–0.12	–	–	–	–
(E,E)-α-Farnesene	tr	–	0–0.02	–	0.04–0.08	tr	–	–	–
(Z)-β-Farnesene	–	–	tr–0.05	0.1	0.07–0.17	0–0.11	–	–	tr
Germacrene B	0.01	–	0–0.01	–	–	–	–	–	–
Germacrene D	0.03	0.03	0–0.05	tr	0.09–0.18	0–0.17	–	–	–
α-Humulene	0.01	0.01	0–0.08	0.1	0.03–0.07	0–0.17	–	–	–
β-Santalene	tr	tr	tr	tr	–	tr	–	–	0.1
Aldehydes									
Aliphatic									
Decanal	0.11	0.08	0.03–0.08	tr	0.03–0.10	0.01–0.33	0.10	0.14	–
(E)-2-Decenal	tr	–	–	–	–	tr	–	–	–
Dodecanal	tr	–	0.02–0.05	tr	0.02–0.05	tr	–	–	tr
Nonanal	–	–	0.01–0.04	tr	0–0.05	0.01–0.05	–	–	–
Octanal	–	–	0.02–0.03	–	0.01–0.03	0.01–0.09	–	–	–
Tetradecanal	–	–	tr–0.01	–	0.04–0.06	tr	–	–	–
Undecanal	–	0.01	tr–0.01	–	0.01–0.03	0.01–0.04	–	–	–
Monoterpene									
Citronellal	–	–	0.02	tr	0.02–0.04	0–0.04	–	–	tr
Geranial	0.39	0.37	0.15–0.43[c]	tr	0.24–0.53	0.14–0.52	0.22	0.22	0.8
Neral	0.25	0.27	0.10–0.30	0.4	0.17–0.34	0.10–0.39	–	–	–
Ketones									
Aliphatic									
6-Methyl-5-hepten-2-one	–	–	tr	–	–	tr	–	–	–
Monoterpene									
Camphor	–	–	tr	–	0–0.01	tr	–	–	0.1

continued

TABLE 1.18 (continued)
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Bergamot Oils (1998–2009)

	Cold Extracted							Distilled	
	1a	**1b**	**2**	**3a**	**4**	**5**	**6a**	**6b**	**3b**
Sesquiterpene									
Nootkatone	0.18	0.07	0.05–0.10	0.1	0.05–0.18	0–0.07	0.25	0.11	–
Alcohols									
Aliphatic									
Dodecanol	–	–	tr	–	–	tr	–	–	–
Octanol	–	–	tr	–	–	tr	–	–	–
Monoterpene									
Citronellol	tr	0.01	0–0.03[d]	–	–	0–0.12[d]	–	–	–
Geraniol	0.09	0.02	tr	–	–	tr	0.10	1.24	1.7
Isopulegol	–	–	tr	–	–	tr	–	–	–
Linalol	4.81	20.02	1.20–10.08	7.9–18.7	2.84–13.57	1.09–29.02	5.22	10.68	20.1
1-Hydroxy linalol	0.01	0.01	–	0.1	–	–	–	–	–
Nerol	0.02	0.07	0–0.03[d]	0.2	0.02–0.06	0–0.12[d]	0.06	0.48	0.8
cis-Sabinene hydrate	0.07	0.03	0.03–0.05	–	–	0–0.06	–	–	–
trans-Sabinene hydrate	tr	–	–	–	0.02–0.04	0.01–0.09	–	–	–
Terpinen-4-ol	0.03	0.55	0.01–0.02	tr	0.02–0.05	0.01–0.05	0.02	0.15	0.5
α-Terpineol	0.19	0.10	0.02–0.05	0.1	0.03–0.07	0.03–0.17	0.04	2.04	3.0
Sesquiterpene									
α-Bisabolol	–	–	0.02	tr	0.01–0.03	0.01–0.03	–	–	–
Campherenol	–	–	0.02	–	0.01–0.02	0.01–0.02	–	–	–
(*E*)-Nerolidol	0.01	0.01	tr–0.01	0.1	0.01–0.05	0–0.16	–	–	–
Norbornanol[e]	–	–	0.01–0.02	–	–	0–0.01	–	–	–
Esters									
Aliphatic									
Decyl acetate	tr	tr	0.01–0.02	0.1	0.03–0.06	tr	–	–	tr

Heptyl acetate	tr	tr	tr	–	–	tr	–	–	–
Hexyl acetate	–	–	tr	0.1	–	tr	–	–	0.1
Nonyl acetate	tr	tr	0.01–0.04	tr	0.02–0.06	tr	–	–	tr
Octyl acetate	0.19	0.16	0.04–0.18	0.1–0.2	0.05–0.15	0.01–0.19	–	–	–
Monoterpene									
Bornyl acetate	–	–	0.01–0.02	tr	0.01–0.03	0.01–0.02	–	–	0.1
Citronellyl acetate	–	–	0.02–0.03	0.1	0.01–0.05	0–0.04	–	–	tr
Geranyl acetate	0.42	0.24	0.15–0.40	0.5–0.7	0.23–0.71	0.02–0.54	0.24	1.27	2.7
Linalyl acetate	30.76	36.37	24.92–37.71	29.5–36.3	28.23–42.10	19.26–41.96	30.50	21.41	17.3
Linalyl propanate	–	–	0.03–0.04	–	0–0.01	0.01–0.19	–	–	–
Methyl geranate	–	–	tr–0.01	–	0.01	tr	–	–	–
Neryl acetate	–	0.01	0.21–0.39	0.7–1.1	0.24–0.64	0.05–0.57	0.25	0.77	2.1
cis-Sabinene hydrate acetate	–	–	–	–	0.01–0.14	tr	–	–	–
trans-Sabinene hydrate acetate	–	–	0.05–0.09	–	–	0.01–0.09	–	–	–
α-Terpinyl acetate	–	–	0.10–0.17	–	0.16–0.30	0–0.16	–	–	–
Ethers and oxides									
Monoterpene									
1,8-Cineole	–	–	tr	tr	–	tr	–	–	tr
cis-Limonene oxide	tr	–	tr	–	0.01–0.02	tr	–	–	–
trans-Limonene oxide	tr	–	tr	–	0.01–0.02	tr	–	–	–
trans-Linalol oxide	tr	–	–	–	–	tr	–	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; [a] limonene + β-phellandrene; [b] β-pinene + sabinene; [c] geranial + perilla aldehyde; [d] citronellol + nerol; [e] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol.

Appendix to Table 1.18

1. Sawamura (2000). (a) One sample hand pressed from fruits of the cv. Fantastico grown in Calabria, Italy, (b) one sample hand pressed from fruits of the cv. Balotin grown in Japan; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.25 μm) coated with Thermon 600T; Sawamura also found α-bergamotene* (0.25%), (*E*)-β-farnesene (0.06%), *trans*-carveol (0.01%), perilla alcohol (0.01%), (*Z*)-nerolidol (0.01%), spathulenol (0.01%), verbenol (0.01%), solanone[t] (0.12%), and trace amounts of β-elemene, δ-muurolene, α-sinensal, *p*-cymen-8-ol, 2,7-dimethyl-2,6-octadien-1-ol, caryophyllene oxide* in sample (a) and α-bergamotene* (0.25%), (*E*)-β-farnesene (0.11%), solanone[t] (0.08%), and trace amounts of verbenol in sample (b).

continued

TABLE 1.18 (continued)
Percentage Composition of the Volatile Fraction of Cold Extracted and Distilled Laboratory Bergamot Oils (1998–2009)

2. Verzera et al. (2000). Sicily, Italy; range of the composition of two samples each hand pressed from the cvs.: Fantastico, Femminello, Castagnaro (typical Calabrian cvs.) and from a new clone of Femminello named "PCF"; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary columns coated with Mega-5MS (30 m × 0.25 mm × 0.25 μm) or Megawax (30 m × 0.32 mm × 0.40–0.45 μm); Adams MS library; relative percentage of peak areas. Ranges reported in table are obtained from the original data provided by the authors and not from the average values reported in the article.
3. Kirbaslar et al. (2001). Turkey; (a) range of the composition of hand pressed oils from fruits picked from December 1998 to February 1999; (b) one sample steam distilled; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.52 μm) coated with HP-Innowax. MS libraries: Wiley and NBS; relative percentage of peak areas. Kirbaslar et al. also found *p*-menth-1-en-9-yl acetate (0.1%–0.2%), 3,7-dimethyl,3-hydroxy,1,6-octadienyl formate (0.1%) in sample (a), 1,3-divinyl benzene (0.2%), α-terpinyl isobutyrate (0.2%), and trace amounts of *m*-cymene in sample (b).
4. Gionfriddo et al. (2003). Calabria, Italy; cvs.: Femminello, Castagnaro, Fantastico; range of the composition of 25 oils cold extracted from fruits picked from December 2000 to February 2001; GC/FID and GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with BP-1 or BP-20; NIST 1.7 MS library; LRI on BP-20 and BP-1 are reported. Gionfriddo et al. also found β-sesquiphellandrene (0.04%–0.07%), tridecanal (0.01%–0.06%).
5. Verzera et al. (2003). Sicily, Italy; 203 samples hand pressed from fruits picked from 1997 to 2000 from plants grafted on different rootstock (Sour orange, Carrizzo citrange, Trifoliate orange, Alemow, Volkamerian lemon, Troyer citrange); GC/FID on capillary column (30 m × 0.32 mm × 0.25 μm) coated with SE-52; GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with MEGA-5MS or Megawax; Adams MS library; relative percentage of peak areas. Ranges reported in table are obtained from the original data provided by the authors and not from the average values reported in the article. Verzera et al. also found trace amounts of dodecane, (*Z*)-α-bisabolene, *cis*-linalol oxide, indole.
6. Kiwanuka et al. (2000). Calabria, Italy; cv. Castagnaro; (a) one sample solvent extracted, (b) one sample steam distilled; GC/FID on capillary column (50 m × 0.32 mm × 0.5 μm) coated with SPB-5; GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with CP-SIL 8CB; Adams MS library; relative percentage of peak areas. Kiwanuka et al. also found β-ocimene* (0.12% in sample (a) and 0.46% in sample (b)).

Verzera et al. (2003) determined some differences of the composition among bergamot oils obtained from fruits grafted on different rootstock; for example, the average value of limonene ranged from 34.2% (sour orange) to 41.1% (Trifoliate orange); the average content of linalol ranged from 8.2% (Trifoliate orange) to 14.8% (Volkamerian lemon) and that of linalyl acetate ranged from 29.9% (Trifoliate orange) to 36.6% (sour orange). In conclusion, the oils obtained using sour orange as rootstock (traditional rootstock) have the average content of oxygenated compounds of 52.1%, the highest average value of linalyl acetate (36.6%) and a good average content of linalol (13.7%). Among the other oils analyzed, the most similar to those grafted on sour orange were the oils obtained from fruits grafted on Alemow and Volkamerian lemon, which can be considered two possible alternative rootstocks.

Kiwanuka et al. (2000) observed, as predicted, higher content of linalol and lower content of linalyl acetate in the oil obtained by distillation compared to the solvent-extracted one. Differences become more evident distilling at decreasing pH values (5.2 and 2.5) which also lead to racemization of linalol and α-terpineol.

1.8 LEMON OIL (*CITRUS LIMON* [L.] BURM)

1.8.1 1979–1999

Table 1.19 summarizes the results relative to articles published between 1979 and 1999 on the composition of the volatile fraction of lemon oil, already revised by Dugo et al. (2002). The data relative to industrially cold-pressed and commercial oils, and laboratory-extracted oils by cold extraction are reported separately within the table.

The data reported in the table do not include among the oils extracted in laboratory, those obtained in this period by distillation techniques, due to the drastic influence on the composition of the different experimental conditions used. These oils were extensively treated in the previous review by Dugo et al. (2002).

1.8.2 1998–2009

1.8.2.1 Industrial Oils

Table 1.20 summarizes the results relative to articles on the composition of lemon oil published in the last decade, not yet revised in the former review (Dugo et al., 2002). Two of these papers (Verzera et al., 1999, 2004) were carried out on very large number of samples of Sicilian lemon oils obtained by different extraction technologies. The first (Verzera et al., 1999) compared four different extraction technologies and the authors asserted that Pelatrice oils had the highest content of oxygenated compounds but had poor grass note, probably due to a high content of chlorophyll extracted from the fruit peel using rasping machines. Sfumatrice oils had a lower content of oxygenated components but the best odor notes. Sfumatrice machine give a lower yield and is more expensive than Pelatrice. FMC oils are a good compromise between cost, yield and oil quality. Oils obtained with Torchi, obtained by the peels previously pressed by Sfumatrice, analyzed in this paper are surely of lower quality than those extracted by the other techniques. The second paper (Verzera et al., 2004) compares the composition of lemon oils produced from fruits cultivated traditionally and organically, obtained in the same period with FMC extractor. The authors found higher monoterpene aldehydes amounts in organic oils. The articles published by Mondello et al. (2003, 2004b) report the composition of only one sample of lemon oil; in fact, these studies focused on the comparison between conventional and fast gas chromatographic analytical methods. The results of Viuda-Martos et al. (2009) are limited to the principal components of one Spanish oil. The results of these papers are in good agreement with those reported in Table 1.19 for cold-pressed oils. The other papers reported in Table 1.20 are carried out on commercial oils, each of them limited to one sample. Baratta et al. (1998) focused on the antimicrobial activity of some essential oils. Sawamura et al. (2004) evaluated the composition of the oil

TABLE 1.19
Percentage Composition of the Volatile Fraction of Lemon Oil (1979–1999)

	Cold-Pressed Oils		Laboratory Oils Cold-Pressed and Solvent Extracted	
	wt%	Relative % of Peak Areas	Relative % of Peak Areas	wt%
		Hydrocarbons		
Monoterpene				
Camphene	0.03–0.07	tr–0.13	0.04–0.08	0–0.07
δ-3-Carene	tr	tr–0.04	tr	–
p-Cymene	0.02–0.14	0.01–1.71	tr–0.03	0.06–0.08
Limonene	59.58–76.17	59.57–71.82	61.13–79.15	70.08–76.50
Myrcene	1.28–1.75	1.05–2.23	1.34–1.63	1.14–1.58
β-Ocimene*	–	0.04–0.08	0.21–0.43	–
(*E*)-β-Ocimene	0.09–0.11	0.07–0.60	tr–0.15	0.11–0.19
(*Z*)-β-Ocimene	0.05	0.03–0.15	0.03–0.09	–
α-Phellandrene	0.04	tr–0.13	0.09–0.18	0–0.02
β-Phellandrene	–	0–0.48	tr	–
α-Pinene	1.47–2.13	0.88–4.40	1.22–3.03	0.39–1.26
β-Pinene	5.96–16.50	8.57–17.79	6.85–18.57	4.10–8.64
Sabinene	1.12–2.59	1.13–2.79	2.32	0.85–1.51
α-Terpinene	0.16–0.32	0–0.25	tr–0.25	0.05–0.14
γ-Terpinene	7.91–9.64	2.88–11.38	6.64–12.53	6.00–9.51
Terpinolene	0.34–0.39	tr–0.50	0.3–0.48	0.38–0.59
α-Thujene	0.35–0.44	0.27–0.54	0.26–0.49	0.07–0.28
Sesquiterpene				
α-Bergamotene*	–	0.33–0.42	0.21	–
cis-α-Bergamotene	0.03	tr–0.04	–	–
trans-α-Bergamotene	0.35–0.39	0.21–0.78	0.30–0.45	0.11–1.76
Bicyclogermacrene	+	0.05–0.07	0.16	–
(*Z*)-α-Bisabolene	+	0.04	–	–
β-Bisabolene	0.52–0.59	0.08–1.00	0.31–0.75	0.20–2.50
(*Z*)-γ-Bisabolene	–	tr	–	–
β-Caryophyllene	0.19–0.24	0.11–0.78	0.15–0.51	0.12–0.93
δ-Elemene	–	0.01–0.03	–	–
γ-Elemene	–	0.01–0.03	tr	–
(*E*)-β-Farnesene	0.03[t]	tr–0.05	0.02	–
(*Z*)-β-Farnesene	–	0.02–0.07	–	–
Germacrene B	–	0.02–0.12	0.02–0.09	–
α-Humulene	0.02[t]	0.01–0.05	tr–0.07	0–0.34
β-Santalene	0.01[t]	tr–0.02	0.05–0.06	–
Valencene	–	tr–0.16	tr–0.03	–
		Aldehydes		
Aliphatic				
Decanal	0.04–0.06	tr–0.10	tr–0.06	0.01–0.10
(*E*)-2-Decenal	–	+	0.01	–
Dodecanal	0.01–0.03	tr–0.06	0.02	–

TABLE 1.19 (continued)
Percentage Composition of the Volatile Fraction of Lemon Oil (1979–1999)

	Cold-Pressed Oils		Laboratory Oils Cold-Pressed and Solvent Extracted	
	wt%	Relative % of Peak Areas	Relative % of Peak Areas	wt%
Heptanal	tr	tr–0.01	–	–
Nonanal	0.06–0.23	0.04–0.19	0.08–0.21	0.02–0.32
Octanal	0.07–0.13	tr–0.42	tr–0.13	0.02–0.11
Undecanal	0.03	0–0.08	tr–0.03	0.02–0.04
Monoterpene				
Citronellal	0.07–0.13	0.03–0.17	tr–0.11	0.06–0.16
Geranial	0.95–2.05	0.60–2.66	0.50–3.25	0.81–3.56
Neral	0.60–1.26	0.34–1.33	0.23–1.97	0.57–1.98
Perilla aldehyde	0.03	tr–0.05	0.04	0.02–0.03
		Ketones		
Aliphatic				
6-Methyl-5-hepten-2-one	tr	0–0.36	tr	–
Monoterpene				
Camphor	0.01	tr–0.01	tr	–
Carvone	0.01	–	–	–
Piperitone	tr	tr–0.01	–	–
Sesquiterpene				
Nootkatone	–	tr–0.03	–	–
		Alcohols		
Aliphatic				
Decanol	–	–	0–0.01	–
Nonanol	–	tr–0.21	–	–
Octanol	0.01–0.05	tr–0.09	tr	0.06–0.15
Monoterpene				
Borneol	0.01	tr–0.02	tr	0.01
Carvacrol	–	+	0.01	–
trans-Carveol	–	+	+	–
Citronellol	0.03–0.06	tr–0.18	tr–0.07	–
p-Cymen-8-ol	–	+	0.01	–
Geraniol	0.02–0.04	tr–0.12	0–0.09[b]	0.02–0.10
Linalol	0.13–0.18	0.05–0.46	0.04–0.2	0.21–0.63
Nerol	0.03–0.06	tr–0.16	tr–0.09	0.04–0.09
Sabinene hydrate*	–	–	0.12–0.66	–
cis-Sabinene hydrate	0.13–0.18	0.01–0.07	0.07–0.10	–
trans-Sabinene hydrate	0.01–0.05	tr–0.07	tr–0.08	–
Terpinen-4-ol	0.05–0.14	0.01–0.10	0–0.04	0.04–0.06
α-terpineol	0.15–0.23	0.05–0.84	0.08–0.30	0.21–0.63
trans-p-Mentha-2,8-dien-1-ol	–	+	0.01	–

continued

TABLE 1.19 (continued)
Percentage Composition of the Volatile Fraction of Lemon Oil (1979–1999)

	Cold-Pressed Oils		Laboratory Oils Cold-Pressed and Solvent Extracted	
	wt%	Relative % of Peak Areas	Relative % of Peak Areas	wt%
Thymol	–	+	0.01	–
Sesquiterpene				
α-Bisabolol	0.04	0.01–0.03	0.01–0.03	0.04–0.05
β-Bisabolol	–	0.01	+	–
Campherenol	0.03	0.01–0.03	0.02–0.03	–
Nerolidol*	tr	–	0.01	–
Norbornanol[a]	0.02–0.03	0.01–0.04	0.01–0.02	–
Esters				
Aliphatic				
Heptyl acetate	–	tr	tr	–
Decyl acetate	0.01	tr–0.01	–	–
Nonyl acetate	tr–0.02	tr–0.02	tr–0.05	–
Octyl acetate	tr–0.01	tr–0.03	tr–0.09	–
Monoterpene				
Bornyl acetate	–	tr–0.01	tr	–
Citronellyl acetate	0.02–0.03	tr–0.10	0.01–0.06	0–0.40
Geranyl acetate	0.20–0.65	0.06–0.86	0–0.87	0.31–3.18
Geranyl propanate	0.01	+	0.01	–
Linalyl acetate	–	0–0.05	0.01	–
Methyl geranate	tr–0.01	tr–0.01	–	–
Neryl acetate	0.46–0.60	0.07–0.88	0.24–1.24	0.57–2.54
Neryl propanate	0.01	+	–	–
Ethers and oxides				
Monoterpene				
1,8-Cineole	0.04–0.05	+	–	–
cis-Limonene oxide	tr–0.01	tr–0.02	0.07	–
trans-Limonene oxide	tr–0.01	tr–0.02	0.04	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; + identified but not quantitatively determined; [a] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol; [b] the high values of geraniol (0.28%–0.70%) reported by Germanà et al. (1987) were not included.

Appendix to Table 1.19

- The results reported in Table 1.19 and in this appendix, for the different categories of lemon oils are taken from the following original papers:
  - Cold-pressed industrial oils (relative % of peak areas): Sanchez et al. (1980); Cappello et al. (1981); Formàček and Kubeczka (1982); Koketsu et al. (1983); Analytical Methods Committee (1984); Licandro et al. (1984, 1987); Lancas et al. (1988); Mazza (1987b); Boelens and Jimenez (1989a); Sawamura et al. (1990); Boelens (1991); Dellacassa et al. (1991, 1995); Dugo et al. (1983, 1984b, 1999); Dugo (1986, 1994); Cotroneo et al. (1986a,b, 1987, 1988); Trozzi et al. (1993); Verzera et al. (1996a); Sawada and Yamada (1997, 1998); Nishida and Acree (1984). Were also included the qualitative studies carried out by Micali et al. (1990); Lanuzza et al. (1991); Mondello et al. (1995b); Chamblee et al. (1997).

TABLE 1.19 (continued)
Percentage Composition of the Volatile Fraction of Lemon Oil (1979–1999)

  - Industrial cold-pressed oils (wt%): Staroscik and Wilson (1982a,b); Chamblee et al. (1991).
  - Cold extracted laboratory oils (relative % of peak areas): Germanà et al. (1987); Dellacassa et al. (1994); Njoroge et al. (1994b); Gazea et al. (1996); Sawamura et al. (1999a).
  - Cold extracted laboratory oils (wt%): Usai et al. (1996).
- Coelutions indicated by one or more authors in chromatographic separations of lemon oils:
  - *p*-Cymene + limonene; α-phellandrene + octanal; limonene + β-phellandrene + 1,8-cineole; limonene + 1,8-cineole + (*Z*)-β-ocimene; β-pinene + sabinene; terpinolene + octanal; (*Z*)-β-farnenese + β-santalene; β-bisabolene + geranial; citronellal + octyl acetate; octanol + *trans*-sabinene hydrate; linalol + *cis*-sabinene hydrate; citronellol + nerol; dodecanal + decyl acetate; *trans*-α-bergamotene + citronelly propanate.
  - Ranges reported in Table 1.19 for some components in cold-pressed oils, coeluted in chromatographic separation reported by few authors (β-phellandrene, sabinene, α-phellandrene, (*E*)-β-farnesene, β-santalene, octyl acetate, citronellol, nerol) are determined considering the results where coelutions did not occur.
- In addition to those listed in Table 1.19, in lemon oil were found the components listed below:
  - Cold-pressed industrial oils: tricyclene (tr–0.01%), (*E*)-α-bisabolene (0.02%), germacrene D (tr–0.02%), γ-muurolene (tr–0.02%), β-sesquiphellandrene (tr–0.04%), tetradecanal (tr–0.02%), exo *cis*-4,7-dimethyl bicyclo (3,2,1)-oct-3-en-6-one (0.01%), germacrene D-4-ol (0.02%), selin-11-en-4-α-ol (0.01%), undecyl acetate (0.01%), terpinyl acetate* (tr–0.45%), and trace amounts of allo-ocimene, γ-(*Z*)-bisabolene, tridecanal, citronellyl propanate and also identified but not quantitatively determined linear chained hydrocarbons C_{21}-$C_{33,}$ and correspondent "iso" isomers C_{23}–C_{31}, *p*-cymenene, β-acoradiene, (*E*)-γ-bisabolene, γ-curcumene, α-selinene, hexadecanal, hexanal, (*E*)-2-nonenal, pentadecanal, (*E*)-2-tetradecenal; myrtenal, isopiperitone, heptanol, *cis*-carveol, isopulegol, γ-isogeraniol, *cis*- and *trans*-isopiperitenol, *cis*-*p*-mentha-2,8-dien-1-ol; *cis*- and *trans*-*p*-mentha-1(7),8-dien-2-ol; *p*-mentha-1,8(10)-dien-9-ol, perilla alcohol, δ-terpineol, *cis*- and *trans*-1-acetoxy-3,7-dimethyl-2,7-octadien-6-ol, *p*-mentha-1,8(10)-dien-9-yl acetate; peryllyl acetate, ethyl benzoate, 1,4-cineole, acetic acid, octanoic acid, methyl jasmonate, methyl epijasmonate.
  - Cold-pressed and solvent-extracted laboratory oils: aromadendrene (0.01%), α-copaene (0%–0.08%), cumin aldehyde (0.02%), menthone (0.01%), pulegone (0%–0.24%), *p*-mentha-4(8)-en-9-ol (0%–0.05%), β-eudesmol (0.01%), spathulenol (0.01%), limonene diol[t] (0.01%), hept-1-en-1-yl acetate[t] (0.01%), geranyl formate (0.01%), terpinyl propanate* (0.01%), *cis*-caryophyllene oxide[t] (0.01%), *trans*-limonene-8,9-oxide (0.01%), and trace amounts of tetradecane, allo-aromadendrene, α-cubebene, sesquiphellandrene[t,]*, tetradecanal, cinnamyl aldehyde, nonan-2-one, *trans*-carvone, cinnamyl alcohol, *trans*-piperitol, elemol, eugenol, (*E*,*E*)-farnesol, (*Z*,*E*)-farnesol, dodecyl acetate, (*E*,*E*)-farnesyl acetate, *trans*-caryophyllene oxide[t], myrcene epoxide, *cis*-limonene-8,9-oxide, decanoic acid, undecanoic acid; also identified but not quantitatively determined: pentadecane, δ-cadinene, β-cubebene, δ-muurolene, longifolene, tetradecenal[t]*, *cis*-carvone, undecanol, α-cadinol, cedryl acetate, tert-butyl benzene[t].
- Some additional papers, published in the same period of those summarized in the table, are relative to oils distilled in laboratory from lemon fruit of different geographical origin: Spain (Melendreras et al., 1985, 1986, 1988; Laencina et al., 1986); Italy (Caccioni et al., 1998); Russia (Kekelidze et al., 1982); India (Kumar et al., 1992); China (Wen et al., 1989); Japan (Yang R-H. et al., 1992); Benin (Ayedoun et al., 1996). The composition of these oils were was strongly influenced by the extraction technology used, determining drastic changes on the natural composition of the oil. All these oils in fact present high values of monoterpene alcohols, and many present high values of *p*-cymene and of 1,8-cineole. Relatively to an Egyptian oil (Haggag et al., 1998) was reported a completely anomalous composition: it was determined 10% of limonene and high values of α-pinene (27%), β-pinene (18%), thymol (3%), eugenol (9%) and very high amounts (16%) of monoterpene alcohols. More information on the composition of these oils is reported in the former review by Dugo et al. (2002).
- Were not included in this table some results on commercial oils published by Prager and Miskiewicz (1982); Sugiyama and Saito (1988); Haubruge et al. (1989); Wen et al. (1989); Yamauchi and Saito (1990); Barth et al. (1994) since they presented some important anomalies on the composition more or less indicating contamination or adulteration. Information on the composition of these oils is reported in the former review by Dugo et al. (2002). Was also not included the value of α-thujene (0.01%), probably due to not optimal chromatographic separation, reported by Sanchez et al. (1980) and Cappello et al. (1981).

continued

TABLE 1.19 (continued)
Percentage Composition of the Volatile Fraction of Lemon Oil (1979–1999)

- The studies carried out in these years on the industrial cold-pressed lemon oils revealed that the composition of these oils is strongly influenced by the following variables:
  - Geographical origin: Staroscik and Wilson (1982b) underline the difference on the composition of the oils obtained from the coast of California and those obtained from the desert area of Arizona. Particularly interesting is the seasonal variation of neral and geranial in the two types of oils. These variations are reported in Figure 1.13. Licandro et al. (1987) studied the differences of the composition in Sicilian oils produced in the same period but in different Sicilian areas. Sawada e Yamada (1997, 1988) reported that Sicilia lemon oils extracted by FMC presented higher amounts of monoterpene aldehydes and esters and of α-terpineol than those produced by the same technology in California.
  - Production season: Dugo et al. (1983, 1984b), Dugo (1986, 1994), Licandro et al. (1984), Cotroneo et al. (1986a,b, 1988), Trozzi et al. (1993), Verzera et al. (1996a) deeply studied the composition of lemon essential oils produced by the common industrial processing technology, during different productive seasons, that in the case of lemon last the whole year. The composition resulted reproducible for all the years of production, with the exception of small differences, due to climate and weather factors. In particular, the oils produced in the period October-March presented only slight variations in composition, while some components or class of component reached their maximum or their minimum value in the summer period (June–August), to go back to their initial values at the end of the season. Such behavior allows to differentiate summer lemon oils, mostly extracted from green lemons (verdelli), from the winter lemon oils. Limonene and monoterpene aldehydes presented their minimum values in the summer oils while, in the same period, β-pinene, aliphatic aldehydes and esters were at their maximum values. In Figure 1.14 are plotted the variations of some classes of components and, of some single components, versus the period of production for two different years. The correlation of the seasonal variation with the different composition parameters can provide, for the evaluation of the genuineness and the quality of lemon oils, a more reliable parameter than the simple ranges of variation of the oils composition (Dugo et al., 1992).
  - Extraction technology: The composition of lemon oil is slightly dependent on the extraction technology. Sawada and Yamada (1998) and Dugo (1994), noticed that the difference between oils extracted with different technologies mostly pertain the alcohols content, that appears higher in oils extracted by Pelatrice and FMC than in those extracted by Sfumatrice and Torchi. This is due to the higher water/oil ratio used by the Sfumatrice and the Torchi extractors. Cotroneo et al. (1987), observed a similar behavior comparing the oil manually extracted with the sponge, without water use, and those produced by mechanical processes. In the oil extracted by the sponge method, the content of aldehydes and alcohol were higher than in mechanically extracted oils, for the inevitable loss of water soluble components even if optimized conditions were used. The only exception was terpinen-4-ol present in both oils at almost identical values. This could be explained by hydration phenomena that caused the formation of terpinen-4-ol compensating the amount lost during the process.

after twelve months of storage in different conditions: two samples stored in an incubator set at 25°C with the cap being opened for three minutes everyday (A) and for three minutes every month (B); two samples stored in an incubator set at 5°C with the cap being opened for three minutes everyday (C) and for three minutes every month (D). Some compositional changes after twelve months under the four storage conditions are reported below.

	Start	A	B	C	D
p-Cymene	1.6	4.8	4.1	5.5	3.3
Limonene	68.5	20.1	51.7	47.4	66.1
β-Pinene	12.2	4.0	9.0	7.2	12.3
γ-Terpinene	7.2	tr	2.7	1.0	5.2
Geranial	0.9	tr	0.5	0.5	0.7
cis-Carveol	tr	0.9	0.1	0.1	tr
cis-Limonene oxide	tr	0.7	0.2	0.2	0.1

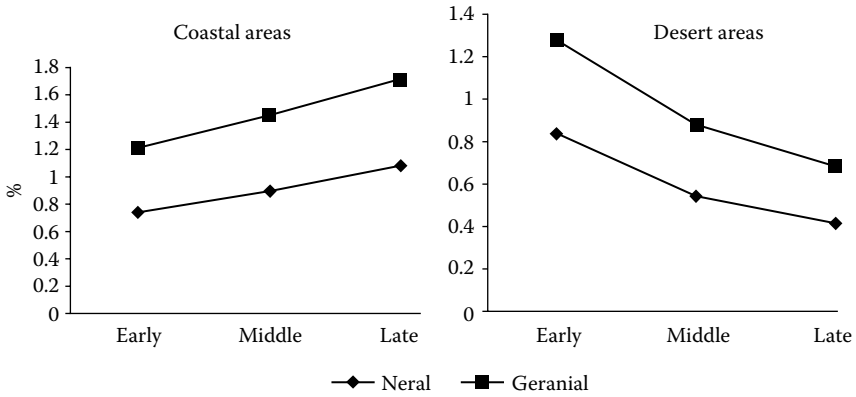

FIGURE 1.13 Variation in average content of neral and geranial for lemon oils produced in coastal area (California, USA) and in desertic areas (Arizona, USA), during the productive season. (From the results reported by Staroscik, J.A., and Wilson, A.A., *J. Agric. Food Chem*. 30, 835–837, 1982b.)

Variations of *trans*-carveol and *trans*-limonene oxide were similar to those of the correspondent *cis* isomers. The high value in the "start" of *p*-cymene (1.6%) indicates that the sample was probably kept under inadequate storage conditions prior to the beginning of this study. It should also be highlighted the high value of (*E*)-β-farnesene (1.0%) reported by the same authors. This component is usually present in lemon oil at trace levels, therefore the anomalous value reported by Sawamura et al. could be due to the overlapping with a different component present at higher levels.

In addition to those summarized in the table, little information is available in literature on lemon essential oils industrially extracted and of secure origin or commercial oils. Oberhofer et al. (1999) analyzed two commercial samples one bought in Austria and one bought in Italy both evidently adulterated or contaminated due to the excessive content of linalyl acetate respectively 1.07% and 0.65%. Wright (1999) limited the data reported to the content of principal components of lemon oil: limonene (63.3%); β-pinene (12.0%); γ-terpinene (9.0%); nonanal (0.1%); citronellal (0.2%); geranial (1.5%); neral (1.0%); linalol (0.2%); geranyl acetate (0.4%); and neryl acetate (0.5%). Veriotti and Sacks (2001), using fast gas chromatography and TOF/MS identified some components in commercial lemon oil. The results obtained by these authors, mainly related to the identification of some components, unusual in lemon oil, do not contribute to the knowledge of the composition of lemon essential oil. Steuer et al. (2001) and Schulz et al. (2002) in two papers aimed at the classification and quantitative analysis of some citrus essential oils by spectroscopic techniques reported the following average composition determined by GC in 14 samples of lemon oil: limonene (68.6%); myrcene (1.5%); α-pinene (1.9%); β-pinene (12.1%); sabinene (1.7%); α-terpinene (8.7%); and total aldehydes (2.6%). Kubeczka and Formàček (2002), in the second edition of their volume on citrus essential oil analysis, reported the same data published in 1982; these were discussed in our previous review (Dugo et al., 2002). Mondello et al. (2005) proposed a comprehensive two dimensional GC method for the study of citrus essential oils using as reference matrix lemon essential oil. The results obtained confirmed the enormous potentiality of the method and the superiority of this approach compared to conventional MD techniques, highlighting the advantages, despite the instrumental complexity, for qualitative and quantitative analysis of the oils, and revealing adulterations. Wilson et al. (2002) proposed a method to determine by near infrared spectroscopy the citral content in oils where these components are present in high amounts (lemongrass) and in oils were these components are scantly present. Gironi and Maschietti (2005), in a paper focused on the fractionation of lemon oil by supercritical CO_2, reported the following content of the principal components in the whole oil: limonene (61.1%); α-pinene (2.2%); β-pinene (14.0%); γ-terpinene (11.7%); β-bisabolene (0.8%); neral + geranial (2.5%); and linalol (0.2%).

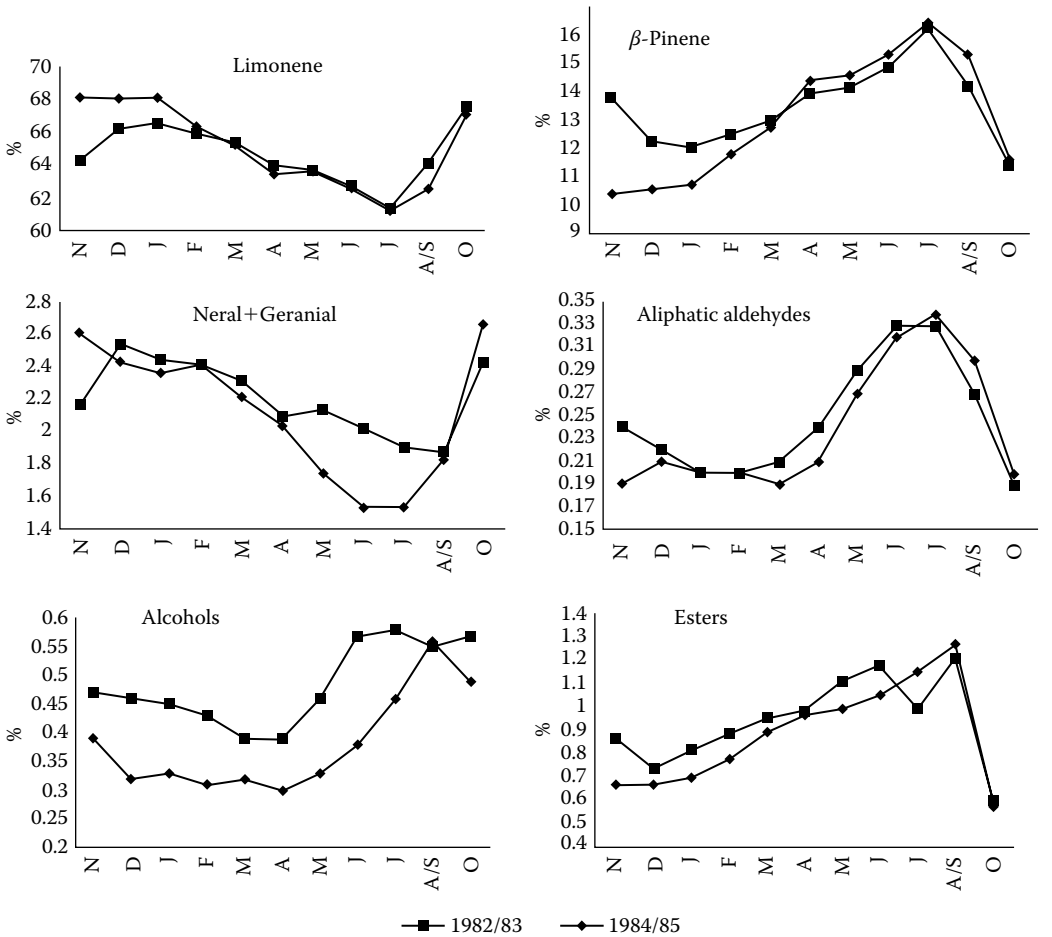

FIGURE 1.14 Variation in average content of limonene, β-pinene, neral + geranial, aliphatic aldehydes, alcohols, and esters for lemon oils produced in Italy during two productive seasons. (From Dugo, G., et al., *Essenz. Deriv. Agrum.* 53, 173–217, 1983; Dugo, G., et al., Indagine sulla composizione media dell'essenza di limone siciliana ottenuta industrialmente durante l'intera stagione produttiva 1982/83. *1° Conferenza Nazionale sugli Aromatizzanti,* Salsomaggiore Terme, Parma, Italy, April 2–3, 1984b; Dugo, G., *Perfums Cosmetiques aromes* 68, 95–105, 1986.)

1.8.2.2 Laboratory Oils

In the period under consideration numerous studies were carried out on lemon oils extracted in laboratory from different cultivars and of different geographic origins. The extraction systems were cold extraction by manual pressure on the fruit skin to break the utricles or by the use of laboratory automatic machines; solvent extraction; distillation process carried out on fruit skin or on the whole fruits after homogenization; microwave accelerated extraction (MAD). Most of these were carried out by cold-extraction techniques, which usually provide the most natural composition of the oil, avoiding artifacts. These results are useful for improving knowledge of the composition of lemon oil. The composition of these oils is reported in the first part of Table 1.21. In the table the oils are divided, when possible, based on the geographic origin and cultivar. Most of the results are relative to Italian oils extracted from different cvs. of Femminello and Monachello

TABLE 1.20
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Lemon Oil (1998–2009)

	1	2a	2b	2c	2d	3,4	5a	5b	6	7
					Hydrocarbons					
Monoterpene										
Camphene	–	0.07	0.07	0.07	0.06	0.06	0.06	0.05	0.1	tr
δ-3-Carene	–	tr	tr	tr	tr	–	0.01	tr	–	–
p-Cymene	0.7	0.12	0.16	0.16	0.12	0.35	0.08	0.07	1.6	0.6
Limonene	68.8	61.84	61.13	60.18	63.31	66.05	65.59	69.64	68.5	69.9
Myrcene	1.3	1.62	1.57	1.52	1.60	1.47	1.70	1.67	1.4	1.3
(*E*)-β-Ocimene	0.1	0.13	0.15	0.13	0.13	0.12	0.13	0.12	–	–
(*Z*)-β-ocimene	tr	0.07	0.08	0.07	0.07	0.08	0.07	0.06	0.1	–
α-Phellandrene	–	0.04	0.04	0.08	0.04	0.12[d]	0.05	0.04	tr	–
α-Pinene	1.8	2.21	2.15	2.23	2.11	1.89	2.07	1.86	1.8	1.9
β-Pinene	12.8	16.56[a]	16.74[a]	18.15[a]	16.04[a]	12.56	12.97[a]	11.18[a]	12.2	11.2
Sabinene	1.2	16.56[a]	16.74[a]	18.15[a]	16.04[a]	1.97	12.97[a]	11.18[a]	2.0	2.0
α-Terpinene	0.1	0.19	0.17	0.16	0.17	0.16	0.21	0.19	0.1	0.1
γ-Terpinene	8.7	10.26	10.42	10.10	10.25	9.16	9.88	9.01	7.2	8.2
Terpinolene	0.2	0.40	0.39	0.36	0.39	0.35	0.41	0.38	0.3	0.3
α-Thujene	0.3	0.48	0.47	0.47	0.46	0.42	0.47	0.44	–	0.4
Tricyclene	–	0.01	0.01	0.01	0.01	0.01	0.01	0.01	–	–
Sesquiterpene										
trans-α-Bergamotene	0.3	0.36	0.38	0.39	0.38	0.38	0.40	0.37	–	–
Bicyclogermacrene	–	0.07	0.06	0.06	0.07	0.04	0.06	0.05	–	–
β-Bisabolene	0.5	0.54	0.56	0.58	0.56	0.57	0.57	0.53	–	0.5
β-Caryophyllene	0.2	0.23	0.23	0.24	0.24	0.21	0.22	0.23	0.1	0.1
(*E*,*E*)-α-Farnesene	–	–	–	–	–	0.06[e]	–	–	tr	–
(*E*)-β-Farnesene	–	0.03	0.03	0.03	0.03	–	–	–	1.0	–
(*Z*)-β-Farnesene	–	–	–	–	–	–	0.04	0.03	tr	–
Germacrene D	–	tr	0.01	0.01	0.01	0.02	0.01	0.01	–	–
α-Humulene	–	0.02	0.02	0.02	0.02	0.05	0.02	0.03	–	–
γ-Muurolene	–	0.01	0.01	0.01	0.04	–	0.01	0.01	–	–
β-Santalene	–	0.01	0.01	0.01	0.01	0.01	0.02	0.01	–	–
Valencene	–	0.02	0.02	0.02	0.03	0.05	0.02	0.02	–	–
					Aldehydes					
Aliphatic										
Decanal	–	0.05	0.05	0.06	0.05	0.05	0.05	0.04	tr	tr
Dodecanal	–	0.01	0.01	0.01	0.01	tr	0.01	0.01	tr	–
Nonanal	–	0.15	0.16	0.16	0.14	0.12	0.09	0.07	0.1	0.1
Octanal	–	0.11	0.11	0.08	0.08	0.12[d]	0.07	0.06	0.2	tr
Tedradecanal	–	tr	0.01	tr	0.01	0.01	0.01	0.01	–	–
Undecanal	–	0.03	0.03	0.04	0.03	0.03	0.02	0.02	–	–
Monoterpene										
Citronellal	–	0.09	0.10	0.10	0.08	0.10	0.13	0.14	tr	tr
Geranial	1.3	1.64[b]	1.74[b]	1.44[b]	1.22[b]	1.29	1.74	1.43	0.9	0.9

continued

TABLE 1.20 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Lemon Oil (1998–2009)

	1	2a	2b	2c	2d	3,4	5a	5b	6	7
Neral	–	0.95	1.00	0.86	0.68	0.77	1.03	0.84	0.5	0.5
Perilla aldehyde	–	b	b	b	b	0.02	tr	tr	tr	–
Ketones										
Aliphatic										
6-Methyl-5-hepten-2-one	–	0.01	0.01	0.01	0.01	–	0.01	tr	tr	–
Monoterpene										
Camphor	–	tr	tr	tr	tr	0.01	0.01	0.01	tr	–
Piperitone	–	tr	tr	tr	tr	–	tr	tr	–	–
Sesquiterpene										
Nootkatone	–	tr	tr	tr	tr	0.01	tr	tr	–	–
Alcohols										
Aliphatic										
Octanol	–	tr	tr	tr	tr	–	tr	tr	–	–
Monoterpene										
Borneol	–	0.01	0.01	0.01	tr	0.01	0.01	0.01	–	–
Citronellol	–	–	–	–	–	0.02	0.03[f]	0.03[f]	tr	–
p-Cymen-8-ol	–	–	–	–	–	0.01	–	–	tr	–
Geraniol	–	0.02	0.04	0.02	0.02	0.03	0.04	0.03	tr	–
Linalol	0.1	0.12	0.12	0.13	0.07	0.12	0.11	0.11	0.1	0.1
Nerol	–	0.03	0.04	0.07	0.02	0.04	0.03[f]	0.03[f]	tr	–
cis-Sabinene hydrate	–	0.03	0.06	0.05	0.01	0.03	0.03	0.03	–	–
trans-Sabinene hydrate	–	0.02	0.04	0.04	0.01	–	0.02	0.02	–	–
Terpinen-4-ol	–	0.07	0.04	0.05	0.04	0.05	0.03	0.03	0.1	tr
α-Terpineol	0.1	0.18	0.21	0.23	0.10	0.17	0.14	0.13	0.2	0.1
Sesquiterpene										
α-Bisabolol	–	0.02	0.02	0.02	0.02	0.03	0.02	0.02	tr	–
Campherenol	–	0.02	0.02	0.02	0.02	0.02	0.02	0.02	–	–
Norbornanol[c]	–	0.02	0.02	0.02	0.02	0.02	0.02	0.01	–	–
Esters										
Aliphatic										
Decyl acetate	–	tr	0.01	0.01	tr	0.02	0.03	0.03	–	–
Nonyl acetate	–	tr	tr	0.01	tr	0.01	tr	tr	–	–
Octyl acetate	–	tr	tr	tr	tr	tr	tr	tr	tr	–
Monoterpene										
Bornyl acetate	–	tr	tr	tr	tr	–	0.01	tr	–	–
Citronellyl acetate	–	0.03	0.03	0.03	0.03	0.03	0.03	0.03	tr	–
Geranyl acetate	0.2	0.39	0.45	0.50	0.41	0.35	0.39	0.33	0.2	0.3
Methyl geranate	–	tr	tr	tr	tr	0.01	tr	tr	–	–
Neryl acetate	–	0.40	0.42	0.49	0.43	0.44	0.44	0.41	0.1	0.3

TABLE 1.20 (continued)
Percentage Composition of the Volatile Fraction of Industrial Cold-Pressed Lemon Oil (1998–2009)

	1	2a	2b	2c	2d	3,4	5a	5b	6	7
					Ethers and oxides					
Monoterpene										
cis-Limonene oxide	–	tr	tr	tr	tr	tr	tr	tr	tr	tr
trans-Limonene oxide	–	tr	tr	0.01	tr	tr	tr	tr	0.1	tr

Notes: tr, traces; *, correct isomer not characterized; [a] β-pinene + sabinene; [b] geranial + perilla aldehyde; [c] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol; [d] α-phellandrene + octanal; [e] (*E*,*E*)-*a*-farnesene + unknown sesquiterpene; [f] citronellol + nerol.

Appendix to Table 1.20

1. Baratta et al. (1998). One commercial sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-1; GC/FID was also performed using a capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax; relative percentage of peak areas.
2. Verzera et al. (1999). Sicily, Italy; average values of (a) 100 samples extracted by Sfumatrice; (b) 59 samples extracted by Pelatrice; (c) 124 samples extracted by FMC on-line; (d) 81 samples extracted by Torchi processing the peels which had been previously cold-pressed by Sfumatrice machine; the oils were produced during an entire productive season; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary columns coated with DB-5 (30 m × 0.25 mm × 0.25 μm) or Megawax (30 m × 0.25 mm × 0.40–0.45 μm); Adams MS library; relative percentage of peak areas. Verzera et al. also found (*E*)-α-bisabolene (0.01%), (*Z*)-α-bisabolene (0.04%).

3 and 4. Mondello et al. (2003, 2004b). Sicily, Italy; one sample; conventional GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX-5MS; Fast GC/FID on capillary column (10 m × 0.1 mm × 0.1 μm) coated with RTX-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. In the table are reported the results obtained with conventional method. The results obtained with fast method were very similar. More information is reported in Chapter 11. Mondello et al. also found (*Z*)-γ-bisabolene (0.02%), sesquithujene (0.02%), linalyl isobutyrate (0.01%).

5. Verzera et al. (2004). Syracuse, Sicily, Italy; (a) ten samples extracted from fruit grown under biological techniques, (b) ten samples extracted from fruit grown with traditional methods by FMC-in line extractor; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with Mega-5MS or Megawax; Adams MS library; relative percentage of peak areas. Verzera et al. also found γ-elemene (0.02%).
6. Sawamura et al. (2004). One commercial sample for aromatherapy; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-Wax; GC/MS analysis was also performed on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-1; wt%, using methyl myristate as internal standard. LRI on DB-Wax and DB-1 are reported; NIST MS library. Sawamura et al. also found β-phellandrene (0.3%), α-bergamotene* (0.4%), 1,2-cyclohexandiol (0.2%), *trans*-pinocarveol (0.1%), 1,8-cineole (0.1%), and trace amounts of undecane, α-santalene, carvone, *p*-mentha-8-one, *cis*- and *trans*-carveol, *p*-mentha-1,8-dien-9-ol, *trans*-*p*-mentha-2,8-dien-9-ol, *cis*- and *trans*-β-terpineol, spathulenol, geranyl propanate, limonene diepoxide, (*E*)-α-bisabolene epoxide.
7. Viuda-Martos et al. (2009). Spain; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5 MS; LRI on HP-5 MS are reported; relative percentage of peak areas. Viuda-Martos et al. also found β-ocimene* (0.1%), α-bergamotene* (0.4%).

lemons. The results are in good agreement with those relative to industrially extracted oils; only a few present anomalies in their composition, such as high values of *p*-cymene (0.43%–2.54%) reported by Geraci et al. (1998) in oils extracted from Femminello lemons, (0.9%) reported by Lota et al. (2002) in oils extracted from Eureka lemons; (1.9%) reported by Kirbaslar et al. (2006) in an oil extracted from lemons cultivated in Turkey; the high values of α-phellandrene (1.62%) in the oil extracted from Eureka lemons; and of bicyclogermacrene (1.84% and 1.56%) in oils

TABLE 1.21
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed												
	Italy												
	Femminello						Monachello				Femminello + Monachello	Other Cultivars	
	1	2a	3a	4a	5a	10	2b	3b	4b	5b	5c	2c	3c
	Hydrocarbons												
Monoterpene													
Camphene	–	0.06	0.06	0.03–0.04	0.03–0.08	0.03–0.06	0.05	0.06	0.04	0.03–0.08	0.06–0.07	0.05	0.04
δ-3-Carene	–	–	tr	tr–0.01	–	0.01	–	tr	tr	0.01	–	–	tr
p-Cymene	0.43–2.54	0.20	–	0.02–0.08	0.06–0.35	–	0.18	–	0.04	0.05–0.67	0.06–0.14	0.19	–
Limonene	60.07–72.23	56.60	61.57	65.96–75.99	60.09–62.91	48.75–67.95	59.34	61.16	67.01	60.40–65.27	59.04–62.13	64.34	62.34
Myrcene	1.08–1.53	1.30	1.46	1.45–1.75	1.03–1.80	1.12–2.01	1.38	1.50	1.52	1.20–1.48	1.40–1.58	1.10	1.42
(*E*)-β-Ocimene	0.10–0.21	–	0.22	0.07–0.12	0.12–0.35	0.09–0.49	–	0.14	0.13	0.16–0.33	0.13–0.14	–	0.14
(*Z*)-β-Ocimene	0.09–0.14	0.10	0.12	0.03–0.06	0.22–0.39	0.05–0.28	0.08	0.08	0.07	0.20–0.40	0.19–0.22	0.06	0.06
α-Phellandrene	0–0.14	0.04	0.04	0.04–0.12	0.05–0.37	0.04–0.06	0.04	0.04	0.09	0.03–0.05	0.04–0.06	0.26	0.04
β-Phellandrene	–	0.55	–	–	–	–	0.39	–	–	–	–	tr	–
α-Pinene	0.94–1.79	2.29	1.92	1.12–1.50	1.08–2.35	0.71–1.87	2.04	2.03	1.51	0.89–2.40	1.91–2.20	2.11	1.27
β-Pinene	6.84–15.41	17.10	14.28	6.66–8.52[c]	12.87–15.55	5.38–12.57	14.46	13.34	9.77[c]	9.38–15.55	14.62–17.21	12.64	9.98
Sabinene	1.11–2.03	2.62	2.28	6.66–8.52[c]	1.68–2.65	1.06–2.11	2.18	2.10	9.77[c]	1.49–2.98	1.98–2.31	1.08	1.63
α-Terpinene	0.10–0.20	0.20	0.22	0.16–0.19	0.11–0.19	0.14–0.22	0.21	0.24	0.23	0.12–0.20	0.14–0.21	0.01	0.21
γ-Terpinene	7.67–10.96	9.08	10.43	7.42–9.82	7.50–10.46	6.66–9.66	10.24	10.99	11.11	9.42–10.58	9.73–10.59	9.78	12.05
Terpinolene	0.34–0.50	0.33	0.44	0.32–0.46	0.30–0.39	0.32–0.46	0.40	0.45	0.48	0.34–0.41	0.34–0.40	0.47	0.56
α-Thujene	0.20–0.35	–	0.41	0.26–0.33	0.12–0.37	0.20–0.44	–	0.48	0.39	0.19–0.50	0.41–0.49	–	0.30
Tricyclene	–	–	0.01	tr–0.03	–	–	–	0.01	tr	–	–	–	tr
Sesquiterpene													
trans-α-Bergamotene	0.37–0.69	0.39	0.36	0.31–0.62	0.55–0.87	0.31–0.79	0.46	0.31	0.42	0.32–0.84	0.26–0.38	0.37	0.50

Bicyclogermacrene	–	tr	0.16	0.04–0.11	–	0.05–0.09	tr	0.05	0.02	–	–	1.84	0.14
(*E*)-α-Bisabolene	–	–	–	0.01–0.03	–	–	–	–	0.02	–	–	–	–
(*Z*)-α-Bisabolene	–	–	–	0.03–0.07	–	tr–0.05	–	–	0.05	–	–	–	–
β-Bisabolene	0.57–1.00	–	0.55	0.46–0.91	0.56–1.22	0.46–0.84	–	0.47	0.63	0.43–0.63	0.40–0.58	–	0.76
δ-Cadinene	–	–	–	–	–	–	–	–	–	–	–	tr	–
β-Caryophyllene	0.18–0.36	0.13	0.16	0.16–0.44	0.12–0.50	0.18–0.81	0.23	0.24	0.28	0.26–0.70	0.20–0.30	0.18	0.32
γ-Elemene	–	tr	0.02	–	–	0.01–0.08	tr	0.01	–	–	–	tr	0.02
δ-Elemene	–	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-β-Farnesene	–	0.04	–	0.03–0.06	–	0.03–0.06	0.06	–	0.04	–	–	0.04	–
Germacrene D	–	–	0.01	0.01	0.01–0.03	0.01–0.02	–	0.01	0.01	0.01–0.03	tr	–	0.01
α-Humulene	–	–	0.02	0.02–0.04	–	0.02–0.07	tr	0.02	0.03	–	–	tr	0.03
γ-Muurolene	–	–	0.01	0.01	–	0.01–0.05	–	0.01	0.01	–	–	–	0.01
β-Santalene	–	tr	0.01	0.01–0.02	–	0.01–0.05	tr	0.01	0.02	–	–	0.01	0.02
Valencene	–	–	0.01	0.01–0.06	0.02–0.09	0.02–0.11	–	0.01	0.01	0.02–0.10	tr	–	–
Aldehydes													
Aliphatic													
Decanal	–	0.08	0.06	0.04–0.08	0.01–0.05	0.02–0.07	0.04	0.04	0.22	0.01–0.10	0.09–0.11	0.05	0.09
Dodecanal	–	–	0.01	0.01	0.01	–	–	0.01	0.01	0.01–0.03	0.02–0.03	–	0.02
Heptanal	–	–	0.01	–	–	–	–	0.01	–	–	–	–	0.01
Nonanal	0–0.18	0.17	0.19	0.05–0.11	0.11–0.26	0.07–0.15	0.18	0.21	0.12	0.13–0.38	0.08–0.16	0.10	0.20
Octanal	–	0.13	0.10	0.03–0.04	0.05–0.06	0.04–0.11	0.06	0.08	0.04	0.05–0.12	0.04–0.07	tr	0.14
Tetradecanal	–	–	0.03	0.01–0.02	–	–	–	0.01	0.01	–	–	–	0.03
Undecanal	–	tr	0.04	0.01–0.04	0.02–0.07	0.02–0.07	tr	0.03	0.02	0.01–0.10	0.07–0.09	0.01	0.05
Monoterpene													
Citronellal	0.10–0.31	0.07	0.13	0.08–0.33	0.11–0.19	0.03–0.15	0.06	0.07	0.10	0.03–0.13	0.07–0.11	0.09	0.16
Geranial	1.22–2.82	2.57	1.48[b]	0.78–4.16[b]	0.87–1.44	1.19–3.77	2.48	1.32[b]	2.42[b]	0.92–1.40	0.79–1.19	0.65	2.57[b]
Neral	0.60–1.49	0.77	0.88	0.48–2.47[d]	0.51–0.68	0.69–2.75	0.59	0.79	1.44[d]	0.61–0.93	0.50–0.78	0.74	1.43
Perilla aldehyde	–	0.07	b	b	–	–	0.09	b	b	–	–	0.03	b
Sesquiterpene													
β-Sinensal	–	–	–	–	–	–	–	–	–	–	–	–	–

continued

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed												
	Italy												
	Femminello						Monachello				Femminello + Monachello	Other Cultivars	
	1	2a	3a	4a	5a	10	2b	3b	4b	5b	5c	2c	3c
Ketones													
Aliphatic													
6-Methyl-5-hepten-2-one	–	–	–	tr	–	–	–	–	tr	–	–	–	–
Monoterpene													
Camphor	–	tr	0.01	0.01	0.01	–	tr	0.01	0.01	0.01–0.02	0.01–0.02	tr	0.02
Carvone	–	tr	–	d	–	–	–	–	d	–	–	–	tr
Piperitone	–	–	tr	tr	–	–	–	tr	tr	–	–	–	tr
Sesquiterpene													
Nootkatone	–	–	–	tr–0.01	0.02–0.03	–	–	–	tr	0.01–0.03	0.01	0.01	–
Alcohols													
Aliphatic													
Hexenol	–	–	–	–	–	–	–	–	–	–	–	–	–
Octanol	–	tr	0.01	0–tr	–	tr–0.03	–	tr	–	–	–	0.01	tr
Monoterpene													
Borneol	–	–	0.02	0.01–0.03	–	0.01–0.08	–	0.02	0.02	–	–	–	0.03
trans-Carveol	–	tr	–	–	–	–	tr	–	–	–	–	tr	–
Citronellol	–	tr	0.03[a]	0.02–0.07[a]	0.01	0.05–0.38[a]	–	0.02[a]	0.03[a]	0.01–0.04	tr	tr	0.05[a]
p-Cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–
Geraniol	0.10–0.19	0.01	0.01	0.01–0.05	0.02–0.10	0.02–0.36	0.02	0.02	0.05	0.02–0.22	0.11–0.12	0.03	0.03
Linalol	0.18–0.37	0.23	0.19	0.13–0.22	0.10–0.19	0.10–1.05	0.27	0.10	0.14	0.12–0.21	0.10–0.14	0.77	0.23

cis-p-Menth-2-en-1-ol	–	–	–	–	–	–	–	–	–	–	–	–	–
Nerol	0.10–0.26	0.03	0.03[a]	0.02–0.07[a]	0.18–0.40	0.05–0.38[a]	0.02	0.02[a]	0.03[a]	0.07–0.27	0.02–0.03	0.06	0.05[a]
Perilla alcohol	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-Sabinene hydrate	–	0.03	0.09	0.05–0.08	tr	0.04–0.25	0.04	0.08	0.08	tr	0.09–0.10	0.03	0.10
trans-Sabinene hydrate	–	0.14	0.07	0.04–0.10	0.05–0.10	0.03–0.22	0.12	0.08	0.10	0.05–0.12	0.11–0.14	0.07	0.12
Terpinen-4-ol	0.10–0.36	0.02	0.03	0.02–0.04	0.02–0.04	0.02–0.15	0.02	0.03	0.03	0.03–0.05	tr–0.02	0.02	0.04
α-Terpineol	0.10–0.13	0.31	0.28	0.16–0.37	0.20–0.39	0.17–1.03	0.26	0.27	0.31	0.16–0.33	0.15–0.23	0.27	0.38
Thymol	–	–	–	–	–	–	–	–	–	–	–	–	–
Sesquiterpene													
α-Bisabolol	–	0.03	0.03	0.02–0.05	–	0.02–0.10	0.03	0.02	0.03	–	–	0.02	0.04
α-Cadinol	–	–	–	–	–	–	–	–	–	–	–	–	–
Campherenol	–	–	0.02	0.02–0.04	–	0.02–0.09	–	0.02	0.03	–	–	–	0.03
(*E*,*E*)-Farnesol	–	–	–	–	–	–	–	–	–	–	–	–	–
Germacrene-D-4-ol	–	–	–	–	–	–	–	–	–	–	–	–	–
Norbornanol[e]	–	–	0.02	0.02–0.03	–	0.01–0.08	–	0.02	0.02	–	–	–	0.02
Spathulenol	–	–	–	–	–	–	–	–	–	–	–	–	–
Esters													
Aliphatic													
Decyl acetate	–	tr	0.02	0.02–0.04	0.01–0.02	–	–	0.02	0.03	0.01–0.02	0.04–0.05	0.02	0.03
Heptyl acetate	–	tr	–	tr	–	–	–	–	tr	–	–	tr	–
Nonyl acetate	–	–	0.01	tr	0.02–0.03	–	tr	0.01	tr	0.01–0.03	0.06–0.09	tr	0.01
Octyl acetate	–	–	tr	tr	0.01–0.02	–	–	tr	tr	0.01	tr	–	tr
Monoterpene													
Bornyl acetate	–	tr	0.02	tr–0.01	0.01–0.02	–	tr	0.01	0.01	0.01–0.06	0.02–0.03	0.01	0.02
Citronellyl acetate	0.10–0.16	0.03	0.03	0.03–0.06	0.02–0.06	0.02–0.09	0.02	0.02	0.02	0.01–0.06	0.08–0.10	0.03	0.03
Geranyl acetate	0.24–1.51	0.68	0.16	0.17–0.45	0.32–0.80	0.21–0.47	0.86	0.38	0.54	0.35–1.66	0.36–0.57	0.58	0.40

continued

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed												
	Italy												
	Femminello						Monachello				Femminello + Monachello	Other Cultivars	
	1	2a	3a	4a	5a	10	2b	3b	4b	5b	5c	2c	3c
Geranyl propanate	–	–	–	–	–	–	tr	–	–	–	–	tr	–
Methyl geranate	–	–	0.02	0.01	–	–	–	0.01	tr	–	–	–	0.02
Neryl acetate	0.38–0.70	–	0.30	0.30–0.60	0.27–0.73	0.28–0.57	–	0.31	0.39	0.20–1.69	0.48–0.83	tr	0.64
α-Terpinyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethers and oxides													
Monoterpene													
cis-Limonene oxide	–	tr	–	tr	0.01	–	tr	–	tr	0.01–0.02	tr	–	tr
trans-Limonene oxide	–	tr	0.01	tr–0.01	0.01–0.02	–	tr	tr	tr	0.01	tr	–	tr
Sesquiterpene													
Caryophyllene oxide*	–	–	–	–	–	–	–	–	–	–	–	–	–
Others													
Palmitic acid	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed									Distilled						
	Italy			Other Geographical Origin												
	Other Cultivars															
	4c	4d	6	2d	2e	7a	7b	7c	8	9a	9b	9c	11a	11b	12	13a	13b
								Hydrocarbons									
Monoterpene																	
Camphene	0.04	0.04	0.07	0.05	0.06	tr	tr	0.1	tr	0.03	0.04	0.04	–	0.05	0.1	0.03	0.05
δ-3-Carene	tr	tr	tr	–	–	–	–	–	0.3	0.07	0.07	0.09	–	0.04	tr	–	0.01
p-Cymene	0.04	0.06	tr	0.04	0.11	0.9	0.1	0.1	1.9	–	–	–	1.02	2.93	0.1	1.84	0.59
Limonene	70.20	70.95	62.8	69.65	59.76	70.5	65.6	62.6	61.8	75.68	69.65	72.90	60.71	56.99	61.8	70.10	64.13
Myrcene	1.54	1.60	1.62	tr	1.61	1.5	1.5	1.4	2.0	1.62	1.09	1.57	1.40	1.34	1.4	1.50	1.51
(*E*)-β-Ocimene	0.29	0.07	0.07	–	–	0.1	0.1	0.1	0.1	–	–	–	0.11	0.19	0.1	0.30	0.18
(*Z*)-β-Ocimene	0.20	0.03	0.03	0.04	0.04	tr	tr	tr	tr	–	–	–	–	–	0.1	0.12	0.09
α-Phellandrene	0.03	0.11	0.06	1.62	0.04	tr	tr	tr	tr	0.03	0.02	0.05	–	0.03	tr	–	–
β-Phellandrene	–	–	–	tr	0.37	0.3	0.4	0.4	–	–	–	–	–	–	–	0.31	0.42
α-Pinene	1.41	1.44	2.19	2.27	2.64	1.6	1.3	1.4	2.2	1.31	1.00	1.34	1.30	1.20	1.8	1.26	1.78
β-Pinene	8.44[c]	10.81[c]	14.68	10.54	14.23	11.7	12.9	14.2	8.1	8.70	6.61	8.58	8.78	9.74	11.9	5.03	10.07
Sabinene	8.44[c]	10.81[c]	2.59	2.20	2.42	2.0	2.1	2.2	2.0	–	–	–	1.30	1.23	1.9	0.71	1.83
α-Terpinene	0.20	0.15	0.20	0.02	0.22	0.1	0.2	0.2	0.1	0.14	0.15	0.21	tr	–	–	0.04	0.11
γ-Terpinene	9.61	7.16	8.93	8.22	9.29	6.3	8.7	11.1	10.6	7.19	6.88	7.77	7.97	4.79	10.7	6.24	8.75
Terpinolene	0.42	0.31	0.38	0.33	0.38	0.3	0.4	0.5	0.6	0.31	0.32	0.39	0.39	0.30	0.5	0.29	0.42
α-Thujene	0.33	0.29	0.43	–	–	0.2	0.3	0.4	0.2	0.28	0.18	0.26	0.28	0.20	0.4	–	–
Tricyclene	tr	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	tr	0.01
Sesquiterpene																	
trans-α-Bergamotene	0.61	0.38	0.27	0.21	0.27	0.3	0.3	0.3	1.0	0.40	0.28	0.25	0.82	0.17	0.3	0.53	0.22

continued

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed										Distilled						
	Italy			Other Geographical Origin													
	Other Cultivars																
	4c	4d	6	2d	2e	7a	7b	7c	8	9a	9b	9c	11a	11b	12	13a	13b
Bicyclogermacrene	0.02	0.05	tr	1.56	–	–	–	–	–	0.02	0.07	0.07	0.12	–	–	0.06	0.03
(*E*)-α-Bisabolene	0.03	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(*Z*)-α-Bisabolene	0.06	0.04	0.03	–	–	–	–	–	–	0.04	0.03	0.03	–	–	–	–	–
β-Bisabolene	0.88	0.54	0.40	–	–	0.4	0.5	0.5	1.6	0.58	0.44	0.37	–	0.29	0.5	0.93	0.47
δ-Cadinene	–	–	–	–	–	–	–	–	–	0.02	–	–	0.42	–	–	0.01	0.02
β-Caryophyllene	0.35	0.17	0.15	0.17	0.16	0.2	0.2	0.2	0.7	0.23	0.27	0.21	0.47	0.23	0.3	0.32	0.16
γ-Elemene	–	–	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
δ-Elemene	–	–	–	–	–	–	–	–	–	0.05	0.05	0.04	–	0.06	–	–	–
(*E*)-β-Farnesene	0.05	0.03	0.04[f]	tr	–	tr	tr	tr	tr	0.17	0.07	0.01	–	–	–	–	–
Germacrene D	tr	0.02	0.01	–	–	–	–	tr	–	–	–	–	0.16	–	–	–	tr
α-Humulene	0.03	0.02	tr	0.02	–	–	–	tr	tr	–	–	0.04	1.07	–	tr	0.03	0.02
γ-Muurolene	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
β-Santalene	0.02	0.01	0.04[ft]	0.01	tr	–	–	–	–	–	–	–	–	–	–	0.03	0.02
Valencene	0.03	0.01	0.04	–	–	–	–	–	–	0.02	0.06	0.08	–	0.24	–	0.13	0.15
Aldehydes																	
Aliphatic																	
Decanal	0.02	0.05	0.04	tr		–	tr	tr	0.1	0.01	–	0.07	–	–	tr	–	–
Dodecanal	0.02	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.02
Heptanal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	0.08
Nonanal	0.01	0.09	0.12	0.04	–	0.1	0.1	0.1	0.1	0.02	–	–	–	–	0.1	0.05	0.13
Octanal	0.03	0.03	0.04	0.01	tr	tr	tr	tr	0.1	–	–	–	–	–	tr	0.03	0.06
Tetradecanal	0.05	0.01	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Undecanal	0.01	0.02	0.02	0.01	–	–	–	–	–	0.01	0.08	0.06	–	–	–	0.05	0.05

Monoterpene																	
Citronellal	0.07	0.19	0.14	0.05	0.04	tr	0.1	0.1	0.1	0.03	0.03	0.05	–	–	0.1	0.07	0.12
Geranial	0.68[b]	2.20[b]	1.69[b]	0.98	4.29	1.3	2.1	1.3	1.3	1.05	0.94	1.22	2.39	5.43	1.6	1.58	1.60
Neral	0.41[d]	1.34[d]	1.03	1.06	2.00	0.6	1.1	0.7	0.7	0.60	0.63	0.95	0.27	1.06	1.2	1.18	1.39
Perilla aldehyde	b	b	b	0.02	0.09	–	–	–	–	–	–	–	–	–	–	–	–
Sesquiterpene																	
β-Sinensal	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.04	tr	–	–
Ketones																	
Aliphatic																	
6-Methyl-5-hepten-2-one	tr	tr	–	–	–	–	–	–	–	–	–	–	0.07	0.20	–	0.01	0.01
Monoterpene																	
Camphor	0.01	tr	0.01	0.01	tr	–	–	–	–	–	0.11	0.01	–	–	–	0.04	0.05
Carvone	d	d	–	–	–	–	–	–	–	–	0.14	0.06	–	–	–	0.03	–
Piperitone	tr	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01
Sesquiterpene																	
Nootkatone	tr	tr	tr	–	–	–	–	–	–	–	0.01	–	–	–	–	0.01	0.03
Alcohols																	
Aliphatic																	
Hexenol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	0.01	0.01
Octanol	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
Monoterpene																	
Borneol	0.01	0.02	0.01	–	–	–	–	–	–	–	0.07	0.02	–	–	–	–	–
trans-Carveol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.01
Citronellol	0.01[a]	0.03[a]	–	tr	tr	–	–	tr	–	–	–	–	–	–	–	0.09	0.09
p-Cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	–	0.02	tr
Geraniol	0.01	0.03	0.04	0.01	0.02	–	tr	0.1	0.1	0.01	0.59	0.64	0.26	0.58	0.9	0.76	0.61
Linalol	0.13	0.15	0.14	0.19	0.28	0.2	0.2	0.2	0.2	0.11	0.19	0.30	0.57	1.29	0.2	0.29	0.25

continued

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

	Cold-Pressed										Distilled						
	Italy			Other Geographical Origin													
	Other Cultivars																
	4c	4d	6	2d	2e	7a	7b	7c	8	9a	9b	9c	11a	11b	12	13a	13b
cis-p-Menth-2-en-1-ol	–	–	–	–	–	–	–	–	–	–	0.05	0.03	–	–	–	0.01	0.01
Nerol	0.01[a]	0.03[a]	0.09	0.02	0.02	–	tr	tr	0.1	0.01	0.44	0.53	1.82	4.26	0.8	0.50	0.44
Perilla alcohol	–	–	–	–	–	–	–	–	–	–	0.06	0.02	–	–	–	0.01	0.01
cis-Sabinene hydrate	0.06	0.07	0.08	0.01	0.02	–	–	–	–	0.02	0.13	–	–	–	–	0.02	tr
trans-Sabinene hydrate	0.05	0.06	0.06	0.06	0.13	tr	0.1	0.1	0.2	–	–	–	–	–	–	0.01	0.02
Terpinen-4-ol	0.03	0.02	0.02	0.02	–	tr	tr	tr	tr	0.01	0.14	0.29	1.17	1.88	0.4	0.30	0.26
α-Terpineol	0.21	0.24	0.23	0.23	0.27	0.2	0.3	0.3	0.1	0.15	0.37	0.39	2.19	2.92	0.6	0.46	0.45
Thymol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	0.01	0.02
Sesquiterpene																	
α-Bisabolol	0.04	0.02	0.02	0.01	0.02	–	–	–	0.1	0.03	0.05	0.03	–	–	–	0.06	0.04
α-Cadinol	–	–	–	–	–	–	–	–	–	–	0.01	0.01	–	–	–	+	0.01
Campherenol	0.04	0.02	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(*E*,*E*)-Farnesol	–	–	–	–	–	–	–	–	–	–	0.02	–	0.05	–	–	–	–
Germacrene-D-4-ol	–	–	–	–	–	–	–	–	–	0.06	0.06	–	–	–	–	0.02	0.01
Norbornanol[e]	0.03	0.02	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spathulenol	–	–	–	–	–	–	–	–	0.1	–	–	–	0.09	–	–	0.03	tr
Esters																	
Aliphatic																	
Decyl acetate	0.04	0.03	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Heptyl acetate	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nonyl acetate	tr	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Octyl acetate	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Monoterpene																	
Bornyl acetate	0.02	tr	–	–	–	–	–	–	–	–	–	–	–	0.07	–	0.01	tr
Citronellyl acetate	0.06	0.04	0.03	0.02	–	tr	tr	tr	tr	0.08	0.14	0.02	–	–	tr	0.06	0.05
Geranyl acetate	0.49	0.31	0.20	0.16	0.30	0.4	0.6	0.8	0.6	0.20	0.31	0.27	0.20	0.26	0.2	1.04	0.57
Geranyl propanate	–	–	–	0.01	tr	–	–	–	tr	0.02	0.10	0.01	–	–	–	0.01	0.02
Methyl geranate	0.02	tr	–	–	–	–	–	–	–	0.01	0.04	0.03	–	–	–	–	–
Neryl acetate	0.38	0.40	0.64	–	–	0.4	0.5	0.5	1.2	0.36	0.52	0.48	–	–	0.3	0.50	0.53
α-Terpenyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	0.26	0.56	tr	–	–
Ethers and oxides																	
Monoterpene																	
cis-Limonene oxide	tr	tr	tr	–	–	tr	–	–	0.1	–	–	–	–	0.11	–	0.24	0.01
trans-Limonene oxide	tr	tr	tr	–	–	–	–	–	tr	–	0.03	–	0.05	0.12	–	0.15	–
Sesquiterpene																	
Caryophyllene oxide*	–	–	–	–	–	–	–	–	0.1	–	–	–	0.18	–	–	0.11	tr
Others																	
Palmitici acid	–	–	–	–	–	–	–	–	–	–	0.36	–	–	–	–	0.07	0.03

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; + identified but not quantified; [a] citronellol + nerol; [b] geranial + perilla aldehyde; [c] β-pinene + sabinene; [d] neral + carvone; [e] 2,3-dimethyl-3-(methyl-3-penthenyl) -2-norbonanol; [f] (*E*)-*b*-farnesene + *b*-santalene.

Appendix to Table 1.21

1. Geraci et al. (1998). Sicily, Italy; solvent extraction (petroleum ether); variability ranges of the oils extracted from fruits harvested in January, May and August from the cvs.: (a) Femminello comune, (b) Femminello precoce; GC/FID on column coated with SE-54; relative percentage of peak areas.
2. Sawamura (2000). One sample each hand pressed from Italian fruits of the cvs.: (a) Femminello, (b) Monachello, (c) Bagheria and from Japanese fruits of the cvs.: (d) Eureka, (e) Lisbon; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with Thermon 600T. Sawamura also found in Italian oils δ-muurolene (tr–0.01%), α-santalene (tr–0.01%), β-selinene (tr–0.01%), *p*-mentha-1,8-dien-9-ol (0%–0.01%), verbenol* (tr–0.01%), δ-cadinol (tr–0.01%), and trace amounts in one or more of the analyzed oils of (*E*)-2-decenal, solanone, hexadecanol, 2,7-dimethyl-2,6-octadien-1-ol, *p*-menth-1-en-9-ol, β-bisabolol, (*Z*)-nerolidol, linalyl anthranilate, 1,8-cineole, neryl acetone; in Japanese oils δ-muurolene (tr–0.01%), α-santalene (tr–0.01%), *p*-mentha-1,8-dien-9-ol (tr–0.01%), verbenol* (0%–0.01%), neryl acetone (tr–0.01%), and trace amounts in one or more of the analyzed samples of hexadecanol, 1,8-cineole, limonene dioxide.

continued

TABLE 1.21 (continued)
Percentage Composition of the Volatile Fraction of Laboratory-Extracted Lemon Oils (1998–2009)

3. Corleone et al. (2000). Sicily, Italy; average values of four samples each hand pressed from fruits harvested in January, February, March, and April from the cvs.: (a) Femminello, (b) Monachello, (c) Lo Porto; GC/FID on capillary column (30 m × 0.25 mm × 0.22μm) coated with DB-5; relative percentage of peak areas. Corleone et al. also found α-bisabolene*(0.04%–0.07%), (*Z*)-β-farnesene (0.03%–0.04%).
4. Verzera et al. (2001). Sicily, Italy; average values of two samples each hand pressed from fruits of the cvs.: (a) Femminello Siracusano, Femminello Continella, Femminello Santa Teresa, Femminello fior d'arancio, Femminello Dosaco, Femminello incappucciato, (b) Monachello, (c) Interdonato, (d) Fino; GC/FID on capillary column (30 m × 0.32 mm × 0.40–0.45 μm); GC/MS on capillary columns coated with MEGA-5MS (30 m × 0.25 mm × 0.25 μm) or Megawax (30 m × 0.32 mm × 0.40–0.45 μm); LRI on MEGA-5 and Megawax are reported; relative percentage of peak areas.
5. Gionfriddo et al. (2004). Sicily, Italy; average values of samples obtained by rasping the fruit peels every 15 days from October 2002 to January 2003 from the cvs.: (a) Femminello comune, (b) Monachello; (c) average values of oils extracted by the same method from mixtures of both cvs. in October 2003. GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; relative percentage of peak areas.
6. Verzera et al. (2005). One sample hand pressed from fruits of the cv. Cavone. For experimental conditions see point 4 of this appendix.
7. Lota et al. (2002). Corsica, France; one sample each hand pressed from the cvs.: (a) Eureka, (b) Limoneire, (c) Lisbon; GC/FID and GC/MS on capillary columns (50 m × 0.22 mm × 0.25 μm) coated with BP-1 or BP-20; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas.
8. Kirbaslar et al. (2006). Antalya, Turkey; one sample cold-pressed from fruits collected in November 2003; GC/FID and GC/MS on capillary columns coated with DB-5 (60 m × 0.25 mm × 0.25 μm) and Innowax (30 m × 0.25 mm × 0.25 μm); MS libraries: Wiley and NIST; relative percentage of peak areas.
9. Ferhat et al. (2007). Algeria; cv. Eureka; (a) one sample cold-pressed using a Schwaub machine; (b) one sample extracted by microwave accelerated distillation; (c) one sample hydrodistilled; GC/FID and GC/MS on capillary columns coated with HP-5MS (30 m × 0.25 mm × 0.25 μm) or with Stabilwax (60 m × 0.2 mm × 0.25 μm); LRI on HP-5MS and Stabilwax are reported; relative percentage of peak areas. Ferhat et al. also found ocimene* (0.01%), (*E*)-γ-bisabolene (0.01%), (*Z*)-γ-bisabolene (0.01%), γ-curcumene (0.03%) in sample (a); ocimene* (0.01%), (*E*)-γ-bisabolene (0.01%), (*Z*)-γ-bisabolene (0.01%), γ-curcumene (0.03%), α-muurolene (0.01%), endo-fenchol (0.01%), epi-α-bisabolol (0.02%), caryophyllene alcohol (0.01%), α-muurolol (0.02%), linoleic acid (0.10%), dill apiole (0.10%) in sample (b); ocimene* (0.03%), (*Z*)-γ-bisabolene (0.02%), γ-curcumene (0.03%), endo-fenchol (0.02%) in sample (c).
10. Badalamenti et al. (2004). Sicily, Italy; range of the composition of oils extracted by pentane/ethyl acetate 90:10, from the cv. Femminello comune from October 2003 to July 2004; GC/FID on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SPB-5; relative percentage of peak areas.
11. Zollo Amvam et al. (1998). Yaoundè, Cameroon; one sample each hydrodistilled from the cvs.: (a) Lisbon, (b) Eureka; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 or Carbowax 20M; GC/MS on capillary column (25 m × 0.23 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found allo-aromadendrene (0.05%), cadina-1,4-diene (1.34%), γ-cadinene (0.42%), β-elemene (0.07%), verbenone (0.05%), dodecanol (0.17%), carveol* (0.14%), isopulegol (0.09%), terpinen-1-ol (0.05%), β-terpineol* (0.21%), eudesmol (0.06%), (*Z*,*Z*)-farnesol (0.08%), pinene oxide (0.33%), 1-methyl naphthalene (0.10%), 2-methyl naphthalene (0.05%) in sample (a), myrtenal (0.04%), menthone (0.03%), verbenone (0.04%), carveol* (0.03%), isopulegol (0.14%), terpinen-1-ol (0.08%), β-terpineol* (0.09%), eudesmol (0.04%), (*Z*,*E*)-farnesol (0.03%), 1-methyl naphthalene (0.03%) in sample (b).

12. Ruberto et al. (1999). Sicily, Italy; one sample extracted by steam distillation; GC/FID and GC/MS on capillary column (25 m × 0.2 mm × 0.33 μm) coated with HP-1; MS libraries: Wiley and NBS; relative percentage of peak areas. Ruberto et al. also found trace amounts of 2-dodecenal*, 2-nonenal*, nonanol.
13. Fujita et al. (2008). Japan; one sample each hydrodistilled from the cvs.: (a) Lisbon, (b) Eureka; GC/FID on capillary column (30 m × 0.25 mm) coated with DB-Wax; relative percentage of peak areas. Fujita et al. also found in sample (a) and (b) respectively *cis*-α-bergamotene (0.08%, 0.07%), α-copaene (0.07%, tr), α-curcumene (0.03%, 0.03%), (*E*,*E*)-α-farnesene (0.01%, tr), germacrene A (0.01%, 0.01%), hexenal (tr, 0.01%), (*E*)-2-hexenal (0.01%, 0.01%), (*E*)-photocitral (0.05%, 0.03%), (*E*)-2-hexen-1-ol (0.02%, tr), (*Z*)-3-hexen-1-ol (0.02%, 0.04%), *cis*-carveol (0.02%, 0.01%), (*Z*)-isogeraniol (tr, 0.01%), *trans*-*p*-mentha-2,8-dien-1-ol (0.07%, 0.03%), *trans*-*p*-menth-2-en-1-ol (0.04%, 0.02%), myrtenol (0.01%, 0.01%), *cis*-verbenol (0.01%, 0.03%), T-muurolol (0.02%, tr), neryl propanate (tr, 0.01%), isocariophyllene oxide (0.03%, 0), geranic acid (0.04%, 0.01%), neric acid (0.04%, 0.02%), phytol (0.01%, tr), and trace amounts in one or both the analyzed samples of *p*-cymenene, undecanol, γ-isogeraniol, (*E*)-isogeraniol, (*E*)-nerolidol, indole, 2,3-dihydro-1,8-cineole.

TABLE 1.22
Stationary Phases Used for GC Analyses of the Volatile Fraction of Citrus Essential Oils

Polarity	Chemical Composition and Commercial Acronym	Coelutions Indicated by One or More Authors[a]
Apolar[b]	*100% dimethylpolysiloxane* OV-1, DB-1, BP-1, HP-1, SPB-1, OV-101, HP-101, SE-30, PETROCOL-DH	*p*-Cymene + limonene + β-phellandrene; limonene + β-phellandrene; δ-3-carene + 1,4-cineole; limonene + 1,8-cineole; myrcene + octanal; β-phellandrene + 1,8-cineole; γ-terpinene + octanol; terpinolene + nonanal; (*Z*)-α-bisabolene + (*E*,*E*)-α-farnesene + β-selinene; (*E*)-γ-bisabolene + germacrene C + 7-epi-α-selinene; β-cubebene + β-elemene; germacrene A + α-selinene; α-cubebene + geranyl acetate; β-copaene + 2-hexylciclopropane acetic acid; dodecanal + *cis*-*p*-mentha-1(2),8-dien-10-yl acetate; nonanal + linalol; citronellal + *cis*-*p*-mentha-1(7),8-dien-2-ol; neral + carvone; perilla aldehyde + decanol; citronellol + nerol; *cis*-*p*-mentha-2,8-dien-9-ol + *cis*-limonene oxide; citronellyl acetate + α-terpinyl acetate.
Slightly polar[b]	*5% phenyl, 95% methylpolysiloxane* SE-52, DB-5, HP-5, RTX-5, SPB-5, MDN-5S, MEGA-5, OPTIMA-5	Nonane + heptanal; p-cymene + limonene + (*Z*)-β-ocimene; *p*-cymene + limonene + β-phellandrene; limonene + (*Z*)-β-ocimene + 1,8-cineole; (*E*)-β-ocimene + (*Z*)-β-ocimene; β-pinene + sabinene; limonene + β-phellandrene + 1,8-cineole; α-phellandrene + octanal; β-bisabolene + (*E*,*E*)-α-farnesene; β-cubebene + β-elemene; (*E*)-β-farnesene + β-santalene; α-humulene + β-farnesene*; α-selinene + valencene; (*E*,*E*)-decadienal + nonyl acetate; dodecanal + decyl acetate; geranial + perilla aldehyde; octanol + *cis*-sabinene hydrate; linalol + *trans*-sabinene hydrate; citronellol + nerol; geraniol + linalyl acetate; δ-elemene + linalyl propanate; citronellyl acetate + α-terpinyl acetate; neral + carvone; linalol + *trans* linalol oxide; nonanal + *trans*-sabinene hydrate; (*Z*)-isocitral + terpinen-4-ol
	silphenylene polymer SLB-5	Limonene + (*Z*)-β-ocimene; octanol + *cis*-sabinene hydrate; linalol + *trans*-sabinene hydrate.
	5% phenyl, 95% polysilphenylene-siloxane BPX-5 *5% phenyl, 1% vinyl, 94% methylpolysiloxane* SE-54	*p*-Cymene + α-terpinene; (*E*)-β-farnesene + (*Z*)-β-farnesene; (*E*,*E*)-farnesol + (*Z*,*E*)-farnesol; *cis*-limonene oxide + *trans*-limonene oxide
Moderately polar	*50% phenyl, 50% methylsilicone* OV-17 *14% cyanopropylphenyl, 86% methylpolysiloxane* OV-1701	Germacrene D + valencene

TABLE 1.22 (continued)
Stationary Phases Used for GC Analyses of the Volatile Fraction of Citrus Essential Oils

Polarity	Chemical Composition and Commercial Acronym	Coelutions Indicated by One or More Authors[a]
Polar	*Polyethylene glycol* PEG, Carbowax, Carbowax-20M, Supelcowax, Stabilwax, Innowax, Mega-wax, DB-wax, BP-20 CP-wax 52-CP	Limonene + β-phellandrene; α-pinene + α-thujene; β-phellandrene + 1,8-cineole; β-bisabolene + geranial; bicyclogermacrene + carvone; (*E*)-β-farnesene + citronellyl acetate; citronellal + octyl acetate; geranial + perilla aldehyde; citronellal + neral; decyl acetate + α-terpinyl acetate.
	Poly(oxethyleneoxypropylene) UCON-LB550X	β-Pinene + sabinene
	Phthalic acid ester Thermon-600T	β-bisabolene + 2,7-dimethyl-2,6-octadien-1-ol[t]; α-terpineol + α-terpinyl acetate.

Notes: *, correct isomer not characterized; t, tentative identification.

[a] The overlapping of two peaks occurs in GC when resolution between two compounds equals zero, under the applied experimental conditions. Resolution (R_S) is dependent on column efficiency (N = theoretical plate number), on column selectivity (α = ratio of two peak retention times) and on the retention factor k which indicates the measure of the molar distribution of the analyte between the stationary and mobile phases (measured by the ratio of the time spent by the component in the stationary phase and the time spent in the mobile phase). The relationship between R_S, N, α, and k is expressed by the resolution master equation which, in its first version (Purnell, 1960), considers N and k values relative to the second compound of the peak-pair of interest:

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

where N represents the number of theoretical plates required (N_{req}) to separate, with resolution R_s, a peak pair with selectivity value α and a retention factor k_2, relative to the most retained component. Therefore, if $\alpha = 1$, resolution will equal zero and the two compounds will undergo complete coelution; if α is higher than 1, the separation will be function of column efficiency. Column selectivity (α) and retention factor (k) vary in function of the temperature.

It is evident, hence, that coelutions reported in this appendix not only depend on the stationary phase used, but also on the experimental conditions and on column characteristics (length, internal diameter, film thickness). It must be noted, moreover, that between stationary phases of essentially the same chemical nature there can sometimes be slight compositional differences, especially for polar phases, influencing α values and improving or worsening the resolution between two components.

[b] The largest number of possible coelution are reported relatively to dimethylpolysiloxane and 5% phenyl, 95% methylpolysiloxane stationary phases. This phenomenon does not mean that these phases are the least adequate for the separation of citrus essential oil volatiles, but simply that these are the most widely employed and, thus, the literature is rich in information on their analytical performances. On the other hand only few or zero coelution are reported relatively to stationary phases scantly used for the separation of citrus essential oil volatiles (e.g., OV-17). This is not due to better separation power given by these columns, but only to the lack of information reported in literature relatively to coelutions or critical pairs.

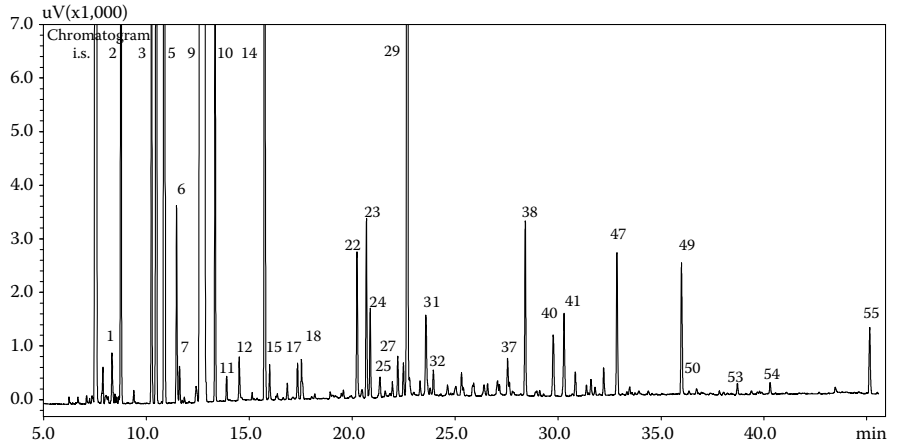

FIGURE 1.15 GC-FID chromatogram of bitter orange oil. Peak identification: **1** α-thujene; **2** α-pinene; **3** sabinene; **4** β-pinene; **5** myrcene; **6** octanal; **7** α-phellandrene; **8** *p*-cymene; **9** limonene; **10** (E)-β-ocimene; **11** γ-terpinene; **12** octanol; **13** terpinolene; **14** linalol; **15** nonanal; **16** *trans*-*p*-mentha-2,8-dien-1-ol; **17** cis-limonene oxide; **18** *trans*-limonene oxide; **19** citronellal; **20** nonanol; **21** terpinen-4-ol; **22** α-terpinenol; **23** decanal; **24** octyl acetate; **25** *trans*-carveol; **26** *cis*-carveol; **27** neral; **28** carvone; **29** linalyl acetate; **30** (*E*)-2-decenal; **31** geranial; **32** perilla aldehyde; **33** perilla alcohol; **34** undecanal; **35** δ-elemene; **36** α-terpinyl acetate; **37** neryl acetate; **38** geranyl acetate; **39** dodecanal; **40** decyl acetate; **41** β-caryophyllene; **42** perillyl acetate; **43** (*E,Z*)-2,6-dodecadienal; **44** (*E*)-β-farnesene; **45** α-humulene; **46** (*E*)-2-dodecenal; **47** germacrene D; **48** bicyclogermacrene; **49** (*E*)-nerolidol; **50** germacrene B; **51** spathulenol; **52** caryophyllene oxide*; **53** dodecyl acetate; **54** (*E*)-2-tetradecenal; **55** nootkatone. (This image will appear in Dugo, G., D. Sciarrone, R. Costa, et al., *J. Essent. Oil Res.* 2010a. In press. Printed with permission of Allured Business Media.)

extracted from Bagheria and Eureka lemons reported by Sawamura (2000). The absence of neryl acetate in two samples of oils extracted from Femminello and Monachello lemons reported by the same author also appear anomalous. The results of three of the oils analyzed by Sawamura (2000) were already reported in a previous paper by Sawamura et al. (1999b). In the cvs. analyzed, Geraci et al. (1998) found the minimum content of neral and geranial in oils produced in winter. However, systematic investigation carried out in the past on many hundreds of samples demonstrated, on the contrary, that the average content of monoterpene aldehydes is at the maximum value in winter, decreasing during the productive season, and reaching the minimum value in summer. The behaviors of limonene (higher value in winter) and of β-pinene (inverse behavior to limonene) appear correct. In addition to the three samples reported in Table 1.21, Lota et al. (2002) analyzed eight more samples of cvs. of *Citrus limon* [L.] Burm. Their composition was not reported in Table 1.20 because six of these (Fino, Berna, Santa Teresa, Corpaci, Lapithon, Menton) presented a high content of *p*-cymene, ranging between 5.4% and 7.8%, in our opinion indicating alteration or peculiar composition of the oils; one (Barum) had a very low value of limonene (52.6%) and high content of linalol (16%) and of linalyl acetate (23.3%), recalling more bergamot oil than lemon oil. The last (Panaché) was discharged since it presented, in our opinion, many anomalies for a lemon essential oil: low content of limonene (48.6%); high content of *p*-cimene (2.2%); of geranyl acetate (3.2%), and of neryl acetate (3.9%). Badalamenti (2004) studied the variation of composition of Femminello comune lemon during the period October 2003 to July 2004. In this article are described the variation of class of compounds and of single components. This author asserted that some differences of composition can occur between oils

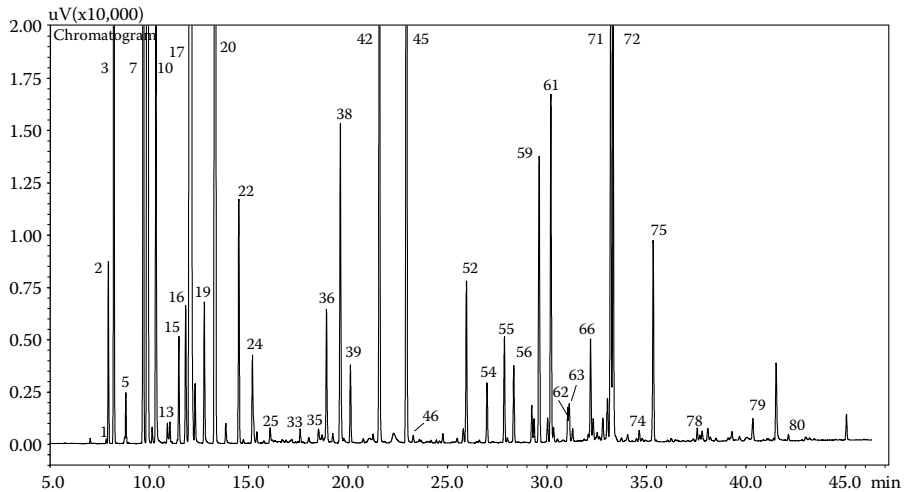

FIGURE 1.16 GC-FID chromatogram of cold-pressed Key lime type B. Peak identification: **1** tricyclene; **2** α-thujene; **3** α-pinene; **4** α-fenchene; **5** camphene; **6** thuja-2,4(10)-diene; **7** sabinene; **8** β-pinene; **9** 6-methyl-5-hepten-2-one; **10** myrcene; **11** decane; **12** octanal; **13** α-phellandrene; **14** δ-3-carene; **15** α-terpinene; **16** *p*-cymene; **17** limonene; **18** (*Z*)-β-ocimene; **19** (*E*)-β-ocimene; **20** γ-terpinene; **21** *cis*-sabinene hydrate; **22** terpinolene; **23** *p*-cymenene; **24** linalol; **25** *trans*-sabinene hydrate + nonanal; **26** fenchol*; **27** *trans*-*p*-mentha-2,8-dien-1-ol; **28** *cis*-*p*-menth-2-en-1-ol; **29** allocimene; **30** *cis*-limonene oxide; **31** *trans*-limonene oxide; **32** *trans*-pinocarveol; **33** citronellal; **34** (*Z*)-isocitral; **35** borneol; **36** isogeraniol + terpinen-4-ol; **37** *p*-cymen-8-ol; **38** α-terpineol; **39** decanal; **40** citronellol; **41** nerol; **42** neral; **43** carvone; **44** geraniol; **45** geranial; **46** perilla aldehyde; **47** bornyl acetate; **48** *trans*-pinocarvyl acetate; **49** tridecane; **50** undecanal; **51** methyl geranate; **52** δ-elemene; **53** citronellyl acetate; **54** neryl acetate; **55** geranyl acetate; **56** β-elemene; **57** dodecanal; **58** *cis*-α-bergamotene; **59** β-caryophyllene; **60** γ-elemene; **61** *trans*-α-bergamotene; **62** (*E*)-β-farnesene; **63** α-humulene; **64** β-santalene; **65** γ-curcumene; **66** germacrene D; **67** *trans*-β-bergamotene; **68** β-selinene; **69** α-selinene; **70** (*Z*)-α-bisabolene; **71** (*E,E*)-α-farnesene; **72** β-bisabolene; **73** (*E*)-γ-bisabolene; **74** (*E*)-α-bisabolene; **75** germacrene B; **76** caryophyllene oxide*; **77** dodecyl acetate; **78** tetradecanal; **79** α-bisabolol; **80** (*E,E*)-farnesal. (From Dugo, G., 2009. Personal communication.)

manually extracted from ripe fruits and those industrially extracted because mixtures of ripe and small unripe fruits can arrive at the transformation industry, resulting in oils with different composition; this explains some apparent anomalies of the final product.

In addition to the results reported in Table 1.21, some other studies are relative to laboratory cold extracted oils. Choi (2006) analyzed an oil extracted from fruits cultivated in Korea, with an exceptional content of camphene (0.74%) and an extremely low content of neral and geranial (total 0.30%). The results by Naef and Jaquierr (2006) are interesting. In a lemon oil extracted by CH_2Cl_2 and fractionated on silica gel, they identified by GC/MS and confirmed the structure by synthesis of the aliphatic aldehydes (*E*)-2-methyl-2-heptenal; (*E*)-2-methyl-2-nonenal; (*E*)-2-methyl-2-undecenal; 4-methyl-nonanal; 4-methyl undecanal; and the aliphatic alcohols 4-methyl nonanol and 4-methyl undecanol. These components contribute positively to the flavor of freshly grated lemon peel.

Less numerous are the articles relative to lemon essential oil extracted in laboratory by distillation techniques. The results of some of these are reported in the final part of Table 1.21. The composition of these oils is characterized by higher amounts of monoterpene alcohols than that seen in the cold-pressed ones due to hydration phenomena of monoterpene hydrocarbons, and by the absence of aliphatic esters that are present in small amounts in cold-pressed oils, surely due to hydrolytic phenomena.

Among other papers not included in Table 1.21 on lemon oils extracted in laboratory by distillation techniques, Vekiari et al. (2002) observed the seasonal variation of oils extracted from

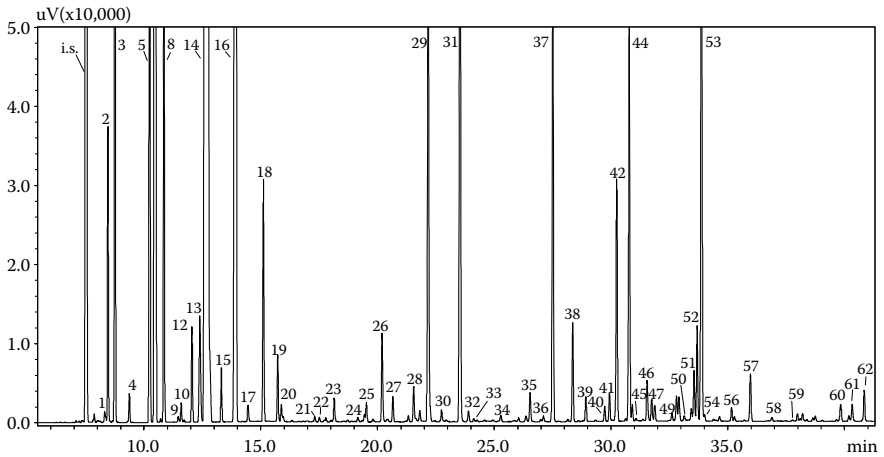

FIGURE 1.17 GC-FID chromatogram of Persian lime oil. Peak identification: **1** tricyclene; **2** α-thujene; **3** α-pinene; **4** camphene; **5** sabinene; **6** β-pinene; **7** 6-methyl-5-hepten-2-one; **8** myrcene; **9** octanal; **10** α-phellandrene; **11** δ-3-carene; **12** α-terpinene; **13** *p*-cymene; **14** limonene + 1,8-cineole + (*Z*)-β-ocimene; **15** (*E*)-β-ocimene; **16** γ-terpinene; **17** *cis*-sabinene hydrate; **18** terpinolene; **19** linalol; **20** nonanal; **21** *cis*-limonene oxide; **22** *trans*-limonene oxide; **23** citronellal; **24** borneol; **25** terpinen-4-ol; **26** α-terpineol; **27** decanal; **28** nerol + citronellol; **29** neral; **30** geraniol; **31** geranial; **32** perilla aldehyde; **33** bornyl acetate; **34** undecanal; **35** δ-elemene; **36** citronellyl acetate; **37** neryl acetate; **38** geranyl acetate; **39** *cis*-β-elemene; **40** dodecanal; **41** *cis*-α-bergamotene; **42** β-caryophyllene; **43** γ-elemene; **44** *trans*-α-bergamotene; **45** (*Z*)-β-farnesene; **46** (*E*)-β-farnesene; **47** α-humulene; **48** β-santalene; **49** γ-curcumene; **50** β-selinene; **51** (*Z*)-α-bisabolene; **52** (*E,E*)-α farnesene; **53** β-bisabolene; **54** (*Z*)-γ-bisabolene; **55** (*E*)-γ-bisabolene; **56** (*E*)-α-bisabolene; **57** germacrene B; **58** caryophyllene oxide*; **59** tetradecanal; **60** 2,3-dimethyl-3-(4-methyl-3-penten-1-yl)-2-norbornanol; **61** campherenol; **62** α-bisabolol. (From Dugo, G. 2009. Personal communication.)

fruits grown in Crete of the Zambetakis variety. The main variations in composition, observed during the period December 1996 and April 1998, were relative to the content of monoterpene aldehydes and esters. The oils analyzed by Vekiari et al. are also characterized by low amounts of limonene (39.22%–45.93%). Three other papers report qualitative and quantitative composition absolutely not compatible with lemon essential oil; they are cited here only for the purpose of providing complete information. Charai et al. (1999) indicated as main components limonene (49%); myrcene (15.3%); δ-3-carene (8%); sabinene (5%); linalol (1.8%); carvone (1.8%); and *trans*-carveol (1.6%). de Gonzales et al. (2002) reported the unusual presence of 2.8% of thymol methyl ether in one lemon oil from Venezuela. In one oil obtained from Indian fruits, Mahalwal and Ali (2003) indicated the presence of L-limonene (37.2%); camphene (12.3%); α-terpineol (11.2%); and a total amount of sesquiterpenes of 14.2%, along with some components normally used as plasticizers.

In conclusion, the results obtained from the study of the composition of lemon oil extracted by hot techniques, such as distillation, in our opinion, are not useful for deepening the knowledge of the natural composition of the oil, but they can be useful in comparing the influence of the different techniques.

1.9 FINAL REMARKS

The most recent results on the composition of the volatile fraction of citrus essential oils, not included in the former review by Dugo et al. (2002) and summarized in Tables 1.2, 1.4, 1.6, 1.7, 1.9, 1.10, 1.11, 1.12, 1.14, 1.15, 1.17, 1.18, 1.20, and 1.21, are taken from 95 original papers. Among these, 42 are relative to industrial oils of secure origin and commercial; 33 are relative to

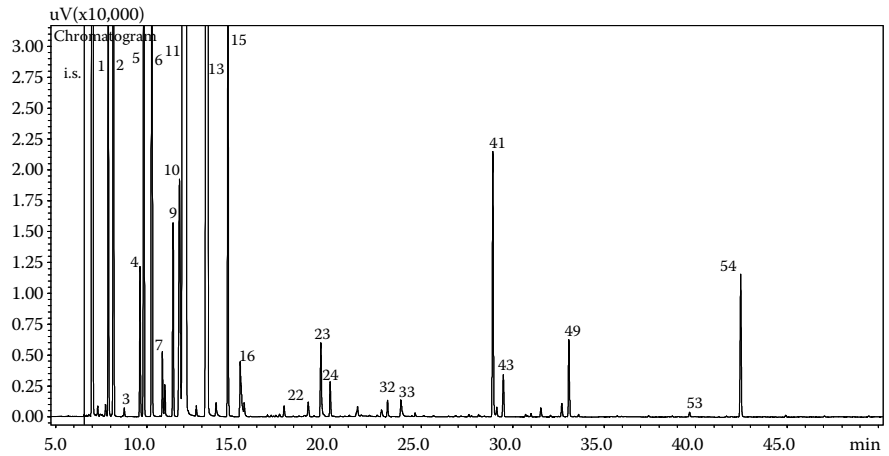

FIGURE 1.18 GC-FID chromatogram of cold-pressed mandarin oil. Peak identification: **1** α-thujene; **2** α-pinene; **3** camphene; **4** sabinene; **5** β-pinene; **6** myrcene; **7** octanal; **8** α-phellandrene; **9** α-terpinene; **10** *p*-cymene; **11** limonene; **12** (*E*)-β-ocimene; **13** γ-terpinene; **14** octanol; **15** terpinolene; **16** linalol + *trans*-sabinene hydrate; **17** nonanal; **18** *cis*-limonene oxide; **19** *trans*-limonene oxide; **20** camphor; **21** citronellal; **22** terpinen-4-ol; **23** α-terpineol; **24** decanal; **25** nerol + citronellol; **26** *cis*-carveol; **27** thymol methyl ether; **28** neral; **29** carvone; **30** geraniol; **31** geranial; **32** perilla aldheyde; **33** thymol; **34** undecanal; **35** (*E,E*)-2,4-decadienal; **36** α-terpinyl acetate; **37** citronellyl acetate; **38** neryl acetate; **39** α-copaene; **40** geranyl acetate; **41** methyl *N*-methyl anthranilate; **42** dodecanal; **43** β-caryophyllene; **44** (*E,Z*)-2,6-dodecadienal; **45** α-humulene; **46** (*E*)-**2**-dodecenal; **47** germacrene D; **48** α-selinene; **49** (*E,E*)-α-farnesene; **50** δ-cadinene; **51** (*E*)-2-tridecenal; **52** tetradecanal; **53** (*Z,E*)-farnesol; **54** α-sinensal. (From Dugo, G., I. Bonaccorsi, C. Ragonese, et al., *Flavour Fragr. J.* 2010. In press. Printed with permission of John Wiley & Sons, Ltd.)

laboratory-extracted oils by cold-extraction procedures, 18 are laboratory extracted by distillation processes; and 2 results were relative to samples extracted by distillation and cold extraction. Some papers on industrial oils are relative to only one sample and do not focus exclusively on the determination of the composition of the oil.

In this chapter are also cited some results relative to 15 studies carried out on commercial samples and to 13 studies on oils extracted in laboratory. These were not included in the relative tables because they concerned only the identification of few principal components, were relative to some peculiar aspects of the composition of the oils, or reported the composition of samples with numerous and important anomalies.

Among the papers relative to the results reported in tables, 43 were carried out in Italy (Sicily or Calabria). Of these, 23 were on oils industrially produced, and many reported sets of a large number of samples; the remaining 20 were relative to studies carried out on samples extracted in laboratory mainly by cold extraction. Only 7 articles were carried out in European nations different from Italy (France, Germany, Austria, Switzerland, United Kingdom, Turkey). Seven papers were from Cuba or Brazil, 1 from Australia, and 1 from the United States. Four papers were written by Indian authors; 10 were from the extreme Eastern Countries (Japan, China, Vietnam, and Korea), and 11 were from Africa (Tunisia, Algeria, Ethiopia, Kenya, Cameroon, Nigeria, South Africa). The 25 last-cited studies were carried out on samples extracted in laboratory by distillation techniques. Many of these articles were focused on the identification of numerous trace levels components in only one cultivar; it is our opinion that in these cases further confirmation is necessary. In fact, the unavailability in many cases of pure standards and the identification performed on the basis of commercial mass spectral libraries suggest great attention when new components are indicated within the composition of essential oils. It must also be highlighted that

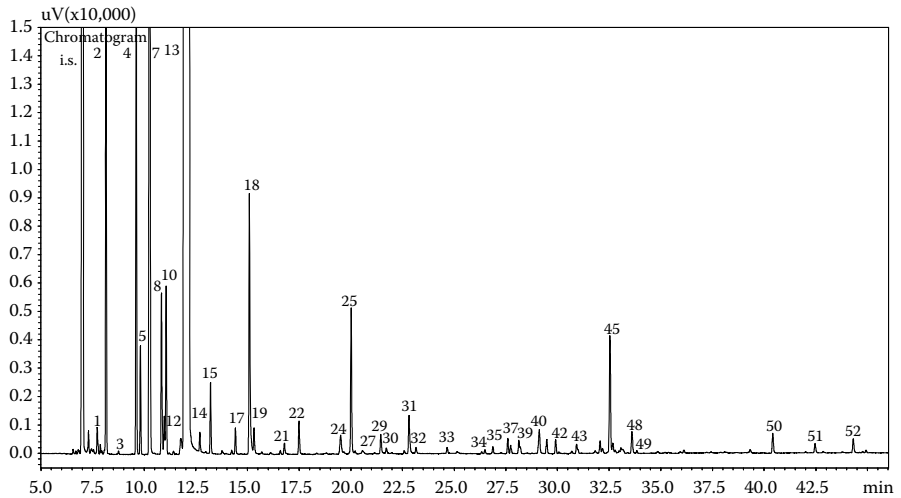

FIGURE 1.19 GC-FID chromatogram of sweet orange oil. Peak identification: **1** α-thujene; **2** α-pinene; **3** camphene; **4** sabinene; **5** β-pinene; **6** 6-methyl-5-hepten-2-one; **7** myrcene; **8** octanal; **9** α-phellandrene; **10** δ-3-carene; **11** α-terpinene; **12** *p*-cymene; **13** limonene + 1,8-cineole + (*Z*)-β-ocimene; **14** (E)-β-ocimene; **15** γ-terpinene; **16** *cis*-sabinene hydrate; **17** terpinolene; **18** linalol; **19** nonanal; **20** *cis*-limonene oxide; **21** *trans*-limonene oxide; **22** citronellal; **23** terpinen-4-ol; **24** α-terpineol; **25** decanal; **26** octyl acetate; **27** citronellol; **28** nerol; **29** neral; **30** geraniol; **31** geranial; **32** perilla aldehyde; **33** undecanal; **34** nonyl acetate; **35** citronellyl acetate; **36** neryl acetate; **37** α-copaene; **38** geranyl acetate; **39** β-cubebene+ β-elemene; **40** dodecanal; **41** β-caryophyllene; **42** *trans*-α-bergamotene; **43** α-humulene; **44** (*E*)-β-farnesene; **45** valencene; **46** germacrene D; **47** (*E,E*)-α-farnesene; **48** δ-cadinene; **49** (*Z*)-nerolidol; **50** β-sinensal; **51** α-sinensal; **52** nootkatone. (From Dugo, G., 2009. Personal communication.)

many of these studies were carried out on cultivars that had never been investigated before or had previously been investigated using inadequate analytical tools, so that these components could represent their peculiarities.

The geographic origin of the oils revised in this chapter prompt the following consideration: the systemic study of citrus essential oils in the last decade is still of scientific interest among Italian researchers, whereas the studies carried out in other European countries and in the United States are scant. It is surprising that in Central and South America the scientific interest in citrus essential oils is rare. It survives in a limited way in Cuba and Brazil but is totally absent in Mexico and in Argentina, where the citrus industry represents a growing commercial reference. A significant scientific interest in this field is noticed in Asia and Africa, mainly on essential oils extracted in laboratory and mostly obtained from cultivars of low industrial interest but characteristic of the country of origin. In many cases the oils were extracted by distillation, a process that can determine more or less drastic changes of the natural composition.

In general, the studies that have been done on citrus essential oils in the last decade are contradictory: on the one hand, there has been great progress in the advanced analytical techniques suitable for the study of these matrices, such as fast gas chromatography, conventional and comprehensive MD techniques eventually coupled to mass spectroscopy, and isotopic ratios determination. It has been also widely demonstrated that GC/MS cannot be reliable without the interactive use of retention parameters (LRI) with the mass spectral information (see Chapter 11). On the other hand, the great majority of the recent studies were carried out by GC/MS using commercial libraries of demonstrated unreliable success. Some of these articles reported large variability ranges of their values where LRI were used for the identification of the components, generating uncertainty for a secure identification, mainly for minor components.

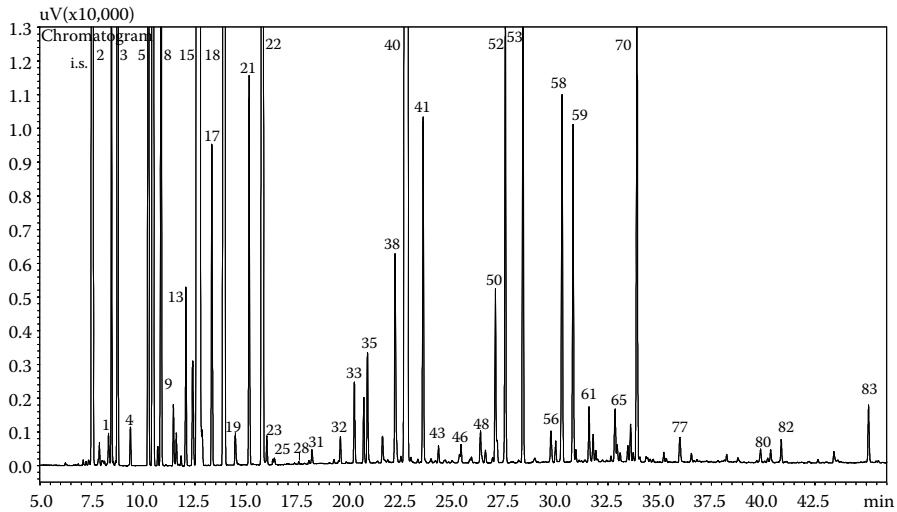

FIGURE 1.20 GC-FID chromatogram of cold-pressed bergamot oil. Peak identification: **1** tricyclene; **2** α-thujene; **3** α-pinene; **4** camphene; **5** sabinene; **6** β-pinene; **7** 6-methyl-5-hepten-2-one; **8** myrcene; **9** octanal; **10** α-phellandrene; **11** δ-3-carene; **12** hexyl acetate; **13** α-terpinene; **14** p-cymene; **15** limonene; **16** (*Z*)-β-ocimene; **17** (*E*)-β-ocimene; **18** γ-terpinene; **19** *cis*-sabinene hydrate; **20** octanol; **21** terpinolene; **22** linalol; **23** nonanal; **24** heptyl acetate; **25** 4,8-dimethyl-1,3(*E*),7-nonatriene; **26** fenchol*; **27** *cis*-limonene oxide; **28** *trans*-limonene oxide; **29** isopulegol; **30** camphor; **31** citronellal; **32** terpinen-**4**-ol; **33** α-terpineol; **34** decanal; **35** octyl acetate; **35** nerol; **37** citronellol; **38** neral; **39** carvone; **40** linalyl acetate; **41** geranial; **42** perillaldehyde; **43** bornyl acetate; **44** ascaridole; **45** undecanal; **46** nonyl acetate; **47** methyl geranate; **48** linalyl propanate; **49** δ-elemene; **50** α-terpinyl acetate; **51** citronellyl acetate; **52** neryl acetate; **53** geranyl acetate; **54** β-elemene; **55** dodecanal; **56** decyl acetate; **57** *cis*-α-bergamotene; **58** β-caryophyllene; **59** *trans*-α-bergamotene; **60** (*Z*)-β-farnesene; **61** (*E*)-β-farnesene; **62** α-humulene; **63** β-santalene; **64** γ-curcumene; **65** germacrene D; **66** *trans*-β-bergamotene; **67** bicyclogermacrene; **68** (Z)-α-bisabolene; **69** (*E,E*)-α-farnesene; **70** β-bisabolene; **71** (*Z*)-γ-bisabolene; **72** δ-cadinene; **73** β-sesquiphellandrene; **74** (*E*)-γ-bisabolene; **75** (*E*)-α-bisabolene; **76** *cis*-sesquisabinene hydrate; **77** (*E*)-nerolidol; **78** spathulenol; **79** *trans*-sesquisabinene hydrate; **80** 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol; **81** campherenol; **82** α-bisabolol; **83** nootkatone. (From Dugo, G., 2009. Personal communication.)

It is auspicious that in future research on citrus essential oils, analytical techniques and methods capable of generating reliable results will be used more widely, not only to confirm the quali-quantitative differences of the main components, but also to identify with certainty minor components as purity markers.

Table 1.22 reports the coelutions indicated by one or more authors on the different stationary phases used for gas chromatographic analysis of citrus oils. Figures 1.15 through 1.21 show the chromatograms of most of the citrus oils discussed in this chapter, obtained on slightly polar stationary phase (5% phenyl, 95% methylpolysiloxane).

Table 1.23 summarizes some of the most relevant quali-quantitative differences determined on the composition of industrial citrus essential oils. These values are relative to the information reported in literature from 1980 as relative percent of peak areas. The table reports components determined in at least two articles.

Limonene is the most abundant component of the volatile fraction of all the cold-pressed citrus peel essential oils, with the exception of bergamot: in bitter orange, grapefruit, tangerine, clementine, and sweet orange, it ranges between 90% and 96%; in mandarin oil it varies between 65% and 78%; in lemon oil the range is 60% to 72%; and in lime oils the range is 49% to 60%, with generally higher amounts in Persian lime than in Key lime oils. In bergamot limonene ranges widely (as it happens for many components of this citrus oil) between 23% and 50%, in

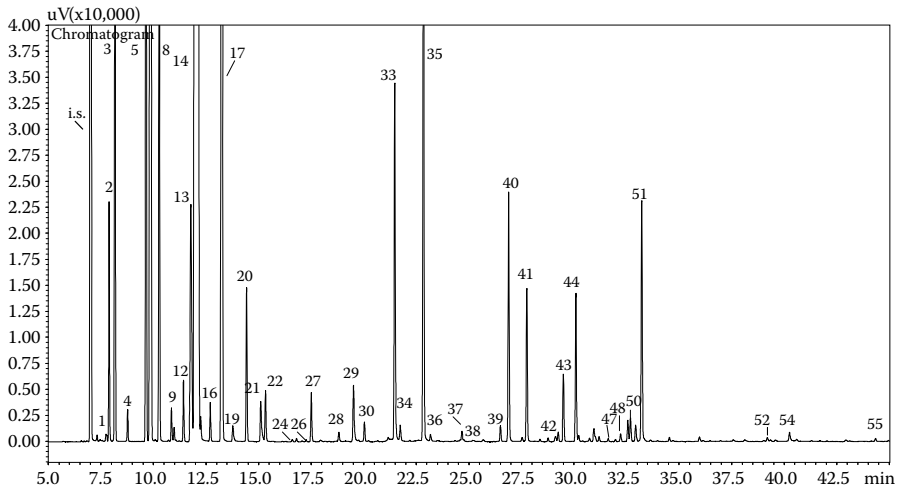

FIGURE 1.21 GC-FID chromatogram of cold-pressed lemon oil. Peak identification: **1** tricyclene; **2** α-thujene, **3** α-pinene; **4** camphene; **5** sabinene; **6** β-pinene; **7** 6-methyl-5-hepten-2-one; **8** myrcene; **9** octanal; **10** α-phellandrene; **11** δ-3-carene; **12** α-terpinene; **13** *p*-cymene; **14** limonene; **15** (*Z*)-β-ocimene; **16** (*E*)-β-ocimene; **17** γ-terpinene; **18** octanol; **19** *cis*-sabinene hydrate; **20** terpinolene; **21** linalol + *trans*-sabinene hydrate; **22** nonanal; **23** borneol; **24** *cis*-limonene oxide; **25** *p*-cymene-**8**-ol; **26** camphor; **27** citronellal; **28** terpinen-**4**-ol; **29** α-terpineol; **30** decanal; **31** octyl acetate; **32** nerol + citronellol; **33** neral; **34** geraniol; **35** geranial; **36** perilla aldehyde; **37** undecanal; **38** nonyl acetate; **39** citronellyl acetate; **40** neryl acetate; **41** geranyl acetate; **42** dodecanal; **43** β-caryophyllene; **44** *trans*-α-bergamotene; **45** (*Z*)-β-farnesene; **46** α-humulene; **47** β-santalene; **48** germacrene D; **49** valencene, **50** bicyclogermacrene; **51** β-bisabolene; **52** 2,3-dimethyl-3-(4-methyl-3-penten-1-yl)-2-norbornanol; **53** campherenol; **54** α-bisabolol; **55** nootkatone. (From Dugo, G., 2009. Personal communication.)

some cases the limonene content in this oil can be lower than linalol and/or linalyl acetate. The essential oils of lemon, bergamot, and lime are characterized by high values of β-pinene and γ-terpinene; in Key lime β-pinene is ca. double the amount of Persian lime, while γ-terpinene has an opposite behavior. Also abundant is γ-Terpinene in mandarin. δ-3-Carene is a key component, absent or present at trace levels in most of the citrus essential oils, with the exception of sweet orange, up to 0.3%. The variability of the percentage of δ-3-carene is determined by the cvs. used to extract the oils as shown by Verzera et al. (1996c). Among oxygenated components, monoterpene aldehydes, mainly geranial and neral, are present at relatively high levels in lime and lemon; in the latter the total aldehyde content (citral) represents a fundamental quality parameter. In bergamot oil, as previously mentioned, linalol and linalyl acetate are the predominant oxygenated components. In all the other citrus oils, these two components almost never exceed 1%, particularly linalyl acetate, which is often absent. Valencene, α- and β-sinensal, and nootkatone are usually found in bitter orange oil at levels lower than 0.1%; Koketsu et al. (1983) reported in a Brazilian oil the value of 0.29% of valencene, and Boelens and Oporto (1991) reported for Spanish bitter orange oil values of nootkatone up to 0.39%. Valencene and α- and β-sinensal are also present in sweet orange and clementine, while valencene and α-sinensal are also present in mandarin, and β-sinensal is present in grapefruit. Nootkatone is present at trace levels in lemon and bergamot. Mandarin oil is characterized by the presence of methyl *N*-methyl anthranilate, thymol, and thymol methyl ether. These last two components are also present in tangerine, and thymol and methyl *N*-methyl anthranilate are also found in clementine. For the quali-quantitative differences of other components, and of the sesquiterpene hydrocarbons in particular, please refer to Tables 1.1 through 1.21 in this chapter.

TABLE 1.23
Main Quali-quantitative Differences among Industrial Citrus Peel Oils

	1	2	3	4	5	6	7	8	9	10
	Bitter Orange	**Grapefruit**	**Key Lime**	**Persian Lime**	**Mandarin**	**Tangerin**	**Clementine**	**Sweet Orange**	**Bergamot**	**Lemon**
δ-3-Carene	tr–0.01	0–0.03	tr–0.01	tr–0.01	0–tr	–	0.04	tr–0.31	tr–0.02	tr–0.04
Limonene	91.54–96.52	92.20–96.20	47.87–51.14	51.47–59.81	65.30–77.82	89.58–90.94	94.82	91.15–96.10	23.5–54.85	59.57–71.82
β-Pinene	0.10–1.28	tr–0.26	18.25–25.45	10.01–12.20[a]	1.0–2.44	0.29–0.34	0.09	tr–0.11[e]	2.97–10.60	8.57–17.79
γ-Terpinene	tr–0.13	tr–0.3	6.19–9.79	12.55–15.65[a]	12.97–22.75[b]	2.15–3.46	0.68	0–0.33	1.15–11.38	2.88–11.38
Valencene	0–0.02[h]	0–tr	–	–	0–tr	–	tr	tr–0.38	–	tr–0.16
Geranial	0.01–0.10	tr–0.10	1.71–2.99	1.70–3.93	0–0.12	0.01–0.02	0.27[d]	tr–0.22	0.14–1.25	0.60–2.66
Neral	tr–0.05	tr–0.2	1.06–1.82	0.48–1.72	tr–0.09	tr–0.01	0.01	tr–0.20	0.05–0.72	0.34–1.33
α-Sinensal	0–tr	–	–	–	0.12–0.53	0.11–0.20	0.19	0–0.04	–	–
β-Sinensal	tr–0.01	tr–0.01	–	–	–	–	0.04	tr–0.10	–	–
Nootkatone	0–0.39[f]	tr–0.84[g]	–	–	–	–	–	–	tr–0.10	tr–0.03
Linalol	0.06–0.37	tr–0.16	0.15–0.24	0.11–0.24	0.02–0.31	0.54–0.74	0.21	0.17–0.8	1.58–36.14	0.05–0.46
Thymol	–	–	–	–	tr–0.18	0.05–0.07	tr	–	–	0–tr
Geranyl acetate	tr–0.20	0.02–0.13	0.18–0.35	0.07–0.63	tr–0.04[c]	tr–0.01	tr	0–0.03	0.11–0.88[j]	0.06–0.86
Linalyl acetate	0.08–1.17	0–0.06	–	tr–0.12	tr–0.04	–	tr	0–0.06	11.80–41.36	–
Neryl acetate	0.01–0.05	tr–0.1	0.07–0.39	0.36–1.11[i]	tr–0.01	0.01–0.03	tr	tr–0.06	tr–0.8	0.07–0.88
Methyl *N*-Methyl anthranilate	–	–	–	–	tr–0.66	–	0.02	–	–	–
Thymol methyl ether	–	–	–	–	0–0.3	0.03–0.05	–	–	–	–

Notes: tr, traces; [a] not included the values of β-pinene and γ-terpinene reported by Koketsu et al. (1983) in Persian lime, also not included are the values of β-pinene when coeluted with sabinene; [b] not included the value (7.21%) of γ-terpinene reported by Pino et al. (2006) and by Pino and Quijano-Celís (2007) in mandarin oil; [c] not considered the value of geranyl acetate in mandarin (0.31%) reported by Oberhofer et al. (1999); [d] geranial+perylla aldehyde; [e] not included results obtained for sweet orange where β-pinene co-eluted with sabinene;

continued

TABLE 1.23 (continued)
Main Quali-quantitative Differences among Industrial Citrus Peel Oils

[f] high values of nootkatone (up to 0.39%) in bitter orange were determined by Boelens and Sindreu (1988), by Boelens and Jimenez (1989), Boelens and Oporto (1991), Boelens (1991) in Spanish oils, in other oils the maximum is 0.03%; [g] nootkatone high values in grapefruit oil were determined by Inoma et al. (1989) in a commercial oil (0.84%), by Boelens (1991) in a sample from Israel (0.30%), by Feger et al. (2001b) in samples from Israel and from Florida (0.28%–0.37%), in all the other oils the maximum was 0.14%. It is possible that the nootkatone content depends on the fruit transformed (white or pink). Shulz et al. determined by HPLC values of nootkatone between 0.2% and 0.7%; [h] Koketsu et al. (1983) reported in bitter orange valencene at 0.29%; [i] not considered the value 1.53% determined by Haro and Faas (1985) in Persian lime oil since neryl acetate was coeluted with an unknown compound; [j] not included the value of geranyl acetate (2.4%) determined by Russo et al. (2001) in bergamot oil.

Appendix to Table 1.23

1. Koketsu et al. (1983); Boelens and Sindreu (1988); Boelens and Jimenez (1989a); Boelens and Oporto (1991); Boelens (1991); Dugo et al. (1993, 1999, 2010a), Dugo (1994); Mondello et al. (2003, 2004b).
2. Cappello et al. (1981); Koketsu et al. (1983); Inoma et al. (1989); Haubruge et al. (1989); Boelens (1991); Dugo et al. (1999); Pino and Sanchez (2000); Feger et al. (2001b); Viuda-Martos et al. (2009).
3. Haro and Faas (1985); Dugo et al. (1997); Dugo et al. (1999, 2010b); Kubeczka and Formáček (2002).
4. Koketsu et al. (1983); Haro and Faas (1985); Lancas et al.(1988); Dugo et al. (1997); Dugo et al. (1999, 2009b); Franceschi et al. (2004); Mondello et al. (2004c).
5. Wilson and Shaw (1981); Koketsu et al. (1983); Dugo et al. (1984, 1990, 1999, 2010c); Dugo (1994); Mazza (1987a); Oberhofer et al. (1999); Mondello et al. (2003, 2004b); Pino et al. (2006); Pino and Quijano-Celís (2007); Bonaccorsi et al. (2009); Viuda-Martos et al. (2009); Schipilliti et al. (2010).
6. Dugo et al. (2005).
7. Dugo et al. (1999).
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2 Volatile Components in Less Common Citrus Species

Estelle Delort, Regula Naef

CONTENTS

2.1 INTRODUCTION

The genus *Citrus* comprises hundreds of species, varieties, and hybrids issued from natural or human-controlled crossbreeding. Conventional fruits such as oranges, lemons, grapefruits, pummelos, limes, mandarins-tangerines, and yuzu are produced for commercial purposes. In addition, new hybrids with increased phytosanitary and organoleptic performances have been created. At the same time, there exists a category of unusual citrus fruits, either growing wild, limited to local interest, or with minor sensory value that are often used as rootstocks for trees with greater tolerance to extreme temperatures, resistance to diseases, and better yield and quality of fruits. Lawrence (2002) has reviewed a series of these less common *Citrus* species: *Citrus aurantium* L. var. *myrtifolia*, *C. clementina*, *C. grandis*, *C. hystrix*, *C. jambhiri*, *C. junos*, *C. madurensis*, *C. medica*, *C. medica* var. *ethrog*, *C. medica* var. *sarcodactylis*, and *C. unshiu*. Meanwhile, the volatile composition of many more of these interesting members of the *Citrus* genus has been investigated and will be discussed in this chapter. Recently, phylogenetic studies of plants of the genus *Citrus* and the related *Fortunella*, *Microcitrus*, *Poncirus*, and *Eremocitrus* have indicated that these genera have very similar genetic systems and can therefore all be classified in the genus *Citrus* (Mabberley, 1998; de Araújo et al., 2003). Some of these species will also be included in this review.

However, we cannot guarantee an exhaustive overview due to the complexity of the subject. In particular, we will not describe the panoply of hybrids, for example, hallabong ([*Citrus unshiu* Marcov x *C. sinensis* Osbeck] x *C. reticulata* Blanco), minneola (*Citrus reticulata* x *C.* x *paradisi*), and so on. In addition, we have decided to focus on both the main constituents and the odor-impact compounds that characterize their aroma. This review will be divided into four parts that summarize the volatile composition of *Fortunella* species (kumquats), Japanese citrus, Australian native citrus, and various other citrus, respectively. Tables 2.1, through 2.4 summarize the relative percentages of the major volatile constituents of cold-pressed peel oils (when available), leaf oils, and juice extracts, present at an abundance of >0.2%. Corresponding references are included. The most recent and/or most representative results have been chosen for comparison. Table 2.5 gives information about the odor-impact compounds of some citrus fruits. Further investigations, mostly steam distillates, are described in the text.

2.2 SELECTED LESS COMMON *CITRUS* SPECIES

2.2.1 *FORTUNELLA* SPECIES

The species *Fortunella*, or kumquat, is a small fruit-bearing tree native to China that spread to Korea, Japan, Florida, California, and the eastern Mediterranean areas. Five species are currently accepted, namely *Fortunella japonica* (Marumi kumquat), *F. margarita* (Nagami kumquat), *F. crassifolia* (Meiwa kumquat), *F. hindsii* (Hong Kong Wild kumquat), and *F. polyandra* (Malayan kumquat). The fruit, with a yellow-to-reddish-orange peel bearing large oil glands, varies in shape from round to oval/oblong. Unlike most of the citrus fruits, kumquat fruits can be eaten whole. Kumquats generally mature from late November to February. They are used as decorative fruits, but also as marmalades, candied juice, and jellies. Their small size makes processing difficult, but the candied orange and flowery notes of kumquat essential oil may be of interest to fine fragrances.

2.2.1.1 *Fortunella japonica* Swingle

Fortunella japonica Swingle (also called the "Marumi kumquat" or "round kumquat") trees grow to about two meters in height, with fine stems, few thorns, and dark green pointed leaves. The fruit varies in shape from round to slightly oval (~2 cm diameter), averaging around 10–12 grams. It

TABLE 2.1
Major Volatile Constituents Reported in *Fortunella* Peel and Leaf Oils

	F. japonica (CPPO: Choi, 2005)	*F. margarita* (CPPO: Delort, 2007)	*F. crassifolia* (S.E.: Hashinaga and Itoo, 1990)	*F. crassifolia* (S.D.: Scora et al., 1969)
	Peel	**Peel**	**Peel**	**Leaf**
		Hydrocarbons		
α-Pinene	0.4	0.5	4.6[a]	
β-Pinene			1.4	
Myrcene	1.8	2.0	14.0	
Limonene	93.7	91.1	58.4	
γ-Terpinene	0.3			
δ-elemene			4.5	
β-Elemene				6.7
β-Caryophyllene				3.8
Germacrene D		1.2	12.3	
Bicyclogermacrene		0.2		
		Aldehydes		
Octanal			0.2	
Citronellal			0.5	
(2*E*)-2-Nonenal			0.2	
Neral			0.6	
Tetradecanal				4.8
		Ketones		
Jasmone				1.0
		Alcohols		
(3*Z*)-3-Hexen-1-ol			1.1	
3-Octanol				0.3
Linalool				1.2
α-Terpineol	0.4			21.9
Nerol			0.5	
Geraniol			0.2	8.9
Undecanol			0.8	
Dodecanol				0.4
Farnesol				0.9
		Esters		
Ethyl acetate	1.1		4.7	
Octyl acetate				9.6
Nonyl acetate				4.5
Octyl butanoate			0.3	
Terpinyl acetate			4.2[b]	4.7
Geranyl acetate		0.2		2.9

continued

TABLE 2.1 (continued)
Major Volatile Constituents Reported in *Fortunella* Peel and Leaf Oils

Phenols	
Thymol	1.5
Carvacrol	3.5
N-Compounds	
Methyl anthranilate	2.5

Notes: Compounds are listed by chemical class and in elution order from a nonpolar column. Only compounds >0.2% are reported. CPPO: cold-pressed peel oil; S.E.: solvent extract; S.D.: steam distillation; [a] α-pinene + ethyl butanoate. [b] terpinyl acetate + geranial.

has a rather sour taste, but its peel is edible. Only a few investigations on the volatile constituents of the peel are known. Umano et al. (1994) prepared the extract of fresh fruits obtained from the Wakayama Prefecture in Japan by steam distillation under reduced pressure. The major components were limonene (87.1%), linalool (1.4%), myrcene (1.3%), geranyl acetate (1.1%), α-pinene (0.8%), and germacrene D (0.8%). Compared to other citrus oils, the oil of *F. japonica* contained a larger variety of terpenyl alcohols and esters, such as terpinen-4-ol, *p*-menth-1-en-9-yl acetate, *trans-p*-mentha-2,8-dien-1-ol and its acetate, *p*-mentha-1,8-dien-9-yl acetate and its propanoate, as well as higher amounts of sesquiterpenoids. In particular, hinesol, a tertiary alcohol with a spirovetivane skeleton, was reported for the first time in a citrus essential oil.

Recently, Choi (2005) analyzed the cold-pressed peel oil obtained from fresh fruit harvested in Jeolla province, Korea (Table 2.1). The major constituents were limonene, myrcene, and α-pinene. The odor activity of each compound was determined by Aroma Extract Dilution Analysis (AEDA). Citronellyl acetate, citronellyl formate, and to a lesser extent camphene and terpinen-4-ol, appeared to be important aroma compounds of *F. japonica* Swingle (Table 2.5).

2.2.1.2 *Fortunella margarita*

Fortunella margarita (also called "Nagami kumquat" or "ovoid kumquat") is the most familiar species in the Western world. It has an oval shape and a smooth, bright orange flavedo. When eaten whole, the orange-like aroma of the peel and woody, floral, sweet notes complement very well the rather sour, acidic pulp. The volatile constituents of *F. margarita* were first reported by Bernhard and Scrubis (1961). The oil was prepared by steam distillation of the epicarp of fruits harvested in California and studied by gas-liquid chromatography. This oil contained large amounts of limonene, some α-pinene, myrcene, aldehydes (decanal, citronellal) and terpenyl esters (geranyl acetate, terpinyl acetate, bornyl acetate, linalyl acetate). In 1983, Koyasako and Bernhard repeated the study using gas chromatographic and mass spectrometric techniques to identify and quantify the volatile components of *F. margarita* from California. The kumquat peel oil was obtained by simultaneous distillation extraction (SDE) of the peel. The major components were limonene (92.7%); sesquiterpene hydrocarbons (0.4%); and oxygenated sesquiterpenoids (2.1%). Both studies showed that, compared to other citrus oils, kumquat oil contains a higher number of esters and less aldehydes. This observation was confirmed by Kwag et al. (1992), who identified limonene (96.5%); β-pinene (1.9%); α-terpineol (0.4%); γ-terpinene (0.2%); β-terpineol (0.1%); geranyl acetate (0.07%); linalool (0.05%); octyl acetate (0.04%); and terpinen-4-ol (0.04%) as major constituents in an extract prepared by SDE. Recently the cold-pressed peel oil of *F. margarita* from Sicily was analyzed by GC-MS and quantified using FID percent with an internal standard and correction factors (Delort, 2007) (Table 2.1). Nonanal and decanal were almost totally absent. Interestingly, a series of less common terpenyl alcohols and acetates was identified in trace amounts: 2,8-*p*-menthadien-1-ol, *trans*-isopiperitenyl acetate, 1,8(10)-*p*-menthadien-9-yl acetate, 1-*p*-menthen-9-yl acetate, and perillyl acetate. These compounds may contribute to the fruity-floral character of the peel.

TABLE 2.2
Major Volatile Constituents Reported in Japanese Citrus Peel and Leaf Oils

	C. aurantium (CPPO: Song et al., 2000a)	*C. aurantium* (S.D.: Huang and Pu, 2000)	*C. depressa* (S.D.: Fujita, 2004)	*C. depressa* (H.D.: Fujita, 2004)	*C. flaviculpus* (S.E.: Katayama and Iwabuchi, 2002; CPPO: Choi, 2001)	*C. inflata* (CPPO: Minh Tu et al., 2003a)	*C. junos* (CPPO: Song et al, 2000b)	*C. kinokuni* (S.E.: Shiota and Ito, 1991)	*C. kinokuni* (S.D.: Huang and Pu, 2000)	*C. sphaerocarpa* (CPPO: Minh Tu et al., 2002)	*C. sudachi* (CPPO: Njoroge et al.,1995)	*C. taguma-sudachi* (CPPO: Njoroge et al., 1996)	*C. tamurana* (CPPO, Choi, 2000)	*C. yuko* (CPPO: Njoroge et al., 1994)
	Peel	Leaf	Peel	Leaf	Peel	Peel	Peel	Peel	Leaf	Peel	Peel	Peel	Peel	Peel
Hydrocarbons														
α-Thujene								0.7	1.3					
α-Pinene	0.6		2.0–4.4	3.5–5.7	2.0/0.8	0.4	2.0	2.3	4.2	0.7	1.6	1.0	1.3	3.3
Camphene									0.2					
Sabinene	0.2	0.3	0.2–0.3	0.3–0.8			0.8		4.7		0.4			0.4
β-Pinene	0.4	1.3	1.2–2.8	2.5–4.3	1.2/0.4			1.9	26.8	0.2	0.5	0.3	0.8	1.8
Myrcene	2.2	2.5	1.6–1.9	0.9–1.8	3.3/1.5	24.6	0.4	1.5	0.8	20.2	1.3	1.8	2.3	1.3
α-Terpinene			0.3–0.6	0.4–0.9			0.2		0.47					0.3
α-Phellandrene				0.0–0.6			0.9				1.3			1.2
p-Cymene			1.4–7.6	2.7–6.2			0.3	1.5	2.2		0.4			0.2
Limonene	92.5	0.6	44.7–72.9	3.0–3.8	61.6/82.4	71.3	77.4	62.0	2.8	70.5	69.1	90.5	82.4	66.6
(*Z*)-β-Ocimene		0.9							0.2					
β-Phellandrene			0.1–0.9	0.0–8.1			2.2				7.2			
(*E*)-β-Ocimene	0.3	2.3				0.9			2.8			0.8		
δ-3-Carene													0.6	
γ-Terpinene			15.2/30.4	29.4–55.0	13.1/8.8		9.4	20.4	13.5	2.6	7.5	4.2	7.7	21.3
α-Terpinolene		0.4	0.9–1.5	1.5–2.2	0.8/0.4			0.3	1.3		0.3	0.2	0.4	0.9
β-Elemene				0.0–3.2							2.0			0.8
β-Caryophyllene		0.2	0.1–0.4	0.5–3.4					0.2	0.2				
α-Humulene				0.0–0.9							0.6			
(*E*)-β-Farnesene					2.9/1.8	0.3	0.5			0.4	0.7	0.8	0.6	0.2
germacrene D			0.4–0.9	0.8–2.0						0.2	1.5			0.2
Valencene					1.3/0.3									
Bicyclogermacrene			0–0.9	0.5–2.2			0.7							
Germacrene A				0.0–3.2										
(*E,E*)-α-Farnesene									0.3		2.2			

continued

TABLE 2.2 (continued)
Major Volatile Constituents Reported in Japanese Citrus Peel and Leaf Oils

	C. aurantium (CPPO: Song et al., 2000a)	*C. aurantium* (S.D.: Huang and Pu, 2000)	*C. depressa* (S.D.: Fujita, 2004)	*C. depressa* (H.D.: Fujita, 2004)	*C. flaviculpus* (S.E.: Katayama and Iwabuchi, 2002; CPPO: Choi, 2001)	*C. inflata* (CPPO: Minh Tu et al., 2003a)	*C. junos* (CPPO: Song et al., 2000b)	*C. kinokuni* (S.E.: Shiota and Ito, 1991)	*C. kinokuni* (S.D.: Huang and Pu, 2000	*C. sphaerocarpa* (CPPO: Minh Tu et al., 2002)	*C. sudachi* (CPPO: Njoroge et al.,1995)	*C. tagumasudachi* (CPPO: Njoroge et al., 1996)	*C. tamurana* (CPPO, Choi, 2000)	*C. yuko* (CPPO: Njoroge et al., 1994)
	Peel	Leaf	Peel	Leaf	Peel	Peel	Peel	Peel	Leaf	Peel	Peel	Peel	Peel	Peel
δ-Cadinene			0.2–2.5	0.4–1.3						0.2				
Germacrene B									0.2					
Aldehydes														
(2*E*)-2-Hexenal				0.0–0.7										
Octanal	0.6									0.7				
Citronellal				0.0–0.2									0.3	
Decanal	0.2		0–0.2					0.2	0.2	0.5		0.3		
Ketones														
l-Carvone													0.4	
Piperitone			0.4–1.3	0.0–17.4										
Alcohols														
(3*Z*)-3-Hexen-1-ol				0.4–1.5										
Octanol								0.7						
Linalool	0.2	39.8	1.0–2.8	10.0–17.4	1.1/0.4	0.2	1.6	0.5	23.0		0.3		1.4	0.2
Terpinen-4-ol			0.2–0.3	0.2–0.4				0.2	0.4					
α-Terpineol		9.9			0.3/0.1			0.8	0.6		0.2			0.5
Citronellol				0.0–0.2										
Nerol		1.7												
Geraniol		5.5												
cis-Carveol											0.6			
(*E*)-Nerolidol				0.0–0.2			0.2							
Spathulenol				0.0–0.4										
Phytol				0.0–0.4										

Esters							
Ethyl acetate	0.3						
Linalyl acetate	1.4	24.8					
Neryl acetate		2.8					
Geranyl acetate	0.2	5.1					
Phenols							
Thymol methyl ether			0–0.9	0.0–7.3			
Thymol			0–2.6	0.0–12.0		0.4	9.8
Methoxythymol				0.0–0.3			
N-Compounds							
Methyl *N*-methyl anthranilate						2.0	

Notes: Compounds are listed by chemical class and in elution order from a nonpolar column. Only compounds >0.2% are reported. CPPO: cold-pressed peel oil; S.D.: steam distillate; S.E.: solvent extract; H.D.: hydrodistillate.

TABLE 2.3
Major Volatile Constituents Reported in Australian Native Citrus Peel and Leaf Oils

	C. australis (S.E.: Craske et al., 2005)	*C. australis* (H.D.: Brophy and Goldsack, 2001)	*C. australasica* (S.E.: Delort and Jaquier, 2009)	*C. australasica* (H.D.: Brophy, 2001)	*C. garrowayi* (H.D.: Brophy 2001, chem1/chem2)	*C. glauca* (H.D.: Brophy, 2001)	*C. gracilis* (H.D.: Brophy, 2001)	*C. inodora* (H.D.: Brophy, 2001)
	Peel	Leaf	Peel	Leaf	Leaf	Leaf	Leaf	Leaf
				Hydrocarbons				
α-Thujene	0.2						0.8	
α-Pinene	1.3	78.4	0.4	5.0	18.2/0.0	40.9	2.2	
Camphene		0.4			0.2/0.0	0.4		
Sabinene	2.2	0.8		0.6		2.0	0.3	
β-Pinene	13.1	2.3	0.4	0.4	0.2/0.0	14.4	2.3	
Myrcene	1.0	3.7	1.7	4.4	1.2/0.0	1.4	0.8	0.2
α-Terpinene							0.3	
α-Phellandrene			0.3	0.9				
p-Cymene				0.5			14.8	
Limonene	35.1	2.1	73.5	12.8	0.4/0.2	1.3	3.7	
(*Z*)-β-Ocimene		0.4	1.5	2.1	0.2/0.2			
β-Phellandrene		1.5		20.2	0.5/0.0	0.6		
(*E*)-β-Ocimene	0.2	0.2	0.3	4.8	0.0/0.5			
δ-3-Carene		0.3		1.0				
γ-Terpinene	11.2			2.6			33.8	
α-Terpinolene	0.4			0.5			1.9	
Bicycloelemene				0.4			0.2	0.6
δ-Elemene			0.2	0.5	1.9/0.2			4.9
α-Cubebene					4.4/0.2			
α-Copaene					0.8/0.7			
β-Bourbonene					0.0/0.2		3.3	
β-Elemene	0.7	0.2			0.3/2.0			0.6
β-Caryophyllene	1.0		0.2	0.5	12.0/17.6	7.4		4.7
α-Bergamotene	1.6							

γ-Elemene								1.3
α-Humulene			0.2		2.7/2.9	1.4	0.2	0.6
Germacrene D	0.5		0.2	7.7	0.0/2.1			23.7
β-Selinene					5.6/8.2			
Cadina-1,4-diene					1.9/1.1			1.0
Viridiflorene				0.5			0.4	
α-Selinene					4.1/5.0			
Bicyclogermacrene		1.7	0.6	19.8	7.0/2.9	6.2	10.2	17.3
(*E*,*E*)-α-Farnesene	3.3	0.2		2.8	2.0/1.7		1.0	
β-Bisabolene	2.8							
δ-Cadinene		0.2		0.6	3.0/1.6			2.6
Calamenene					0.0/0.5			
Germacrene B	0.9							8.1
Ethers								
Caryophyllene oxide					0.4/1.8			
Aldehydes								
Nonanal						0.8		
Citronellal			2.6	4.3				
Decanal	0.8					0.4		
Neral	4.5							
Geranial	7.3							
Ketones								
Isomenthone			7.5					
Piperitone			0.5					
Alcohols								
Linalool oxide (furanoid) isomer 1						9.6		
Linalool oxide (furanoid) isomer 2						5.0		
Linalool	0.3	1.0	1.8			0.3	0.3	3.0
Terpinen-4-ol								
α-Terpineol	0.7	0.3	0.2			0.4		

continued

TABLE 2.3 (continued)
Major Volatile Constituents Reported in Australian Native Citrus Peel and Leaf Oils

	C. australis (S.E.: Craske et al., 2005)	*C. australis* (H.D.: Brophy and Goldsack, 2001)	*C. australasica* (S.E.: Delort and Jaquier, 2009)	*C. australasica* (H.D.: Brophy, 2001)	*C. garrowayi* (H.D.: Brophy 2001, chem1/chem2)	*C. glauca* (H.D.: Brophy, 2001)	*C. gracilis* (H.D.: Brophy, 2001)	*C. inodora* (H.D.: Brophy, 2001)
	Peel	**Leaf**	**Peel**	**Leaf**	**Leaf**	**Leaf**	**Leaf**	**Leaf**
Citronellol			0.4	0.9				
(*E*)-Nerolidol							20.4	
Spathulenol				0.5	0.0/0.3	1.8	1.8	2.7
Ledol		0.4			1.9/2.1			
Globulol		1.4		0.6	9.3/9.6	0.5	0.4	1.0
Viridiflorol		3.2		0.8	9.2/9.2	0.3	0.3	0.8
epi-Cubenol					0.0/0.6			
Cubenol					0.0/0.3			
T-cadinol					0.0/1.3			
α-Bisabolol					0.0/2.7			
Phytol				5.0	0.0/1.9		3.9	
				Esters				
Neryl acetate	0.4							
Geranyl acetate	0.4							
				Coumarins				
7-Methoxycoumarin	1.1							
5,7-Dimethoxycoumarin	3.4							
Iso-bergaptene	0.2							
Bergaptene	0.4							
Isopimpinellin	3.5							

Notes: Compounds are listed by chemical class and in elution order from a nonpolar column. Only compounds >0.2% are reported. S.E.: solvent extract; H.D.: hydrodistillate; chem: chemotype.

TABLE 2.4
Major Volatile Constituents Reported in Other Citrus Peel, Leaf, and Juice Oils

	C. ichangensis (CPPO: Lota et al., 2002/ Sawamura, 2000)	*C. ichangensis* (H.D.: Lota et al., 2002)	*C. limetta* (S.E.: Naef and Velluz, 2003/ S.D.: Sattar, 1992)	*C. nobilis* (CPPO: Dharmawan et al., 2009)	*C. nobilis* (S.E.: Dharmawan et al., 2009)	*Poncirus trifoliata* (S.D.: Scora et al., 1966)	*Poncirus trifoliata* (S.D.: Scora et al., 1969)	*Poncirus trifoliata* (S.D.: Scora et al., 1966)
	Peel	Leaf	Peel	Peel	Juice	Peel	Leaf	Juice
				Hydrocarbons				
α-Thujene	0.5/0	1.3			0.2			
α-Pinene	1.4/3.3	3.5	0.8/0.9	0.4	0.6	1.2		0.4
Camphene								0.3
Sabinene	0.9/0.9	1.8	0.2/0.0					
β-Pinene	4.5/1.8	14.0	2.9/2.1	0.4	0.5		0.8	
Myrcene	1.6/0.7	1.2		2.2	0.4	20.4	29.8	0.4
α-Terpinene	0.4/2.4	0.8						
α-Phellandrene	0.6/0	0.7	0.0/0.2			3.8		
p-Cymene	0.3/0	2.1	0.0/1.4			1.9		1.5
Limonene	63.8/68.8	3.5	85.1/60.2	95.7	89.6	40.7		1.8
(*Z*)-β-Ocimene	0.3/0							
β-Phellandrene	4.2/3.7	5.1						
(*E*)-β-Ocimene		4.0					13.2	
γ-Terpinene	18.0/16.0	36.1	0.0/11.8		0.3	13.5	4.3	1.6
α-Terpinolene	0.8/0.7	1.8	0.0/3.0		0.6			
β-Caryophyllene		0.2	0.0/0.4					
α-Bergamotene			0.8/0.0					
α-Humulene	0.2/0							
Germacrene D	0.4/0.2		0.8/0.0					
β-Bisabolene	0.2/0		1.0/0.0					
δ-Cadinene					0.3			

continued

TABLE 2.4 (continued)
Major Volatile Constituents Reported in Other Citrus Peel, Leaf, and Juice Oils

	C. ichangensis (CPPO: Lota et al., 2002/ Sawamura, 2000)	*C. ichangensis* (H.D.: Lota et al., 2002)	*C. limetta* (S.E.: Naef and Velluz, 2003/ S.D.: Sattar, 1992)	*C. nobilis* (CPPO: Dharmawan et al., 2009)	*C. nobilis* (S.E.: Dharmawan et al., 2009)	*Poncirus trifoliata* (S.D.: Scora et al., 1966)	*Poncirus trifoliata* (S.D.: Scora et al., 1969)	*Poncirus trifoliata* (S.D.: Scora et al., 1966)
	Peel	Leaf	Peel	Peel	Juice	Peel	Leaf	Juice
Ethers								
trans-8,9-Epoxy-1-*p*-menthene			0.3/0.0					
Aldehydes								
Furfural							1.1	1.1
Octanal			0.0/0.6			0.8		0.3
Nonanal				0.2	0.2	0.4		1.6
Citronellal		1.7	2.6/0.0		0.5	2.4		0.2
Decanal				0.2	0.5			0.3
Neral		2.3	0.0/0.6		0.4			0.7
Geranial		3.4	0.0/1.3		0.2	0.2		0.4
Undecanal					0.3			
Ketones								
l-Carvone					0.5			
6-Methyl-hept-5-en-2-one		0.6						
Piperitone								
Alcohols								
Octanol								0.5
cis-Linalool oxide (furanoid)					0.3			
Linalool	0.2/0.2	4.6	0.6/1.9	0.5	0.6	0.7		13.0
Isopulegol								0.7

Nonanol					1.7		9.8
Terpinen-4-ol		0.4		0.2			
α-Terpineol	0.0/0.3	0.3	0.0/1.3	1.0	0.9	2.4	1.7
Citronellol		1.1	0.2/0.0	0.8			
Nerol		2.5			0.6	0.4	
Geraniol		0.7	0.0/0.2				
(*E*)-Nerolidol		0.2					
Esters							
3-Octyl acetate						0.5	
Octyl acetate					0.4		0.4
Linalyl acetate			0.0/0.2			0.3	
Neryl formate					1.9		0.5
Nonyl acetate							0.4
Terpinyl acetate	0.5/0.0					0.3	
Citronellyl acetate	0.2/0.0	0.3	0.3/0.0		0.3		0.5
Neryl acetate	0.3/0.0	4.4	0.0/0.5			25.2	0.4
Geranyl acetate		0.4	0.0/0.3	0.4	0.4	2.6	0.5
Neryl butyrate						3.7	
Geranyl butyrate						1.4	
Phenols							
Thymol						1.2	
Carvacrol						0.8	
p-Vinylguaiacol				0.4			
N-Compounds							
Methyl anthranilate						1.0	

Notes: Compounds are listed by chemical class and in elution order from a nonpolar column. Only compounds >0.2% are reported. CPPO: cold-pressed peel oil; S.E: solvent extract; S.D.: steam distillate; H.D.: hydrodistillate.

TABLE 2.5
Selection of Key Odor-Impact Compounds Found by Means of AEDA and GC-Olfactometry in Peel Oils of Less Common *Citrus* Species

	F. japonica (Choi, 2005)	*C. aurantium L. var. cyathifera* (Song, 2000a)	*C. flaviculpus* (Choi 2002; Katayama and Iwabuchi, 2002; Choi, 2001)	*C. inflata* (Minh Tu et al., 2003b; Njoroge, 1994)	*C. junos* (Miyazawa et al., 2009; Yukawa et al., 1994; Escher et al., 2006; Song, 2000b)	*C. kinokuni* (Shiota, 1991)	*C. sphaerocarpa* (Minh Tu et al., 2003b; Minh Tu et al. 2002; Yang, 1992)	*C. sudachi* (Yang, 2000; Mookdasanit et al., 2003)	*C. tamurana* (Choi, 2001)	*C. limetta* (Naef and Velluz, 2003)	*C. nobilis* (Fisher et al., 2008; Dharmawan et al., 2009)	*Poncirus trifoliata* (Starkenmann et al., 2007)
Hydrocarbons												
α-Pinene												
Camphene	×											
Myrcene				×			×				×	
Limonene									×		×	
(*E*)-β-Ocimene				×			×					
Tetradecane			×									
β-Elemene				×								
β-Copaene							×					
(*E*)-β-Farnesene								×				
(*E*,*E*)-α-Farnesene								×				
Aldehydes												
(3*Z*)-3-Hexenal			×									
Octanal		×						×			×	
Nonanal								×				
Citronellal			×				×[a]	×				
Decanal								×				
(2*E*,4*E*)-2,4-Nonadienal											×	
Cumin aldehyde								×				
Neral							×		×			
(2*E*)-2-Decenal		×					×				×	
Geranial		×										
Perillaldehyde				×			×					
(2*E*,4*E*)-2,4-Decadienal											×	
(2*E*)-2-Undecenal			×		×							
(5*Z*)-5-Dodecenal											×	

Dodecanal									×				
(2*E*,6*Z*)-2,6-Dodecadienal													×
(2*E*)-2-Dodecenal			×										×
Tridecanal											×		
β-Sinensal												×	
α-Sinensal												×	
Ketones													
Carvone									×				
Raspberry ketone			×										
Methyl epijasmonate			×									×	
Nootkatone			×									×	
(6*Z*,8*E*)-6,8,10-Undecatrien-3-one (yuzunone)					×[b]								
Alcohols													
Octanol					×					×			
cis-Linalool oxide (furanoid)				×			×		×				
trans-Linalool oxide (furanoid)				×			×						
Linalool			×						×	×			×
Borneol					×								
Terpinen-4-ol	×												
α-Terpineol			×		×								
trans-Carveol										×			
Citronellol			×						×			×	
Nerol													×
Geraniol		×											×
Perillyl alcohol				×			×						
β-Elemol		×											
(*E*)-Nerolidol										×			
Cedrol		×											
(*E*,*E*)-Farnesol										×			
(6*Z*,8*E*)-6,8,10-Undecatrien-4-ol (yuzuol)					×[b]								
Esters													
Ethyl 2-methylbutanoate			×										
Octyl acetate		×					×						
Citronellyl formate	×		×										
Geranyl acetate													
Neryl acetate										×			

continued

TABLE 2.5 (continued)
Selection of Key Odor-Impact Compounds Found by Means of AEDA and GC-Olfactometry in Peel Oils of Less Common *Citrus* Species

	F. japonica (Choi, 2005)	*C. aurantium L. var. cyathifera* (Song, 2000)	*C. flaviculpus* (Choi 2002; Katayama and Iwabuchi, 2002; Choi, 2001)	*C. inflata* (Minh Tu et al., 2003; Njoroge, 1994)	*C. junos* (Miyazawa et al., 2009; Yukawa et al., 1994; Escher et al., 2006; Song 2000)	*C. kinokuni* (Shiota, 1991)	*C. sphaerocarpa* (Minh Tu et al., 2003b; Minh Tu et al., 2002; Yang, 1992)	*C. sudachi* (Yang, 2000; Mookdasanit et al., 2003)	*C. tamurana* (Choi, 2001)	*C. limetta* (Naef and Velluz, 2003)	*C. nobilis* (Fisher et al., 2008; Dharmawan et al., 2009)	*Poncirus trifoliata* (Starkenmann et al., 2007)
Linalyl acetate		×					×					
Citronellyl acetate	×		×							×		
(*E,E*)-Farnesyl acetate									×			
Phenols												
Eugenol					×							
Carvacrol					×							
Lactones												
Wine lactone			×									
Yuzu lactone			×		×							
Ethers												
Dill ether								×				
1,8-Cineole											×	
cis-Limonene oxide		×						×				
trans-Limonene oxide												
N-Compounds												
Methyl *N*-methyl anthranilate						×						

Sulfur compounds					
Methyltrisulfide	×				
Diisopropyl disulfide			×		
1-Phenylethanethiol				×[b]	
p-Menth-1-ene-8-thiol	×				
3-Mercapto-3-methyl-1-butanol					×[b]
4-Mercapto-4-methyl-2-pentanone	×				
3-Mercapto-1-hexanol					×[b]
3-Methyl-3-mercaptobutyl acetate					×[b]
3-Methyl-3-mercaptobutyl butanoate					×[b]
3-Mercaptohexyl acetate					×[b]
3-Mercaptohexyl butanoate					×[b]

Notes:
[a] Isomer (*R*)-(+)-citronellal; [b] Newly identified.

2.2.1.3 *Fortunella crassifolia*

Fortunella crassifolia (or "Meiwa kumquat") may be a hybrid of *F. margarita* × *F. japonica*. It has a round-to-oval shape and a smooth, thick orange-yellow skin. Its taste is sweet and acidic. It is eaten as the whole fruit in China, where it is cultivated in the southern provinces.

A diethyl ether extract of the peel of kumquat (*F. crassifolia*) revealed limonene, α-pinene, myrcene, and linalool to be the major constituents (Hashinaga and Itoo, 1990) (Table 2.1, fruits harvested in November). Some aldehydes, common in citrus chemistry, such as hexanal, octanal, decanal, undecanal, and the powerful (2*E*)-hexenal, (2*E*)-2-nonenal, (2*E*)-2-dodecenal, as well as neral and geranial were identified. More recently, the chemical constituents of *F. crassifolia* were described by Huang et al. (2007) in a paper that should be regarded with caution. The peel oil was obtained by steam distillation of fresh peel. The study showed high amounts of sesquiterpenoids (46.4%) compared to monoterpenoids (8.9%), and also esters, alcohols, aldehydes, and ketones as minor constituents. The leaf oil prepared by steam distillation was analyzed by Scora et al. (1969) (Table 2.1).

2.2.1.4 *Fortunella hindsii* and *Fortunella polyandra*

Fortunella hindsii (also called "Hong Kong kumquat" or "wild kumquat") is native to Hong Kong and the adjacent mountainous regions of the Guangdong and Zhejiang provinces of China. Its pea-size fruits (<2 cm) are edible. The very thorny shrub is mainly used as an ornamental plant.

Fortunella polyandra, native to Malaysia, is also cultivated as an ornamental plant. The oval-shaped fruit is edible. To the best of our knowledge, the volatile constituents of both *F. hindsii* and *F. polyandra* have never been reported.

2.2.2 Japanese *Citrus* Species

Most Japanese *Citrus* species that we have selected in this review belong to the sour citrus fruits: *C. depressa*; *C. inflata* Hort. *ex* Tanaka; *C. junos* Sieb. *ex* Tanaka; *C. nagato-yuzukichi*; *C. sphaerocarpa* Hort. *ex* Tanaka; *C. sudachi* Hort. *ex* Shirai; *C. yuko* Hort. *ex* Tanaka; and *C. taguma-sudachi* Hort. *ex* Y. Tanaka. *Citrus kinokuni* Hort. *ex* Tanaka is mandarin-like and *C. auranthium* L. var. *cyathifera* Y. Tanaka belongs to the family of bitter oranges. *C. flaviculpus* and *C. tamurana* are sweet and orange-like. Species such as *C. hassaku* or *C. natsudaidai*, although not common in the western world, can be considered commodities due to their high yearly production—62,500 tons and 74,900 tons, respectively (Horticulture Statistics Section, 2003)—which exceeds the frame of "less common" species. They will therefore not be discussed here.

2.2.2.1 *Citrus aurantium* L. var. *cyathifera* Y. Tanaka, Japanese Name: *Daidai*

Citrus aurantium L. var. *cyathifera* Y. Tanaka (*daidai* in Japanese) is an Asian variety of bitter orange that is highly cultivated. The fruit is very bitter, and its juice and peel are used for salad dressings and seasonings. Song et al. (2000a) determined the volatile constituents of the cold-pressed peel oil of fruits obtained from the Fruit Experimental Station in Kōchi (Shikoku Island) (Table 2.2). The major volatile components were limonene, myrcene, linalyl acetate, and β-pinene. Njoroge et al. (1994) and Sawamura (2000) identified the same components in slightly different concentrations. In addition, Song et al. (2000a) performed AEDA, GC-olfactometry, and omission tests to understand the flavor profile of *daidai* and composed a model flavor using in correct proportions the most potent ingredients: geranial, octyl acetate, geraniol, octanal, cedrol, *cis*-limonene oxide, (2*E*)-2-decenal, linalyl acetate, and β-elemol (Table 2.5). The leaf oil, prepared by steam distillation from leaves collected in Chengdu, China, is described by Huang and Pu (2000) (Table 2.2). Previously, Kamiyama (1970a) had

already realized that the characteristic *daidai* leaf aroma was due to linalool, linalyl acetate, neryl, and geranyl acetate.

2.2.2.2 *Citrus depressa* Hayata, Japanese Name: *Shiikuwasha*, Hirami Lemon; English Name: Flat Lemon

This fruit grows in the Kagoshima prefecture (Kyushu Island) and on Okinawa Island. It is small, yellow-orange colored, and highly depressed on both ends. It has a thin, aromatic peel, which is used to flavor food. The juice is very sour and sold as a beverage. *C. depressa* is rich in flavonoids and is a source of pectins. Citracridone, an alkaloid effective as antispasmodic, was isolated from this species. The peel oil composition was analyzed by Fujita and Furuta (2004) and Saotome et al. (2004, solvent extract) (Table 2.2). Its main constituents are limonene, γ-terpinene, and α-pinene. The leaf oil was investigated by Hokama (1966), Kamiyama (1970b), Fujita and Yamashita (1974), and Fujita and Furuta (2004). Despite the fact that different extraction methods were applied to prepare the extracts, all analyses showed α-pinene, γ-terpinene, and linalool as major constituents (Table 2.2). The concentration of thymol, a phenol, particularly depends on the extraction method. Linalool, (*Z*)-jasmone, phenylacetaldehyde, methyl anthranilate, farnesol, and indole contribute to the odor character of a solvent extract of the flowers (Saotome et al., 2004).

2.2.2.3 *Citrus flaviculpus* Hort. ex Tanaka, Japanese Name: *Ki-mikan;* English Name: Golden Orange

This fruit, resistant to cold temperatures, is yellow and round shaped, with a diameter of 4 to 5 centimeters and a weight of 60 to 80 grams. There is a small volume production on the island of Shikoku (Japan) and it is of interest due to its well-balanced juicy, sweet and sour, and refreshing flavor. Choi et al. (2001) analyzed the cold-pressed peel oil, whereas Katayama and Iwabuchi (2002) worked with a diethyl ether extract. Both compositions were very similar apart from the amount of limonene (82.4% and 61.6% respectively), a difference that heavily influences the amounts of the other compounds (Table 2.2). The divergence in the amounts of (*E,E*)-farnesol and nootkatone may be due to different GC-conditions. As minor components with high odor impact, Choi and Sawamura (2001) mentioned citronellal, citronellol, its formate and acetate, as well as (2*E*)-2-undecenal and (2*E*)-2-dodecenal. Katayama and Iwabuchi (2002) identified ethyl 2-methylbutanoate, (3*Z*)-3-hexenal, citronellal, linalool, methyl epijasmonate, nootkatone, and raspberry ketone, together with wine lactone and yuzu lactone as potent odorants (see Chapter 10). In an AEDA study, Choi et al. (2002) came to the conclusion that tetradecane, linalool, and α-terpineol were the key odorants of *ki-mikan* (Table 2.5). Katayama and Iwabuchi (2002) studied the composition of a diethyl ether extract of the juice, which was rich in ethyl acetate (47.4%), ethyl butanoate (12.5%), limonene (9.1%), 3-hydroxy-2-butanone (8.3%), acetic acid (2.9%), ethyl 3-hydroxyhexanoate (2.2%), and valencene (1.5%). More interesting were their results on the volatiles released from the intact fruit and captured by solid-phase micro extraction (SPME). The main constituents were valencene (26.6%), ethyl octanoate (23.3%), ethyl hexanoate (13.0%), limonene (4.1%), and a whole series of sesquiterpene hydrocarbons (caryophyllene, β-elemene, 7-*epi*-α-bisabolene) and mixed esters (butyl butanoate, butyl hexanoate, hexyl butanoate, etc.). Noteworthy is the presence of the two isomers (3*E*)-4,8-dimethyl-1,3,7-nonatriene (0.6%) and (3*Z*)-4,8-dimethyl-1,3,7-nonatriene (0.01%) (see Chapter 10).

2.2.2.4 *Citrus inflata* Hort. ex Tanaka, Japanese Name: *Mochiyu, Mochiyuzu*

Mochiyu grows on the island of Shikoku. It is popular among the Japanese sour citrus fruits for culinary purposes. The peel oil, extracted by hand-squeezing the flavedo, was investigated by Minh Tu et al. (2003a) (Table 2.2). Minor amounts of citronellal, decanal, neral, neryl acetate, citronellol, nerol, geraniol, and nerolidol were identified, apart from limonene, γ-terpinene, β-phellandrene and α-pinene. AEDA and gas chromatography-olfactometry (GC-O) revealed

myrcene, (*E*)-β-ocimene, *cis*- and *trans*-linalool oxides, β-elemene, perillaldehyde, and perillyl alcohol as contributors to the typical *mochiyu* aroma. Some years before, Njoroge et al. (1995) presented similar results, mentioning that myrcene was the flavor impact chemical of the *mochiyu* fruit (Table 2.5).

2.2.2.5 *Citrus junos* Sieb. ex Tanaka, Japanese Name: *Yuzu*

Detailed comments about this Japanese sour citrus fruit, mainly produced in Shikoku, have been already summarized by Lawrence (2002). *p*-Menth-1-ene-8-thiol was described as being the compound responsible for its sulphurous note (Yukawa et al., 1994). Song et al. (2000b) analyzed the peel oil, prepared by hand-pressing the flavedo (Table 2.2). Despite their AEDA and GC-O experiments, no significant new key components could be identified. However, in 2006, Escher et al. mentioned the potent 4-mercapto-4-methyl-2-pentanone as key ingredient of *yuzu*. Recently, Miyazawa et al. (2009) isolated two new compounds, (6*Z*,8*E*)-6,8,10-undecatrien-3-one (Yuzunone) and (6*Z*,8*E*)-6,8,10-undecatrien-4-ol (Yuzuol) as new character impact compounds (see Chapter 10).

2.2.2.6 *Citrus kinokuni* Hort. ex Tanaka, Japanese Name: *Shima-mikan*

Shima-mikan is a rare *Citrus* species grown locally at Kagoshima on the island of Kyushu. Though well known in the nineteenth century, it was replaced by the Satsuma mandarin (*Citrus unshiu*). The small fruit is oblate, depressed, of orange color, and glossy. The thin peel is fragrantly aromatic and the flesh is firm and pleasantly sweet. The solvent extract of the peel has been analyzed by Shiota and Ito (1991) (Table 2.2). Its characteristic feature is the presence of methyl *N*-methylanthranilate (2%) in unripe fruits picked in August, which decreases to 0.8% in fruits picked in January. This substance, otherwise absent in *Citrus* fruits grown in Japan, shows a slightly higher abundance than in Mediterranean mandarins, which contain α-sinensal, not identified in *Shima-mikan*. The leaf oil, prepared by steam distillation, has been analyzed by Huang and Pu (2000) (Table 2.2).

2.2.2.7 *Citrus nagato-yuzukichi* Tanaka, Japanese Name: *Nagato-yuzukichi*

This Japanese sour citrus fruit was discovered in the Yamaguchi Prefecture (in the extreme east of the island of Honshu). The fruit, of 4 to 6 centimeters diameter and deep green, is very juicy. The whole fruits were submitted to a SDE and the resulting oil contains limonene (69.1%); γ-terpinene (9.0%); β-phellandrene (5.9%); myrcene (3.0%); α-terpineol (2.3%); (*E,E*)-α-farnesene (1.2%); (*E*)-β-farnesene (1.9%); decanal (0.7%); octanal (0.9%); (*E*)-ocimene (0.6%); terpinen-4-ol (0.5%); and linalool (0.3%) as main constituents (Akakabe et al., 2008).

2.2.2.8 *Citrus sphaerocarpa* Hort. ex Tanaka, Japanese Name: *Kabosu*

Kabosu is produced in the Oita Prefecture in northern part of Kyushu. The juicy, green, sour citrus fruit, of a weight of 100 to 140 grams, is used instead of vinegar to season many dishes, especially fish. Cold-pressed peel oils have been studied by Minh Tu et al. (2002) (Table 2.2), confirming the results of Njoroge et al. (1994), who, in addition, identified the fatty acid aldehydes C_9 to C_{14} and the heptyl, octyl, nonyl, decyl, and dodecyl acetates as compounds present in amounts < 0.1%. Akakabe et al. (2008) analyzed an extract from whole fruits prepared by SDE. In the profile of the oil, the amount of myrcene (up to 20%) was high enough to induce the formation of a myrcene dimer (0.02%). Yang et al. (1992) identified citronellal, (2*E*)-2-decenal, and geranyl acetate as the compounds that contributed to the characteristic aroma of *kabosu*. Chiral GC-analysis revealed that (*R*)-(+)-citronellal (the only enantiomer detected in the oil) was the compound essentially responsible for the typical odor of the *kabosu* peel oil (Minh Tu et al., 2002). In addition, octyl acetate, citronellal, linalyl acetate, neral, myrcene, (*E*)-ocimene, *cis*-linalool oxide, *trans*-linalool oxide, β-copaene, perillaldehyde, and perillyl alcohol showed organoleptic properties reminiscent of *kabosu* (Minh Tu, 2003b) (Table 2.5).

2.2.2.9 *Citrus sudachi* Hort. ex Shirai, Japanese Name: *Sudachi*

This sour citrus fruit is the symbol of the Tokushima Prefecture in Shikoku, where a slice is served with many traditional dishes. It is small (28 to 40 g, diameter 4.5 cm), round, and green. *Sudachi*-flavored products, such as ice cream, coolers, and soft drinks, are found all over Japan. Sugisawa et al. (1989) prepared a peel extract by SDE and determined, by GC-sniffing of fractions obtained by chromatography on silica gel, that carvone, carveol, perillaldehyde, and citronellal constituted the significant volatiles of *sudachi*. However, they were well aware that the character impact compounds were still unknown. Njoroge et al. (1995) prepared the peel oil by hand-pressing of the flavedo (Table 2.2). Citronellal (0.06%), decanal (0.06%), and undecanal (0.02%) constitute the aldehydic components, whereas *cis*-carveol (0.6%), linalool (0.3%), and α-terpineol (0.2%) were the most abundant alcohols. Tamura et al. (1999) studied the volatiles of *sudachi* peel during maturation using the "purge and trap" headspace technique. Limonene oxides, citronellal, decanal, and carvone were formed during the ripening process. Yang (2000) reconstituted the *sudachi* aroma by combining the linalool oxides, linalool, citronellal, citronellol, carvone, octanal, nonanal, decanal, and dodecanal in appropriate concentrations given by the GC peak area of an extract prepared from the peel by SDE. Mookdasanit et al. (2003) prepared the peel extract in a semi-preparative procedure using a mixture of pentane and dichloromethane, and selected (*E,E*)-α-farnesene, (*E*)-β-farnesene, diisopropyl disulfide, cumin aldehyde, and dill ether as the aroma-active compounds (Table 2.5). A juice extract, prepared by SDE under reduced pressure, was separated on silica gel into several fractions by Yang and Sugisawa (1990). Among the 29 compounds identified in the juice extract, α-terpineol, decanal, and dodecanal were the main compounds. They reported that decanal, dodecanal, and octyl acetate contributed to the sweet aroma of *sudachi* juice. However, the series of (2*E*)-2-alkenals C_6 to C_{12}, as well as damascenone, rarely found in citrus extracts, may certainly have a positive contribution to the flavor.

2.2.2.10 *Citrus taguma-sudachi* Hort. ex Tanaka, Japanese Name: *Naoshichi*

Naoshichi, cultivated in Shikoku, is a large sour citrus fruit (diameter 6 to 8 cm), which is of potential commercial value due to its high content of mild sour juice. Njoroge et al. (1996) compared the volatile constituents of its cold-pressed peel oil with Tahiti lime (*Citrus latifolia* Tanaka) and found that the oil composition showed major differences. *Naoshichi* contained limonene (90.5%), γ-terpinene (4.2%), myrcene (1.8%), and (*E*)-β-farnesene (0.8%) as the main components, whereas the Tahiti lime oil was rather poor in limonene (52.2%), but contained γ-terpinene (17.0%) and β-pinene (13.0%) in much higher amounts (Table 2.2). The characteristic flavor chemicals of lime—namely geranial, neral, geranyl acetate, and neryl acetate—were practically absent. Moreover, these two species could be distinguished by their pattern of sesquiterpene hydrocarbons: (*E*)-β-farnesene (0.8%) bicyclogermacrene (0.1%) for *naoshichi*; and α-bergamotene (0.8%), caryophyllene (0.3%), and β-bisabolene (0.8%) for Tahiti lime (see also Lota et al., 2002).

2.2.2.11 *Citrus tamurana* Hort. ex Tanaka, Japanese Name: *Hyuganatsu*

Hyuganatsu is mainly cultivated in the Kochi Prefecture of Shikoku, where the production reached 5,500 tons per year in 1999. The fruit, weighing 160 to 200 grams and above, has a smooth, thin skin and a fresh, juicy, sweet and slightly sour taste. The albedo is eaten together with the flesh. Choi and Sawamura (2000) determined the composition of the oil extracted by hand-pressing of the flavedo of fruits harvested in March (Table 2.2). Octanal, decanal, dodecanal, (2*E*)-2-decenal, (2*E*)-2-undecenal, (2*E*)-2-dodecenal, and various acetates such as linalyl acetate, citronellyl acetate, decyl acetate, neryl acetate, geranyl acetate, and sesquiterpenoids [(*E*)-β-farnesene, nerolidol, globulol] were described as minor constituents. In 2001, Choi et al. refined their study: AEDA experiments indicated that limonene, linalool, octanol, neral, neryl acetate, tridecanal, *trans*-carveol, (*E*)-nerolidol, (*E*,*E*)-farnesyl acetate, and (*E*,*E*)-farnesol were key compounds. Omission

tests showed that diluted solutions of linalool and octanol at 2 ppm gave a fresh and fruity aroma similar to the *hyuganatsu* flavor (Table 2.5).

2.2.2.12 *Citrus yuko* Hort. ex Tanaka, Japanese Name: *Yuko*

Yuko is an astringent citrus fruit native to Nagasaki (Kyushu) and Tokushima prefectures (Shikoku), resembling in its appearance the *yuzu* and *kabosu* fruits. In the 1960s, this fruit tree was nearly extinct due to the introduction of the Satsuma mandarin. Only recently has it become a protected species. The sweetly scented peel, when ripe, is bright yellow, and the flavor of the juice is sharp, juicy, and well rounded. Njoroge et al. (1994) have analyzed the cold-pressed peel oil (Table 2.2).

2.2.3 Australian Native *Citrus* Species

The native *Citrus* species of Australia occur in the rainforests of Queensland and on their margins. Previously, they were classified in the genera *Microcitrus* and *Eremocitrus*, to distinguish between their occurrence in rainforests and arid areas. However, recent taxonomic work has led to their reclassification and their integration into the genus *Citrus* (Mabberley, 1998; de Araújo et al., 2003). Mabberley, in the same reference, describes how the individual species can be distinguished morphologically and includes a key for their identification. The names *Microcitrus* and *Eremocitrus* are still retained in some publications. Recently, there has been increasing interest in wild native Australian citrus fruits and other crops for the development of so-called bushfood, or food native to Australia Aborigines. As harvesting of wild fruit is troublesome and as the demand of new bushfood flavors has increased, the trend to commercially cultivate these species is growing. To date, three of them are traded: the round lime (*C. australis*), the finger lime (*C. australasica*), and the desert lime (*C. glauca*). Interestingly, the volatile constituents of these particular fruits have only been rarely described in the literature.

2.2.3.1 *Citrus australis*

Citrus australis (previously *Microcitrus australis*), or Australian round lime, is the most vigorous of the Australian native citrus, growing to a height of 5 meters with glossy, dark green leaves. It is endemic to the drier rainforest margins north of Brisbane in Queensland. The round fruit of a diameter of 2 to 5 centimeters is green-to-yellow-colored, and the pulp is pale-green. The skin is very thick (up to 7 mm) and has commercial potential for culinary use, such as grating into spice pastes, candied peel, beverages, and marmalade, as well as for the extraction of the essential oil.

In 2005, Craske et al. reported a comparative study of the peel oil components of Australian native lime (*Microcitrus australe*) and Mexican lime (*C. aurantifolia* Swingle) prepared by solvent extraction of the pericarp with dichloromethane (Table 2.3). No significant difference in the composition and the organoleptic evaluation of round lime and Mexican lime could be found. In particular, the content of geranial/neral, otherwise absent in the Australian limes, slightly exceeds the values determined for the Mexican lime. The leaf oil prepared by hydrodistillation was extremely rich in α-pinene (78.4%) and viridiflorol (3.2%) (Brophy and Goldsack, 2001) (Table 2.3).

2.2.3.2 *Citrus australasica*

Citrus australasica (previously *Microcitrus australasica*), or finger lime, occurs as an understory shrub or tree in rainforests in southern Queensland and northern New South Wales. Production is usually biennial. There are a number of commercial plantations that produce small quantities of fruit. The fruits are finger shaped, up to 10 centimeters long, with a green or yellow skin and green-yellow vesicles that tend to burst out when the skin is cut (Figure 2.1). The fruit is therefore also called "lemon caviar." The finger lime shows a wide genetic diversity. The fruits vary

FIGURE 2.1 (**See color insert following page 462.**) *Citrus australasica*. (Photo: A. Jaquier, Firmenich S.A.)

significantly in skin color, ranging from yellow to green, red, purple, and even black (*C. australasica* var. sanguinea). Pulp colors can be green, yellow, white, or pink. Finger limes can be used as a fresh fruit to garnish seafood (oysters, caviar, sushi, etc.), as flavorings for champagne and gin and tonics, and for processing into a wide range of value-added products. In the literature, analyses of the volatiles of the species *C. australasica* are scarce. In 2000, Ruberto et al. reported the chemical composition of the peel essential oil of *Microcitrus australasica* var. *sanguinea* Swingle acclimatized in Sicily. In their extract, obtained by SDE, 65 components were identified, the main compounds being bicyclogermacrene (25.9%), α-pinene (10.2%), spathulenol (9.8%), and (*Z*)-β-ocimene (5.1%), while limonene was found at only 1.2% of the total oil. In 2002, Lota et al. investigated the chemical composition of the peel and leaf oils of 43 lemons and limes, all of them cultivated in Corsica to take advantage of the same climatic environment and growth conditions. In this study, the cold-pressed peel oil of *C. australasica* showed an original pattern. It differed from all other cultivars in the occurrence of sabinene (19.6%) as the second major component after limonene (51.1%); the leaf oil obtained by water distillation contained even higher amounts of sabinene (42.5%), together with limonene (18.4%), citronellal (11.5%), and citronellol (8.4%). Brophy and Goldsack (2001) identified β-phellandrene (20.2%) and bicyclogermacrene (19.8%) as main compounds in leaves originated from Australia and extracted by hydrodistillation (Table 2.3). Delort and Jaquier (2009) studied a solvent extract (dichloromethane) of freshly grated peel and identified 165 compounds (Table 2.3). Noteworthy was the presence of high amounts of isomenthone (7.5%), a terpenoid that rarely occurs in citrus extracts. Powerful constituents were a series of (2*E*)-2-alkenals and some branched saturated aldehydes known in *yuzu*, orange, and lemon (6-methyloctanal, 4-methylnonanal and 8-methyldecanal, see Chapter 10). Additionally, the structures of six new terpenyl esters, citronellyl 2-methylbutanoate, 1,8(10)-*p*-menthadien-9-yl propanoate, 1,8(10)-*p*-menthadien-9-yl 2-methylbutanoate, 1,8(10)-*p*-menthadien-9-yl 3-methylbutanoate, 1-*p*-menthen-9-yl 2-methylbutanoate, and 1-*p*-menthen-9-yl 3-methylbutanoate, were confirmed by synthesis.

2.2.3.3 *Citrus garrowayi*

Citrus garrowayi (previously *Microcitrus garrowayi*), or Mount White lime, occurs in the rainforest of Cape York Peninsula, Queensland, the most northern point of Australia. It is classified as a rare and protected species. Similar to *C. australasica*, the fruit is finger shaped, with a thick, pale, yellow-green skin with large oil glands and a light green pulp. The leaves, which are broader than in *Citrus australasica*, were the subject of the analysis of Brophy and Goldsack (2001), who could distinguish two different chemotypes (Table 2.3). Studies on the composition of the fruit could not be found.

2.2.3.4 *Citrus glauca*

The thorny shrub of *Citrus glauca* (previously *Eremocitrus glauca*), also known as Australian desert lime or native kumquat, grows wild in the semi-arid regions of Queensland, New South Wales, and rarely in central South Australia. Unlike other members of the citrus family, it is extremely tolerant to drought and salty soil. It can withstand extremes of heat (45°C) and cold (–24°C and lower) without damage and is therefore an interesting specimen for hybrids. The fruit, with a globular shape and a diameter of 3 centimeters, can be picked when still green. Appreciated for their strong lime-like, tangy, and refreshing taste, desert lime fruits are popular in Australia and are used in highly priced bushfood, beverages, and marmalade. The constituents of a leaf extract prepared by hydrodistillation have been investigated by Brophy and Goldsack (2001) (Table 2.3).

2.2.3.5 *Citrus gracilis* Mabb

Citrus gracilis, also called "Humpty Doo" or Kakadu lime tree, is a thorny, straggling shrub that grows wild in the Eucalypt woodland in the Northern Territory, where the fruit is eaten by the Aborigines. Its round fruit, of a diameter of 8 centimeters is not traded commercially. Only the volatile constituents of its leaf extract were described (Brophy and Goldsack, 2001) (Table 2.3).

2.2.3.6 *Citrus inodora*

Citrus inodora (previously *Microcitrus inodora*), also called "Russel River" or Large-leaf Australian wild lime, has a limited distribution in the lowland rainforest near Cairns in northern Queensland. The Australian wild lime tree is a shrub up to 3 meters high and produces elliptical fruits of about 5 × 3 centimeters in diameter. *C. inodora* is quite distinct from other Australian native citrus, with larger leaves up to 15 centimeters long and odorless flowers—a remarkable feature in a genus noted for scented flowers. The fruits are not commercially traded. In 2000, Shaw et al. identified 53 volatile constituents in a juice extract prepared by extraction with dichloromethane. The major constituents were limonene (68.5%), ethanol (14.6%), acetaldehyde (9.4%), myrcene (1.4%), hexanal (0.6%), (3*Z*)-3-hexen-1-ol (0.4%), β-pinene (0.3%), hexanol (0.2%), and linalool (0.1%) (area percentages determined by headspace GC of the juice sacs). The components of the peel oil were not quantified. Besides the usual monoterpenes, it contained linalool, neral, geraniol, carvone, and perillaldehyde. The leaf oil was rich in germacrene D (23.7%) and bicyclogermacrene (17.3%) (Brophy and Goldsack, 2001) (Table 2.3).

2.2.4 Others

2.2.4.1 *Citrus ichangensis*, English Name: Ichang Lemon or Lemon Papeda

The Ichang lemon, native to East Asia, is reputed to be one of the hardiest citrus plants, and can therefore be cultivated even in the south of the British Isles. The fruit is greenish-yellow and 5 × 4 centimeters in size. The taste of the flesh, which is packed with large seeds, is extremely bitter due to a series of limonoids (Herman et al., 1989). The peel oil was analyzed by Sawamura (2000) (Table 2.4), and the enantiomeric distribution of α-pinene, β-pinene, and sabinene was determined by Mitiku et al. (2001). Lota et al. (2002) studied a hydrodistillate of leaves grown in Corsica (Table 2.4).

2.2.4.2 *Citrus limetta* (Risso), English Name: Sweet Lemon

Sweet lemon is the general name of non-acid lemons growing in the Mediterranean regions and South India. The ripe fruit has a diameter of 5 to 8 centimeters and is bright yellow. The peel is composed of a thin, smooth flavedo and a level of albedo. If damaged, the flavedo exhibits a floral and terpenic odor quite special for a citrus fruit. The juice sacs are abundant, and the juice itself is limpid and slightly sweet, with a pleasant but insipid aroma and a bitter aftertaste.

The peel oil, produced by manual sponge extraction, was investigated by Di Giacomo et al. (1969), who identified limonene, linalyl acetate, linalool, bisabolene, myrcene, and geranial, in descending order of their content. Sattar et al. (1992) prepared an extract of the variety *Citrus*

limetta var. Mitha from Pakistan by steam distillation (Table 2.4). Naef and Velluz (2003) prepared a solvent extract of freshly grated zest of fruits from Lebanon. The oil composition was interesting: limonene (85%), myrcene (2.9%), citronellal (2.6%), β-bisabolene (1.0%), germacrene D (0.8%), (*E*)-α-bergamotene (0.8%), linalool (0.6%), citronellyl acetate (0.3%), geranyl acetate (0.3%), and citronellol (0.2%). Organoleptically important contributions were given by α-sinensal, β-sinensal, nootkatone, citronellol, and citronellyl acetate, and trace amounts of methyl jasmonate and methyl epijasmonate (Table 2.5). The juice extract prepared by continuous extraction with diethyl ether was weak and contained limonene, acetoin, and citronellol (Naef and Velluz, 2003).

2.2.4.3 *Citrus nobilis* Lour. var. *microcarpa* Hassk., English Name: Pontianak Orange

The Pontianak orange was originally cultivated in Kalimantan (Borneo, Indonesia). Its juice is appreciated for its pleasant, sweet taste with a slightly sulphurous note. Fischer et al. (2008) investigated an oil prepared by solvent extraction followed by Solvent Assisted Flavor Evaporation (SAFE) distillation, and described it as fresh, citrus-like, flowery, and sulphurous. They succeeded in isolating a new natural compound, 1-phenylethanethiol, which, according to AEDA experiments, proved to be one of the most odor-active compounds of Pontaniak together with linalool, myrcene, limonene, α-pinene, octanal, (2*E*,4*E*)-2,4-decadienal, (2*E*)-2-dodecenal, geraniol, and nerol (Table 2.5). Dharmawan et al. (2009) reported the composition of the hand-pressed peel oil (Table 2.4). Undecanal, nerol, dodecanal, and the powerful aldehydes (2*E*)-2-decenal, (2*E*,4*E*)-2,4-decadienal, (2*E*)-2-dodecenal, (5*Z*)-5-dodecenal, and (*2E,6Z*)-2,6-dodecadienal were identified in amounts of 0.1% and below. They confirmed the importance of 1-phenylethanethiol with aroma reconstitutions and omission tests, and recognized the positive impact of (5*Z*)-dodecenal on the characteristic Pontianak flavor (Table 2.5). The same group investigated the juice extract prepared by continuous liquid–liquid extraction with diethyl ether (Dharmawan et al., 2007) (Table 2.4).

2.2.4.4 *Poncirus trifoliata* [L.] Raf

Poncirus trifoliata (Figure 2.2) is native to China. Its hardiness toward temperatures (as low as –20°C) makes it an interesting species for rootstocks of many cultivated citrus fruits to improve resistance to frost and to specific viral diseases of citrus plants. The spiny shrub has trilobate leaves and bears yellow fruit of the size of a golf ball with a special fluffy skin. Its taste is incredibly bitter due to the presence of poncirine, a polyphenol. The strong odor of the peel is reminiscent of jasmine flowers and overripe mango fruits. Scora et al. (1966) prepared essential oils by steam distillation of the flavedo, the pulp, and the leaves, in a Clevenger apparatus for taxonomic studies (Table 2.4). Their

FIGURE 2.2 (**See color insert following page 462.**) *Poncirus trifoliata*. (Photo: C. Starkenmann, Firmenich S.A.)

conclusion was that the "finger print" characteristics of *Poncirus trifoliata* consisted of α-pinene, nonanol, neryl formate, α-terpineol, and citronellal. Starkenmann et al. (2007) specifically investigated the sulfuric notes, and located seven typical zones by GC-sniffing of the peel oil prepared by maceration in dichloromethane. They identified the two alcohols 3-mercapto-3-methyl-1-butanol and 3-mercapto-1-hexanol and a series of their esters, the carboxylate moieties being the even-numbered, linear saturated fatty acids, C_4 to C_{18} (Table 2.5). Both free alcohols are reminiscent of tropical fruits, especially mango. The esters exhibit fruity notes and turn into "fatty, meaty, smoky" when the alkyl-chain is longer (see Chapter 10). Precursors of the two alcohols were isolated from the peel in the form of cysteine-*S*-conjugates. The high amounts of myrcene (29.8%) and neryl acetate (25.2%) found in the leaf oil prepared by steam distillation are remarkable (Scora et al., 1969) (Table 2.4).

2.3 DISCUSSION AND CONCLUSION

We tried in this review to give a global view on the analytical studies of less common citrus species. First a general comment: a comparison of volatile compositions based on relative percentages reported by different working groups at different times can be misleading. Small changes in the amounts of major components, such as limonene, create large fluctuations in the values of minor constituents. Moreover, to be able to identify trace components, the quantities of the oil injected into the GC- or GC-MS-system may easily lead to saturated peaks of the abundant compounds, which results in erroneous integration values. However, identifying new molecules exhibiting interesting properties and characterizing odor-active compounds is essential of the knowledge of the flavors and fragrances of rare citrus fruits. Table 2.5 presents a selection of components with high odor-impact identified in peel extracts, though detailed studies are unfortunately scarce and exclude the Australian species. Aldehydes, either saturated or unsaturated, linear or branched, or aliphatic or terpenic, remain key compounds. Aliphatic and terpenic esters are abundant in Australian and Japanese species, but appear to be also key in kumquat aroma. Kumquats and some Australian fruits, mainly *Citrus australasica*, contain alcohols and esters with a *p*-menthane skeleton, in contrast to Western *Citrus* species whose volatile constituents have structures generally based on open-chain terpenoids such as nerol and geraniol. Comparison of the abundant analytical data of the volatile fractions of these fruits reveals that the prototype of a lemon-lime flavor composed of geranial/neral and geranyl acetate/neryl acetate is restricted to lemon (*Citrus limon*) and lime (*C. aurantifolia* and *C. latifolia*), whereas the aromas of the Australian "limes" (with the exception of *C. australis*) and the Japanese "sour citrus" are based on citronellal/citronellol and its acetate. It should be emphasized that powerful odoriferous compounds may be rediscovered in many other citrus species, since in-depth analyses of specific organoleptic key notes were only performed on a small number of citrus species, and most of these analyses were undertaken for taxonomic profiling. Excellent examples are the analyses of extracts of *Citrus nobilis* (Fischer et al., 2008), *Citrus trifoliata* (Starkenmann et al., 2007) and *Citrus junos* (Miyazawa et al., 2009), where powerful, new constituents could be discovered. Although this inventory of less common *Citrus* described herein is far from being exhaustive, we hope to have given a picture of the impressive chemical diversity of this genus. Despite the multitude of scientific investigations of the volatile fractions of these wonderful fruits, there is still room for further important discoveries, especially by taking advantage of new sophisticated analytical tools.

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3 Composition of Distilled Oils

Luis Haro-Guzmán

CONTENTS

3.1 INTRODUCTION

Distillation is the second most common method used for the recovery of citrus oils. Distillation may be defined as the separation of the components of a mixture of two or more liquids by virtue of the difference in their vapor pressure (Miall in Guenther, 1948). It takes advantage of two characteristics that make the recovery of the oil more feasible:

1. The volatility of most of the components of the citrus oils
2. The immiscibility of the oils with the water-based emulsions resulting in the processing of citrus fruits

Dalton's law states that "the total pressure exerted by a gaseous mixture is equal to the sum of the partial pressures of each individual component of the mixture." Thus, the boiling temperature for any two-phase liquid will be lower than either of the boiling points of the components in such a blend. The water-based citrus oil emulsions boil at temperatures below 100°C at atmospheric pressure, well below the boiling points of the citrus oils' components in the range of 150°C to 280°C. This method, however, produces some changes in the composition of the oil:

- The nonvolatile part of the oil, composed of coumarins, psoralens, pigments, and waxes, is not present in the distilled oil. Consequently, the latter is relatively unstable because it lacks the antioxidants present in the nonvolatile part of cold-pressed oils.
- Some distilled oils obtained from a juice–oil mixture contain volatile components of the juice not present in cold-pressed oils (Shaw, 1977). The presence of such compounds generally imparts to the distilled oils a desirable juicy note.
- They also lack a heavy flavor note that contributes to the characteristic flavor of the corresponding cold-pressed oil (Shaw, 1977).

- There are some chemical transformations that take place because of three main factors. These are temperature, acidity, and duration of the exposure. These transformations have an effect on the composition and characteristics of the oils, including odor and flavor. The extent of such transformations depends on the intensity of the factors. In the citrus industry, a wide range of conditions are used. They go from the very mild (low acidity; low temperature; and short distillation time, as in the vacuum distillation of the water–oil emulsion from the pelatrice oil extractors) to the very severe, such as when the juice–oil emulsion is distilled for 6 to 10 hours at 95°C to 105°C and a pH of 2.2 to 2.4 to recover Key lime oil.

When reference is made to distilled oils, there is considerable confusion in understanding which product is being described (Redd and Hendrix, 1996). Distilled oils are obtained from a variety of emulsions containing it. We can classify the distilled citrus oils according to four main groups depending on the severity of conditions during distillation: going from strong to mild, we have

- High acidity, high temperature: distilled Key and Persian lime oils
- High acidity, low temperature: lemon and lime essence oils, "Peratoner" oil of lemon and bergamot
- Low acidity, high temperature: distilled (at atmospheric pressure) oils of orange and grapefruit
- Low acidity, low temperature: "Peratoner" oil of mandarin, essence oils of orange, grapefruit, and tangerine

3.2 OILS DISTILLED AT ATMOSPHERIC PRESSURE

3.2.1 Key and Persian Lime Oils

Key lime oils are obtained from the small seeded lime fruit known as Key, Mexican, or West Indian lime (*Citrus aurantifolia* Swingle), while Persian lime oil is obtained from the bigger seedless lime fruit known as Persian or Tahitian lime (*Citrus latifolia* Tanaka). Both lime oils are obtained by distilling at atmospheric pressure the juice–oil emulsion (press liquor) obtained by squeezing the whole fruits in a press, most commonly a screw press. It is erroneously mentioned in the literature that the "whole fruits are distilled." Only the emulsion is distilled, while the peel is sent to a washing and drying process to be used as raw material for pectin extraction. Distilled Key lime oil has been a popular citrus essential oil since the invention of the first cola-flavored soft drink in the mid-1880s, and it has conserved its position to the present day. The production of distilled Persian lime oil remained very limited until some 15 years ago when its production was increased. In the beginning it was utilized to adulterate ("extend") Key lime oil, but little by little it has been finding its own place in the market.

Processed flavors and oils such as distilled lime oil are the result of acid-catalyzed terpene reactions, which occur during production or isolation and usually result in unique and desirable flavors (Clark and Chamblee, 1992). Distilled lime oils differ very noticeably in aspect, flavor, and odor from the corresponding centrifuged oils. Almost colorless or pale yellow, with a sharp and terpenic odor, distilled oils have few points in common with centrifuged (also called "cold-pressed" or "expressed") oils, which are brownish-to-green and have a fruity smell. Arctander (1960) describes the odor of distilled oil of Key lime as: "sharp, fresh, terpene-like, somewhat perfumey-fruity citrus-type." Distilled Persian lime oil, while having an odor similar to that of Key lime, shows a less sharp, harsher, more terpenic and, in general, flatter odor. The composition of distilled Key lime oil can vary widely, but in all cases much of the monoterpene component is lost to hydration to give a variety of ethers, oxides, and alcohols. Many of these, such as fenchol, borneol, and β-terpineol, have musty odors, and together with β-terpineol are regarded as off-odors in citrus products. Many of them play a role in changing the aroma of distilled lime oil compared with that of the cold-pressed oil. "More piney" and "less fragrant" were phrases used to describe distilled lime oil (Chisholm

et al., 2001). Even if its odor and flavor are very different from that of the natural fruit, the distilled oil of Key lime remains a major item in the market of citrus oils due to its important role in the "cola" and "lemon-lime" flavors, the two most common soft drink flavors in the world. They both hold over 64% of the total world's market of soft drinks (Buchel, 1989).

During distillation, lime oil undergoes important modifications in its chemical composition. Placed during several hours at a temperature equivalent to the boiling point of the juice–oil emulsion and in a very acidic environment, many constituents are transformed, generating other more stable compounds but no longer keeping the same odor and flavor. The major reactions are the acid-catalyzed hydrolysis and rearrangement reactions of the bicyclic hydrocarbons α- and β-pinene, sabinene, and α-thujene. The major products of these reactions are alcohols (α-terpineol, terpinen-4-ol, endo-fenchol, borneol, isoborneol) and hydrocarbons (terpinolene, limonene, fenchene, camphene, γ-terpinene, α-terpinene) (Chamblee and Clark, 1997). Of course, nonvolatile compounds, such as wax, coloring matters, coumarins, and psoralens, do not enter in the distillate. Buiarelli et al. (2002) have found small amounts of some furocoumarins in samples of commercial distilled "Limette" oil. They report 141 mg/l of citropten, 15 mg/l of 5-geranoxy-7-methoxycoumarin, and no bergapten in the distilled oil, compared with 4,000, 30,000, and 1,945 mg/l, respectively, in a cold-pressed oil. The comparative composition of groups and some components of cold-pressed and distilled Key lime oils is shown in Table 3.1, where it can be observed how deep the changes are. Finally, the enantiomeric distribution of some components changes with the distillation. Mondello et al. (1998) found that with the exception of β-pinene, whose distribution remains unchanged, that of the other studied components changed tending to be racemic. Table 3.2 shows the values found.

The first systematic research on the composition of distilled Key lime oil was made by Ernest Guenther and Edward Langenau, who published their findings in 1943 (Guenther and Langenau, 1943). They identified 22 constituents. In 1963, Kovats published the results of his investigation on the composition of distilled Key lime oil (Kovats, 1963). He identified 44 components. Additional investigations increased the knowledge on how distilled Key lime oil is composed. The composition of distilled oils of Mexican and Persian limes is shown in Table 3.3. The oils obtained from Key and Persian limes show basically no qualitative differences but remarkable quantitative ones: Persian

TABLE 3.1
Quantitative Composition (wt%) of Cold-Pressed and Distilled Key Lime Oils

	Cold Pressed	Distilled
Monoterpene hydrocarbons	78.95	75.44
Bicyclic	25.36	3.31
Monocyclic	53.59	72.13
Sesquiterpene hydrocarbons	5.70	3.48
Aldehydes	5.00	0.48
Neral + geranial	4.77	0.16
Alcohols	1.05	13.41
α-Terpineol	0.25	7.44
Esters	0.44	0.15
Ketones and ethers	0.04	3.96
Unidentified	0.76	2.38
Total volatiles	91.94	99.30

Source: Clark, B.C., and Chamblee, T.S., *Off-flavors in Foods and Beverages,* Elsevier, Amsterdam, 1992.

TABLE 3.2
Enantiomeric Distribution of Some Components of Cold-Pressed and Distilled Key Lime Oils (Average Values for Mexican Oils)

	Cold-Pressed Type A		Distilled	
	(+)	(–)	(+)	(–)
β-Pinene	3.5	96.5	3.5	96.5
Limonene	2.8	97.2	6.2	93.8
Linalool	70.7	29.3	49.9	50.1
Terpinen-4-ol	29.4	70.6	43.1	56.9
α-Terpineol	*84.8*	15.2	54.6	45.4

Source: Mondello, L., et al., *J. Microcolumn Separat.* 10, 203–212, 1988.

TABLE 3.3
Composition of Distilled Lime Oils

	Key Lime	Persian Lime
	Hydrocarbons	
Aliphatic		
Nonane	0.06[a], X[c], 0.02–0.04[d], 0.03[f]	
Tridecane	0.02[a], tr–0.02[d]	
Undecane	0.03[a], 0.01–0.03[d], 0.19[f] (incl. linalool)	
Monoterpene		
Bornylene	X[c], 0.02[f]	
Camphene	0.77[a], 0.42[b], X[c], 0.68–0.79[d] (incl. α-fenchene), 0.48[e], 1.3[g], 0.53[f], 0.49[h]	0.25[e], 0.7[g]
δ-3-Carene	0.21[b], tr–0.03[d], 0.01[f]	0.39[e] (incl. 1,4-cineol)
p-Cymene	11.59[a], 3.88[b], X[c], 1.81–4.28[d], 1.79[e], 1.89[f], 1.6[g], 2.51[h]	1.51[e], 1.5[g]
p-Cymenene	0.49[a], 0.14[b], 0.08–0.16[d], 0.4[g]	0.2[g]
α-Fenchene	X[c], 0.68–0.79[d] (incl. camphene), 0.21[f], 0.19[h]	
Limonene	59.95[a], 38.99[b], X[c], 38.95–42.24[d], 48.85[e] (incl. 1,8-cineole), 46.86[f] (incl. 1,8 cineole), 40.4[g] (incl. 1,8-cineole), 47.56[h]	59.02[e] (incl. 1,8-cineole), 55.6[g] (incl. 1,8-cineole)
p-Mentha-3,8-diene	0.01–0.02[d], tr[g]	tr[g]
p-Mentha-1,3,8-triene	tr-0.01[d]	
Myrcene	0.77[a], 1.20[b], X[c], 0.85–1.10[d], 1.28[e], 1.34[f] (incl. 2,3-dehydro-1, 8-cineole), 2.1[g], 1.25[h]	1.56[e], 2.6[g]
Allo-ocimene	tr[d]	
Neo-allo-ocimene	tr[d]	
(*E*)-β-Ocimene	0.20–0.50[d], 0.68[f] (incl. 2,2-dimethyl-5(1-methylpropenyl)-tetrahydrofuran), 0.5[g], 0.46[h]	tr[g]
(*Z*)-β-Ocimene	0.15–0.20[d], 0.20[f], 0.21[h]	

TABLE 3.3 (continued)
Composition of Distilled Lime Oils

	Key Lime	Persian Lime
α-Phellandrene	X[c], 0.34–0.48[d], 0.37[e], 0.46[f], 0.34[h]	0.20[e]
β-Phellandrene	0.34[h]	
α-Pinene	0.84[a], 0.85[b], X[c], 0.94–1.26[d], 1.19[e], 1.02[f], 2.1[g], 1.22[h]	2.25[e], 1.8[g]
β-Pinene	0.90[a], 0.42[b], X[c], 0.77–1.90[d], 2.23[e], 1.34[f], 2.9[g], 1.95[h]	6.04[e], 1.8[g]
Sabinene	tr[d], 0.02[f]	
α-Terpinene	2.47[b], X[c], 1.47–2.35[d], 2.53[e], 2.84[f], 2.07[h]	1.05[e]
γ-Terpinene	0.63[a], 12.41[b], X[c], 8.51–13.35[d], 10.99[e], 11.02[f], 9.5[g], 10.71[h]	16.09[e], 11.8[g]
Terpinolene	0.82[a], 9.52[b], X[c], 6.91–9.70[d], 7.76[e] (incl. nonanal), 8.43[f] (incl. *trans*-linalool oxide and monoterpene hydrocarbon), 8.7[g], 8.05[h]	2.69[e] (incl. nonanal), 5.2[g]
α-Thujene	tr–0.02[d], 0–02[e], 0.01[f], tr[g]	0.21[e], tr[g]
Tricyclene	0.02–0.03[d], 0.02[f]	
Sesquiterpene		
cis-α-Bergamotene	X[c], 0.04–0.09[d], 0.06[f], 0.06[h]	tr[g]
trans-α-Bergamotene	0.52[a], X[c], 0.45–0.80[d], 0.83[e], 0.70[f], 0.9[g], 0.81[h]	0.56[e], 0.4[g]
(*Z*)-α-Bisabolene	tr–0.14[d], 0.17[f] (incl. α-selinene)	tr[g]
β-Bisabolene	0.94[a], X[c], 1.09–2.47[d] (incl. (*E*,*E*)-α-farnesene), 1.04[f], 1.6[g], 1.78[h]	0.63[e], 0.6[g]
(*E*)-γ-Bisabolene	0.01–0.04[d], 0.01[f]	
(*Z*)-γ-Bisabolene	tr–0.01[d], 0.04[f]	
δ-Cadinene	0.03–0.08[d], 0.05[f] (incl. *epi*-α-selinene), 0.16[h]	
γ-Cadinene	X[c]	
β-Caryophyllene	0.27[a], X[c], 0.42–0.57[d], 0.62[e], 0.60[f], 0.7[g], 0.63[h]	0.29[e], 0.1[g]
β-Chamigrene	0.1[g]	tr[g]
Calamenene	0.05[h]	
γ-Curcumene	0.07–0.11[d]	
β-Elemene	0.04–0.08[d], X[f], 0.07[h]	
δ-Elemene	X[c], 0.04–0.08[d], 0.06[f], 0.1[g], 0.08[h]	0.54[e] (incl. neryl acetate)
γ-Elemene	tr[d]	
(*E*,*E*)-α-Farnesene	X[c], 1.09–2.47[d] (incl. β-bisabolene), 1.11[e], 0.68[f], 0.89[h]	0.18[e]
β-Farnesene	X[c]	
(*E*)-β-Farnesene	0.1[g]	tr[g]
(*Z*)-β-Farnesene	tr–0.09[d], 0.10[f]	
Germacrene b	0.01–0.08[d], 0.03[f], 0.04–0.08[i]	
cis-β-Guaiene	0.08–0.16[d]	
α-Humulene	X[c], 0.08–0.15[d], 0.09[f], 0.1[g], 0.13[h]	
Isocaryophyllene	tr–0.09[d]	
α-Muurolene	0.03[f]	
β-Santalene	X[c], 0.02–0.08[d], 0.04[f]	tr[g]
Selina-3,7(11)-diene	0.03–0.17[d], 0.05[f]	
Selina-4,11-diene	0.13[f]	
α-Selinene	0.06–0.15[d], 0.17[f] (incl. (*Z*)-α-bisabolene)	
epi-α-Selinene	0.05[f] (incl. δ-cadinene)	
β-Selinene	0.01[f]	
δ-Selinene	0.20[f]	
trans, *trans*-Sesquicitronellene	0.20[a]	

continued

TABLE 3.3 (continued)
Composition of Distilled Lime Oils

	Key Lime	Persian Lime
	Alcohols	
α-Bisabolol	X[c], 0.02–0.06[d], 0.06[f]	
epi-α-Bisabolol	0.01–0.06[d]	
Borneol	0.60[a], X[c], 0.59–0.82[d], 0.42[e], 0.69[f] (incl. nonanol), 1.4[g], 0.47[h]	0.10[e], 0.3[g]
α-Cadinol	0.01–0.03[d], 0.04[f]	
epi-α-Cadinol	0.02[f] (incl. *epi*-α-muurolol)	
Campherenol	X[c], 0.01–0.05[d], 0.04[f]	
cis-Carveol	0.1[g]	0.2[g]
trans-Carveol	tr[f], 0.1[g]	0.1[g]
Caryophyllene alcohol	0.02–0.09[d], 0.07[f]	
Citronellol	0.02[f]	
p-Cymen-8-ol	X[c], 0.11–0.28[d], 0.13[f] (incl. *p*-methylacetophenone), 0.16[h]	
Decanol	0.06[a], tr–0.04[d], 0.09[f] (incl. geranial)	
Dihydrocarveol	1.07–1.31[d] (incl. γ-terpineol)	
Dodecanol	tr–0.04[d], 0.04[f]	
Elemol	tr–0.04[d]	
γ-Eudesmol	0.03–0.10[d], 0.05[f]	
Endo-fenchol	1.19[a], X[c], 0.66–0.83[d], 0.66[e], 0.79[f], 1.4[g], 0.60[h]	0.30[e], 1.1[g]
Exo-fenchol	tr–0.02[d]	
Geraniol	tr–0.04[d], 0.02[f]	
Isoborneol	0.05–0.15[d] (incl. (*E*)-ocimenol), 0.08[f], tr[g]	tr[g]
Isopulegol	tr–0.08[d]	
Linalool	0.10[a], X[c], 0.11–0.22[d], 0.05[e], 0.19[f] (incl. undecane), 0.3[g], 0.15[h]	0.05[e], 0.1[g]
cis-*p*-Menth-2-en-1-ol	tr[f]	
trans-*p*-Menth-2-en-1-ol	0.02[f]	
epi-α-Muurolol	0.02[f] (incl. *epi*-α-cadinol)	
Myrcenol	tr–0.08[d], 0.08[f]	
Nerol	tr–0.02[d]	
Nerolidol	0.01[f]	
Nonanol	0.01[a], 0.69[f] (incl. borneol)	
(*E*)-Ocimenol	0.05–0.15[d] (incl. isoborneol), 0.06[f]	
(*Z*)-Ocimenol	0.60–0.91[d] (incl. cis-β-terpineol), 0.09[f]	
Octanol	0.01[a], 0.01–0.02[d], 0.02[f]	
trans-Pinene hydrate	tr–0.03[d]	
Selin-7(11)-en-4-ol	0.01[f]	
Terpinen-1-ol	0.68[a], X[c], 0.74–1.27[d], 0.84[e], 1.20[f], 2.3[g], 0.97[h]	0.12[e], 1.9[g]
Terpinen-4-ol	1.61[a], X[c], 0.56–0.88[d], 0.80[e], 0.97[f], 1.9[g], 0.65[h]	0.46[e], 1.3[g]
α-Terpineol	5.86[a], X[c], 7.00–8.04[d], 6.26[e], 7.9[f], 12.7[g], 5.38[h]	2.15[e], 6.6[g]
β-Terpineol	0.70[a], 0.69[e]	0.19[e]
cis-β-Terpineol	0.60–0.91[d] (incl. (*Z*)-ocimenol), 0.22[f], 2.2[g], 0.54[h]	2.2[g]
trans-β-Terpineol	X[c], 0.10–0.20[d], 0.81[f], tr[g], 0.17[h]	0.1[g]
δ-Terpineol	X[c]	
γ-Terpineol	X[c], 1.07–1.31[d] (incl. dihydrocarveol), 0.94[e], 1.21[f], 1.6[g], 0.77[h]	0.19[e], 0.4[g]
2,4,6-Trimethylbenzyl alcohol	0.56[a]	
Undecanol	tr–0.01[d], 0.03[f]	

TABLE 3.3 (continued)
Composition of Distilled Lime Oils

	Key Lime	Persian Lime
	Aldehydes	
Decanal	0.09[a], X[c], 0.02–0.27[d], 0.20[f], 0.09[h]	
Dodecanal	0.01[a], X[c], tr–0.10[d], 0.08[f]	
Furfural	0.01[a]	
Geranial	X[c], 0.02–0.04[d], 0.04[e], 0.09[f] (incl. decanol)	0.12[e]
Neral	X[c], tr–0.03[d], 0.02[e], 0.02[f]	0.09[e]
Nonanal	0.01–0.04[d], 0.03[f], 0.08[h]	2.69[e] (incl. terpinolene)
Octanal	0.03[a], 0.02–0.05[d], 0.04[f], 0.19[h]	
Perillaldehyde	X[c], 0.01–0.05[d], 0.02[f]	
α-Terpinen-7-al	tr–0.02[d]	
Tetradecanal	0.01–0.04[d], 0.02[f]	
Undecanal	X[c], 0.01–0.06[d], 0.03[f]	
	Esters	
Bornyl acetate	tr–0.02[d]	
Citronellyl acetate	tr–0.01[d]	
Decyl acetate	tr[d]	
Geranyl acetate	0.28[a], X[c], 0.06–0.13[d], 0.08[e], 0.10[f], 0.1[g]	0.09[e], 0.1[g]
Neryl acetate	0.01[a], X[c], 0.02–0.07[d], 0.12[e] (incl. δ-elemene), 0.04[f] (incl. decanoic acid), tr[g]	0.54[e] (incl. δ-elemene), 0.5[g]
trans-Pinocarvyl acetate	tr–0.04[d]	
Sabinyl acetate	0.01[f]	
	Ketones	
Carvone	tr–0.02[d], 0.02[e], 0.01[f], 0.1[g]	0.01[e], tr[g]
Methylheptenone	0.01[a], tr-0.01[d]	
p-Methylacetophenone	tr–0.02[d], 0.13[f] (incl. *p*-cymen-8-ol)	
cis-Pinocamphone	0.01–0.05[d]	
trans-Pinocamphone	0.02–0.06[d]	
Piperitone	tr[d], 0.01[f]	
	Oxides	
Caryophyllene oxide	tr–0.05[d]	
1,4-Cineole	1.75[a], X[c], 2.43–3.72[d], 1.96[e] (incl. δ-3-carene), 2.32[f], 2.0[g], 3.00[h]	0.39[e] (incl. δ-3-carene), 1.8[g]
1,8-Cineole	0.70[a], X[c], 4.35–9.47[d], 46.86[f] (incl. limonene), 1.79[h]	59.02[e] (incl. D-limonene)
2,3-Dehydro-1,8-cineole	0.19–0.29[d], 1.34[f] (incl. myrcene)	
Dihydrocaryophyllene oxide	tr–0.01[d]	
4,8-Epoxy-*p*-menth-1-ene	tr–0.03[d]	
cis-Limonene oxide	tr–0.03[d]	
trans-Limonene oxide	tr–0.04[d]	
cis-Linalool oxide	0.02[f]	
trans-Linalool oxide	8.43[f] (incl. monoterpene hydrocarbon and terpinolene)	

continued

TABLE 3.3 (continued)
Composition of Distilled Lime Oils

	Key Lime	Persian Lime
	Others	
Decanoic acid	0.04[f] (incl. neryl acetate)	
2,2-Dimethyl-5-(1-methyl-1-propenyl)-tetrahydrofuran	0.30[a], 0.14–0.25[d], 0.68[f] (incl. (*E*)-β-ocimene)	
p-Menth-1-en-8-yl vinyl ether	tr–0.01[d]	
1,1,3a,7-Tetramethyl-1*H-1*a,2,3,3a,4,5,6,7b-octahydro-cyclopropa [a]-naphtalene	0.13–0.24[d]	
2,6,6-Trimethyl-tetrahydropyran	X[c]	
2,6,6-Trimethyl-2-ethenyl-tetrahydropyran	0.16[a], 0.18–0.24[d], 0.22[f], 0.5[g], 0.21[h]	0.6[g]

Notes: tr: traces; [a] Kovats (1963). Relative percentage of peak areas; [b] Tápanes et al. (1971). Values reported (monoterpenes = 100%) were corrected estimating 70.5% of monoterpenes in the oil; [c] Chamblee et al. (1985). X = present; [d] Dugo et al. (1998). Relative percentage of peak areas; [e] Haro and Faas (1985). Relative percentage of peak areas; [f] Chamblee and Clark (1997). wt%; [g] Pino and Rosado (2001). Relative percentage of peak areas; [h] Kubeczka and Formàček (2002). Relative percentage of peak areas; [i] Feger et al. (2001). Relative percentage of peak areas.

lime oil contains higher contents of α-thujene, α-pinene, β-pinene, limonene, γ-terpinene, neral, geranial, and neryl acetate. On the other hand, 1,4-cineole, α-terpinene, terpinolene, terpinen-1-ol, α-, β- and γ-terpineol, and *E*,*E*-α-farnesene show higher levels in Key lime oil (Haro and Faas, 1985). In this work, *E*,*E*-α-farnesene was erroneously reported as β-sesquiphellandrene. The correct identification was made by Moshonas and Shaw (1980). As it will be seen later in this chapter, some of the components mentioned (i.e., β-pinene, α-terpineol) show big variations depending on the distillation conditions and cannot be used to differentiate both lime oils. Some components, such as γ-terpinene, neryl acetate, δ-elemene, and *E*,*E*-α-farnesene, show a more stable behavior in face of the distillation conditions and could be used as indicators to determine the type of lime used to obtain the oil. In particular, the ratio of two compounds, neryl acetate and δ-elemene, has been used to differentiate distilled Key lime oil from Persian. The relative amounts of these compounds vary little with the change in distillation conditions. The values of the ratio are less than 1.2 for Key and more than 10.0 for Persian. This ratio is very helpful in the detection of Persian lime oil being added to Key lime oil (Haro-Guzmán, 1999). The quantitative differences between both oils are large enough to explain those found in odor and flavor.

After reviewing the compositions and characteristics of both "West Indian" and "Floridian" Persian lime oils, Kesterson et al. (1971) concluded that the oils were quite different and could not be expected to meet the same requirements. Since they were made from different varieties of limes, they should probably have different quality standards.

In a study of Key lime distilled oil by GC-Olfactometry, Chisholm et al. (2003) evaluated the odor of the components and found that the most important contributors to the odor of the oil are geranial, perilla aldehyde, nonanal, linalol, nerol, citronellol, and neral. Among the sesquiterpenes,

caryophyllene and humulene oxides make important contributions. Many of the oxygenated components formed during the distillation, such as endo-fenchol, borneol, isoborneol and β-terpineol, have musty, camphor-like odors and, together with α-terpineol and carvone, are regarded as off-odors in citrus products.

In Table 3.4, the composition of distilled Key lime oils of different origins is compared to its corresponding cold-pressed type A.

TABLE 3.4
Composition of Key Lime Oils[a]

	Cold-Pressed	Distilled		
	Type A (Avg)[b]	Mexico (Avg)[c]	Peru[c]	Ivory Coast[c]
α-Thujene	0.39	0.01	0.01	tr
α-Pinene	2.45	1.03	0.99	1.26
α-Fenchene	–	0.71[e]	0.79[e]	0.68[e]
Camphene	0.12			
Sabinene + β-pinene	23.36	1.39	0.77	1.90
Myrcene	1.26	0.98	1.00	1.17
α-Phellandrene	0.03	0.42	0.44	0.48
δ-3-Carene	0.01	0.02	0.03	tr
1,4-Cineole	–	2.84	3.52	3.72
α-Terpinene	0.26	1.89	2.35	1.47
p-Cymene	0.32	2.97	2.30	2.17
Limonene	49.35	41.50	39.19	38.95
1,8-Cineole	–	7.27	4.35	9.47
γ-Terpinene	7.80	10.22	13.35	11.71
Terpinolene	0.42	8.01	9.70	7.28
Linalool	0.17	0.15	0.16	0.14
Nonanal	0.02	0.02	0.01	0.03
Endo-fenchol[d]	0.01	0.73	0.82	0.71
Citronellal	0.01	–	–	–
Borneol	0.03	0.64	0.82	0.64
Terpinen-4-ol	0.52	0.75	0.71	0.88
α-Terpineol	0.28	7.43	7.00	7.36
γ-Terpineol	–	1.16	1.24	1.07
Decanal	0.21	0.12	0.11	0.27
Nerol	0.04	0.01	0.02	0.02
Neral	1.15	0.01	0.03	0.01
Geraniol	0.05	0.03	0.01	tr
Geranial + perillaldehyde	1.86	0.07	0.07	0.06
Undecanal	0.03	0.03	0.01	0.02
δ-Elemene	0.31	0.06	0.05	0.04
Neryl acetate	0.08	0.04	0.06	0.04
Geranyl acetate	0.24	0.08	0.07	0.10
β-Elemene	0.18	0.06	0.06	0.04
Dodecanal	0.12	0.03	0.06	–

continued

TABLE 3.4 (continued)
Composition of Key Lime Oils[a]

	Cold-Pressed	Distilled		
	Type A (Avg)[b]	Mexico (Avg)[c]	Peru[c]	Ivory Coast[c]
cis-α-Bergamotene	0.09	0.06	0.06	0.04
β-Caryophyllene	1.07	0.55	0.42	0.43
trans-α-Bergamotene	1.36	0.65	0.72	0.62
(*Z*)-α-Bisabolene	0.15	0.08	0.11	0.08
(*E*,*E*)-α-Farnesene + β-Bisabolene	3.18	1.81	2.07	1.99
Germacrene B	0.50	0.06	0.05	0.01

Notes: tr: traces; [a] In the original papers, in addition to those listed in this table, were reported numerous minor components; [b] Dugo et al. (1997). Relative percentage of peak areas; [c] Dugo et al. (1998). Relative percentage of peak areas; [d] In the original paper it was wrongly reported as exo-fenchol; [e] α-fenchene + camphene.

3.2.2 Other Oils Distilled at Atmospheric Pressure

Oils distilled at atmospheric pressure are obtained from different effluents during the processing of citrus fruits. These effluents (i.e., recirculation water from the oil extractors, several centrifuge discharges, etc.) show a great variety of compositions, hence a wide pH range that produce a wide range of oil's compositions.

Pino et al. (1999) studied the composition of a grapefruit oil obtained by distilling at atmospheric pressure a mixture of the solids separated by the finisher of the emulsion coming from the oil extractor and the water discharged from the centrifuge used to separate such emulsion. The composition of the resulting oil is detailed in Table 3.5. For comparison purposes, the composition of a cold-pressed grapefruit oil has been listed (Pino and Sanchez, 2000). The samples come from two different years and are not directly comparable. However, some observations can be made. A couple of alcohols (α-terpineol and terpinen-4-ol) show a marked increase in the distilled oil, while on the other hand citronellal and the sesquiterpene hydrocarbons show a reduction. Mandarin, tangerin, lemon, bergamot, and sweet and bitter orange laboratory-distilled oils at atmospheric pressure are detailed described in Chapter 1.

3.3 VACUUM-DISTILLED (PERATONER) OILS

Peratoner and Scarlata were the first to use vacuum distillation for the recovery of lemon oil. This method, known as a "Peratoner," consists of the fragmentation and pressing of the fruits and the distillation of the resulting liquid at reduced pressure with steam, at a temperature that should be kept around 50°C to 60°C. The separation of the condensed water is achieved by simple decantation (Di Giacomo, 1966). Different emulsions resulting in the processing of citrus are distilled in the same way.

Peratoner and cold-pressed essential oils show notable differences, if not as marked, as in the case of lime oil. The distillation temperature is lower, the contact time shorter, and the emulsion less acidic. There are also clear differences in the organoleptic characteristics.

Both products show evident differences in composition: the distilled oils practically do not contain the components of the fixed residue. Sesquiterpenes and some aldehydes are in smaller amounts in distilled oils. Some alcohols, such as terpinen-4-ol and α-terpineol, are formed under the distillation conditions.

TABLE 3.5
Composition of Grapefruit Oils

	Cold-Pressed[a]	Distilled[b]
α-Pinene	1.70	3.80
Sabinene	–	0.20
β-Pinene	0.10	–
Myrcene	6.90	13.60
Limonene	84.80	70.90
Octanol	–	0.90
cis-Linanool oxide	–	1.00
trans-Linanool oxide	–	0.40
Linalool	0.50[c]	1.60
Nonanal		–
cis-Limonene oxide	0.10	0.10
trans-Limonene oxide	0.10	tr
Citronellal	0.20	–
cis-β-Terpineol	–	0.10
Terpinen-4-ol	–	0.80
α-Terpineol	0.20	2.30
Dihydrocarveol	–	0.10
Neodihydrocarveol	–	tr
Decanal	1.40	0.10
trans-Carveol	–	0.10
cis-Carveol	–	tr
Neral	0.20	0.30
Carvone	–	tr
Carvotanacetone	–	0.10
Geranial	0.40	0.30
Neryl acetate	0.10	0.10
α-Copaene	0.40	0.20
β-Cubebene	0.50	–
Longifolene	–	0.10
β-Caryophyllene	1.10	0.60
α-Humulene	0.20	0.10
Germacrene D	0.30	–
Bicyclogermacrene	0.10	–
δ-Cadinene	0.40	0.10
Elemol	0.20	–
(*E*)-Nerolidol	tr	–
Germacrene D-4-ol	tr	–
Nootkatone	tr	–

Notes: tr: traces; [a] Pino and Sanchez (2000). Relative percentage of peak areas; [b] Pino et al. (1999). Relative percentage of peak areas; [c] linalool + nonanal.

3.3.1 Lemon Peratoner Oil

Table 3.6 shows the composition of a distilled Peratoner oils of Italian lemon and its cold-pressed counterpart. If we use the average of the values cited for the distilled oil to compare with the values given for the cold-pressed oil, a reduction in the contents of α-thujene, sabinene, and citronellal will be noticed. There is a slight increase in terpinolene, linalol, nerol/citronellol, and geraniol, and a bigger increase in the amounts of terpinen-4-ol and α-terpineol. All these changes correspond well with the changes we have observed in other distilled oils. However the changes are smaller, as could be expected of a vacuum-distilled oil. Also, the sesquiterpene content is lower than in the distilled oil.

3.3.2 Mandarin Peratoner Oil

In Table 3.7, the components of Italian (Peratoner) and Brazilian (hydrodistilled) mandarin oils are listed together with their respective cold-pressed oils. As ranges of values are given, it is not easy

TABLE 3.6
Composition of Lemon Oils

	Cold-Pressed[a]	Distilled (Peratoner)[b]
α-Thujene	0.43	0.24–0.49
α-Pinene	1.95	1.10–2.11
Camphene	0.06	0.04–0.06
Sabinene	2.02	0.78–1.73
β-Pinene	13.01	8.22–13.99
Myrcene	1.44	1.36–1.58
Limonene	65.23	64.01–70.49
γ-Terpinene	9.54	8.37–10.72
Terpinolene	0.38	0.37–0.51
Linalool	0.11	0.15–0.31
Nonanal	0.11	0.06–0.13
Citronellal	0.09	0.01–0.04
Terpinen-4-ol	0.04	0.22–0.88
α-Terpineol	0.17	0.27–0.70
Decanal	0.04	0.02–0.06
Nerol + citronellol	0.04	0.02–0.37
Neral	0.83	0.51–1.45
Geraniol	0.02	0.02–0.43
Geranial + perillaldehyde	1.39	0.69–2.20
Neryl acetate	0.40	0.23–0.52
Geranyl acetate	0.42	0.18–0.59
β-Caryophyllene	0.23	0.10–0.23
trans-α-Bergamotene	0.34	0.12–0.33
α-Humulene	0.02	0.01
β-Bisabolene	0.51	0.16–0.50

Notes: [a] Verzera et al. (1996). Average values for oil produced with FMC machines, 1984–1993; relative percentage of peak areas; [b] Dugo et al. (1983). Relative percentage of peak areas.

TABLE 3.7
Composition of Mandarin Oils

	Italian[a]		Brazilian[b]	
	Cold-Pressed	Distilled (Peratoner)	Cold-Pressed	Hydrodistilled
α-Thujene	0.73–1.06	0.47–1.86	0.10–0.53	0.38–0.50
α-Pinene	2.03–2.74	1.41–4.87	0.26–1.53	1.04–1.44
Camphene	0.01–0.02	0.01–0.04	tr–0.01	0.01
Sabinene	0.23–0.34	0.09–0.33	0.11–0.17	0.17–0.19
β-Pinene	1.39–2.10	1.17–2.60	0.50–1.17	1.01–1.11
Myrcene	1.57–1.84	1.56–2.01	1.33–1.78	1.53–1.71
Octanal	0.06–0.20	0.09–0.29	0.06–0.19	0.25–0.38
α-Phellandrene	0.02–0.09	0.03–0.10	–	–
α-Terpinene	0.26–0.52	0.25–0.60	0.23–0.27	0.32–0.38
p-Cymene	0.10–1.38	0.20–0.94	–	–
Limonene	65.30–74.26	66.28–73.33	69.29–75.04	70.54–76.41
(*E*)-β-Ocimene	0.01–0.04	0.01–0.03	0.02–0.03	0.01–0.02
γ-Terpinene	16.40–22.75	16.37–21.79	15.41–19.52	13.76–15.80
Octanol	tr–0.01	tr–0.09	0–0.01	0.06–0.11
cis-Sabinene hydrate	tr–0.07	tr–0.02	0.05–0.09	0.03–0.06
Terpinolene	0.72–1.01	0.69–1.02	0.73–1.11	0.66–0.83
Linalool	0.04–0.19	0.15–0.34	0.12–0.19	0.34–0.69
trans-Sabinene hydrate	0.01–0.10	tr–0.04	0.12–0.19	0.07–0.14
Nonanal	0.01–0.04	0.02–0.06	0.04–0.08	0.04–0.06
cis-Pinene hydrate	–	–	0–0.01	0.03–0.05
p-Methen-2-en-1-ol	–	–	–	0.01–0.04
Camphor	–	–	0–tr	0.01–0.03
Citronellal	0.02–0.05	tr–0.04	0.04–0.10	0.04–0.07
Borneol	–	–	0–tr	0.01–0.02
Terpinen-4-ol	0.01–0.08	0.18–0.48	0–tr	0.32–0.75
α-Terpineol	0.03–0.27	0.20–0.49	0.29–0.53	0.61–1.46
Decanal	0.05–0.12	0.05–0.12	0.12–0.30	0.11–0.15
trans-Carveol	–	–	0.01–0.09	0.02–0.03
Octyl acetate	–	–	0–tr	0.02–0.04
Citronellol	tr–0.05[c]	0.02–0.06[c]	0.04–0.12	0.10–0.20
Nerol	–	–	–	–
Thymol methyl ether	–	–	–	0.01–0.03
Neral	tr–0.05	0.01–0.05	0–0.02	0.03–0.06
Piperitone	–	–	0–tr	0.01–0.02
Geraniol	tr–0.01	tr–0.01	0–tr	0.02–0.04
Geranial	0.01–0.10	0.03–0.11	0–tr	0.01–0.04
Perillaldehyde	–	–	0.08–0.16	0.16–0.27
p-Cymene-7-ol	–	–	tr–0.05	0.03–0.06

continued

TABLE 3.7 (continued)
Composition of Mandarin Oils

	Italian[a]		Brazilian[b]	
	Cold-Pressed	Distilled (Peratoner)	Cold-Pressed	Hydrodistilled
Thymol	0.01–0.11	0.01–0.13	0.16–0.32	0.20–0.67
Carvacrol	–	–	tr–0.05	0–0.01
Undecanal	tr–0.02	tr–0.01	0.01–0.04	0.01
(*E*,*E*)-2,4-Decadienal	–	–	0–0.01	tr–0.01
Citronellyl acetate	–	–	0–tr	0–0.01
Neryl acetate	–	–	0–tr	0–0.01
α-Copaene	–	–	tr–0.05	0–0.02
Geranyl acetate	–	–	0.01–0.04	0.01
β-Cubebene	–	–	0–0.01	0–0.01
Methyl *N*-methyl anthranilate	0.26–0.66	0.14–0.79	0.41–1.05	0.59–1.45
Dodecanal	–	–	0.03–0.16	0.02–0.04
β-Caryophyllene	0.07–0.14	0.03–0.09	0.11–0.27	0.05–0.10
α-Humulene	tr–0.02	tr–0.01	0.01–0.06	0.01
3-Dodecenal	–	–	0.02–0.12	0.01–0.02
Germacrene D	–	–	tr–0.03	0–0.01
α-Selinene	–	–	tr–0.18	0.03–0.05
α-Farnesene	0.06–0.32	0.02–0.16	0.31–0.84	0.14–0.22
δ-Cadinene	–	–	0.01–0.05	0.01
Tetradecanal	–	–	tr–0.03	0–0.01
α-Sinensal	0.12–0.53	0.01–0.21	0.55–1.05	0.05–0.28

Notes: tr: traces; [a] Verzera et al. (1992). Relative percentage of peak areas; [b] Frizzo et al. (2004). Results for Cai variety; relative percentage of peak areas; [c] Citronellol + thymol + thymol methyl ether.

to see small changes. However, it is clear that terpinen-4-ol and α-terpineol are in higher percentage in both distilled oils. This is more for the hydrodistilled oil, given it was distilled at a higher temperature. Both distilled oils also show a smaller sesquiterpene content as compared to their corresponding cold-pressed oils.

3.3.3 Bergamot Peratoner Oil

Given the composition of photosensitizing components (coumarins and fourocoumarins) in cold-pressed bergamot oils, several types of so called "bergapten-free" oils (distilled or alkali treated oils) have been proposed with the objective of eliminating such components. The range of the composition of same distilled "bergapten-free oils" (Ferrini et al., 1998; Verzera et al., 1998; Poiana et al., 2003; Belsito et al., 2007) is reported in Table 3.8, and so is the composition of a commercial Peratoner oil (Pizzimenti et al., 2006) and that of three samples of Peratoner oil industrially processed under the control of the authors (Costa et al., 2009). Also in the same table is reported the composition of Calabrian cold-pressed bergamot oils. With the exception of nonvolatile constituents, the composition of the distilled "bergapten-free" oils remains practically the same of that of

TABLE 3.8
Composition of Bergamot Oils

	Cold-Pressed[a]	Bergapten Free[b]	Peratoner[c]	Peratoner[d]
Tricyclene	tr–0.01	tr	tr	0.02
α-Thujene	0.19–0.49	0.14–0.4	0.12	0.11–0.18
α-Pinene	0.72–1.84	1.16–1.4	0.45	0.46–0.72
Camphene	0.02–0.05	tr–0.04	0.02	0.01–0.02
Sabinene	4.81–12.80[e]	0.91–1.1	4.24[e]	0.43–0.59
β-Pinene	4.81–12.80[e]	5.54–6.7	–	3.39–4.82
6-Methyl-5-hepten-2-one	tr–0.01	0.01	0.01	0.01–0.03
Myrcene	0.63–1.57	0.9–1.47	0.71	0.62–0.82
Octanal	0.02–0.08	tr–0.06[i]	0.02	0.01–0.02
α-Phellandrene	0.02–0.06	tr–0.06[i]	–	0.01–0.02
β-Phellandrene	tr	tr	tr	–
δ-3-Carene	tr–0.01	tr	0.01	–
Hexyl acetate	0–tr	tr	–	0–0.01
α-Terpinene	0.08–0.28	0.13–0.3	0.13	0.10–0.13
p-Cymene	0.01–0.89	0.09–0.5	–	0.13–0.16
Limonene	25.38–53.19	32.4–46.29	23.14	36.21–45.14
(*Z*)-β-Ocimene	0.01–0.07	tr–0.08	0.12	0.01–0.03
(*E*)-β-Ocimene	0.10–0.36	0.26–0.4	0.28	0.19–0.21
γ-Terpinene	5.27–11.38	5.95–8.7	5.99	6.20–7.55
1,8-Cineole	0.01–0.02	–	–	–
cis-Sabinene hydrate	0.01–0.06	tr–0.08	0.01	0.01–0.03[j]
Octanol	tr–0.02	tr	0.02	0.01–0.03[j]
cis-Linalool oxide (furanoid form)	0–tr	tr	–	–
Terpinolene	0.21–0.47	0.27–0.5	0.31	0.23–0.27
trans-Linalool oxide (furanoid form)	0–tr	tr	–	–
Linalool	1.74–22.68	7.81–13.57	36.88	18.38–33.14
Nonanal	0.01–0.08	tr–0.03	–	0.01–0.11
Heptyl acetate	tr–0.02	0.01	0.01	–
cis-Limonene oxide	tr–0.02	tr	0.01	–
trans-Limonene oxide	tr–0.01	tr	0.01	–
Isopulegol	0–tr	tr	tr	–
Camphor	tr–0.01	tr	0.01	–
Citronellal	tr–0.03	0.01	tr	–
Terpinen-4-ol	0.01–0.04	0.03–0.1	0.35	0.13–0.15
α-Terpineol	0.03–0.13	0.03–0.1	1.30	0.30–0.42
Dodecane	0–0.01	tr	0.01	–
Decanal	0.04–0.10	0.04–0.1	0.17	0.03–0.03
Octyl acetate	0.07–0.20	0.10–0.13	0.01	0.06–0.09
Citronellol	0.01–0.11[f]	0.05[g]	0.13[f]	–
Nerol	0.01–0.11[f]	tr–0.1	0.13[f]	0–0.07

continued

TABLE 3.8 (continued)
Composition of Bergamot Oils

	Cold-Pressed[a]	Bergapten Free[b]	Peratoner[c]	Peratoner[d]
Neral	0.10–0.34	0.10–0.21	0.09	0.12–0.15
Carvone	0–tr	tr	–	0–0.01
cis-Sabinene hydrate acetate	–	0.1	–	–
trans-Sabinene hydrate acetate	0.05–0.13	0.04	–	–
Linalyl acetate	15.62–41.36	26.31–33.6	22.76	16.81–18.71
Geraniol	0–0.01	tr	–	–
(*E*)-2-Decenal	0–0.01	tr	–	–
Geranial	0.20–0.49[h]	0.17–0.3	0.12[h]	0.13–0.19
Perillaldehyde	0.20–0.49[h]	–	0.12[h]	–
Bornyl acetate	0.01–0.04	0.02–0.05	0.02	0.01
Indol	0–0.01	tr	tr	–
Undecanal	tr–0.02	0.01	0.04	–
Nonyl acetate	0.01–0.05	0.02–0.04	0.04	0.01
Methyl geranate	tr–0.01	tr	tr	–
Linalyl proponate	0.01–0.07	0.03–0.1	0.03	0.02
δ-Elemene	0–0.01	tr	tr	–
α-Terpinyl acetate	0.07–0.26	0.12–0.2	0.14	0.05–0.09
Citronellylacetate	0.01–0.05	0.02	0.03	0.01
Neryl acetate	0.13–0.67	0.14–0.4	0.40	0.28
Geranyl acetate	0.11–0.80	0.19–0.5	0.45	0.31
Dodecanal	tr–0.05	tr–0.02	0.04	–
Decyl acetate	0.01–0.05	0.01	0.02	0.01
cis-α-Bergamotene	0.02–0.05	0.03	–	0–0.01
β-Caryophyllene	0.15–0.52	0.26–0.5	0.28	0.10–0.11
trans-α-Bergamotene	0.16–0.44	0.24–0.5	0.27	0.07
(*Z*)-β-Farnesene	0.03–0.09	0.04–0.12	0.04	–
α-Humulene	0.01–0.04	0.02	0.02	0.01
β-Santalene	tr–0.02	0.01	0.01	–
Dodecanol	0–0.01	tr	tr	–
Germacrene D	0.03–0.11	0.04	0.03	0.01
Bicyclogermacrene	0.01–0.08	0.02	0.01	–
(*Z*)-α-Bisabolene	0–tr	tr	–	0.01
(*E*,*E*)-α-Farnesene	0–tr	tr	–	–
β-Bisabolene	0.21–0.65	0.25–0.5	0.36	0.09–0.10
(*Z*)-γ-Bisabolene	tr–0.01	tr	0.01	–
Germacrene B	0–0.01	tr	tr	–
(*E*)-Nerolidol	0.01–0.04	tr	0.01	–
Tetradecanal	0–0.01	tr	–	–
2,3-Dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.01–0.02	tr	tr	–
Campherenol	0.01–0.02	tr	tr	–

TABLE 3.8 (continued)
Composition of Bergamot Oils

	Cold-Pressed[a]	Bergapten Free[b]	Peratoner[c]	Peratoner[d]
α-Bisabolol	0.01–0.03	tr	tr	–
Nootkatone	0.01–0.09	0.01	tr	–

Notes: tr traces; [a] Verzera et al. (1998); [b] Verzera et al. (1998), Ferrini et al. (1998), Poiana et al. (2003), Belsito et al. (2007), ranges are relative to those oils where the components were identified; [c] Pizzimenti et al. (2006); [d] Sciarrone (2009); [e] sabinene + β-pinene; [f] nerol + citronellol; [g] nerol + citronellol (value reported by Verzera et al., 1998); [h] geranial + perillaldehyde; [i] α-phellandrene + octanal; [j] octanol + *cis*-sabinene hydrate.

cold-pressed oils. The Peratoner oils, mainly the commercial sample, show an increase of alcoholic components (linalol, α-terpineol, and terpinen-4-ol) coming from hydration of monoterpene hydrocarbons. The bergamot "bergapten-free" oils are also treated in Chapter 1.

3.4 ESSENCE OILS

Another type of citrus oil that is increasingly used to flavor citrus juice products is essence oil. Essence oils are obtained from evaporators during the concentration of citrus juices (Kesterson and Braddock, 1976). This operation is carried out under vacuum and during a very short period of time. During the process of juice evaporation, many of the natural flavor components are removed with the water. This includes the small amount of peel oil remaining in the juice as well as juice–oil constituents. The volatiles recovered during the manufacture of juice concentrates are referred to as "essence." These essences are divided into two groups, water soluble and oil soluble. The oil-soluble fraction is called "essence oil." It has flowery or fruity aroma notes that are characteristic of the freshly squeezed juice from which it originates (Redd and Hendrix, 1996). Essence oils are normally retrieved in an "essence recovery unit" installed in the evaporator. The principle of essence recovery from citrus juices is based on vaporization of a part of the water present in the juices and the tendency of this vapor to contain both the oil and the aroma and flavor-bearing aqueous components. The concentration and removal of the essence and oil are obtained by use of a stripping column or flash chamber, a reflux column and condenser, and a chilled product condenser and receiver (Kesterson and Braddock, 1976). When there is not an essence recovery unit, the essence is recovered by decanting the condensed vapour from the juice evaporator.

Essence oils are clear, colorless distilled oils that lack natural antioxidants such as the carotenoids and tocopherols present in cold-pressed oils, and thus are stabilized by blending with peel oils (Shaw, 1977).

Essence oil from tangerine can be separated during the preparation of concentrated tangerine juice. However, it is rarely collected because it often has a fishy aroma that does not enhance the flavor of tangerine concentrate (Shaw, 1979a).

In Table 3.9, the composition of orange, lime, lemon, and grapefruit essence oils is reported.

Key lime essence is obtained by decanting the condensate from the evaporator, as the aqueous essence is not a common commercial item and most factories do not have a essence recovery unit. Lime essence oil is highly aromatic, with good odor characteristic of the fresh fruit. However, this fraction has not been widely used to flavor citrus products. Perhaps it is because the stronger flavor of distilled lime oil is so firmly entrenched as the typical lime flavor (Shaw, 1979a). With respect to their corresponding cold-pressed oils (see Chapter 1), Key lime and lemon essence oils show a similar pattern of compositional changes. Among the more important flavor-contributing compounds in orange essence oil are ethyl acetate, acetal, ethyl butyrate, hexanal, *trans*-2-hexenal, octanal, decanal, neral, and geranial. Valencene is the major sesquiterpene present in orange essence oil

TABLE 3.9
Composition of Essence Oils

	Orange				Key Lime	Lemon	Grapefruit
	a	**b**	**c**	**d**	**e**	**f**	**g**
Acetone	–	0.35	–	X	–	–	–
Hexane	–	–	–	X	–	–	–
1,1-Diethoxyethane	–	–	–	X	–	–	–
Ethanol	0.10	–	–		–	–	0.03
Heptane	–	–	–	X	–	–	–
Acetaldehyde	–	–	–	X	–	–	–
Ethyl acetate	tr	–	–	X	–	–	–
Acetal	tr	–	–		–	–	–
Hexanal	0.02	–	–		–	–	–
Methyl butyrate	–	–	–	X	–	–	–
Ethyl vinyl ketone	–	–	–	X	–	–	–
Ethyl propionate	–	–	–	X	–	–	–
Ethyl butyrate	0.10	tr	0.4	X	–	–	–
Furfural	–	–	0.7[h]		–	–	–
trans-2-Hexenal	tr	–	–		–	–	–
α-Thujene	–	–	–		0.22	0.39	–
α-Pinene	0.40	0.01	0.4	X	1.48	1.42	0.05
Camphene	–	–	–		0.08	0.09	–
Sabinene	0.40	tr	0.2	X	0.52	0.50	–
β-Pinene	–	–	–		16.23	8.30	–
6-Methyl-5-hepten-2-one	–	–	–		tr	–	–
Myrcene	1.80	0.04	2.9	X	1.16	1.45	–
Octanal	0.50	–	tr	X	0.05	0.04	0.09
α-Phellandrene	–	–	–		0.08	–	–
1,4-Cineole	–	–	–		0.01	–	–
α-Terpinene	–	–	–		0.54	0.39	–
p-Cymene	–	0.95	–	X	0.51	0.53	–
Limonene	93.60	84.72	93.4	X	55.16	66.60	99.18
1,8-Cineole	–	–	–		0.07	–	–
γ-Terpinene	–	–	–		10.94	10.10	–
n-Octanol	–	tr	–	X	–	–	–
Terpinolene	–	–	–		0.86	0.74	–
p-Mentha-1,8-dien-9-ol	–	tr	–	X	–	–	–
p-Mentha-1,8-diene-9-yl acetate	–	–	–	X	–	–	–
trans-Linalool oxide	–	–	–	X	–	–	–
Linalool	0.50	0.06	0.7[h]	X	0.53	0.30	0.08
Nonanal	–	–	0.1		0.04	–	0.03
trans-*p*-Mentha-2,8-dien-1-ol	–	–	–	X	–	–	–
cis-*p*-Mentha-2,8-dien-1-ol	–	–	–	X	–	–	–

TABLE 3.9 (continued)
Composition of Essence Oils

	Orange				Key Lime	Lemon	Grapefruit
	a	**b**	**c**	**d**	**e**	**f**	**g**
Citronellal	–	–	–		–	0.02	–
n-Nonanol	–	0.01	–	X	–	–	–
Terpinen-4-ol	–	–	0.1		2.26	1.02	–
α-Terpineol	–	–	0.4[i]	X	1.42	1.34	0.07
Decanal	0.60	–	0.7[h]	X	0.32	0.07	0.05
trans-Carveol	–	4.90	–	X	–	–	–
Octyl acetate	–	–	–	X	–	–	–
cis-Carveol	–	–	–	X	–	–	–
Citronellol	–	–	–	X	–	–	–
Nerol	–	–	–	X	0.03	0.07	–
Neral	0.20	–	0.1		1.70	1.22	–
Carvone	–	7.80	–	X	–	–	–
Geraniol	–	–	tr	X	0.03	–	–
Geranial	0.100	–	0.8[j]	X	2.46	2.01	0.10
Decanol	–	0.01	–	X	–	–	–
Perillaldehyde	–	0.01	–	X	–	–	–
Undecanal	–	–	0.1		0.02	–	–
Piperitone	–	0.03	–	X	–	–	–
Neryl acetate	–	–	–		0.13	0.60	–
Undecanol	–	0.20	–	X	–	–	–
Geranyl acetate	–	–	0.8[j]		0.10	0.56	–
α-Copaene	–	0.01	–	X	–	–	–
β-Cubebene	–	–	–	X	–	–	–
β-Elemene	–	0.02	–	X	–	–	–
Dodecanal	–	–	–	X	0.05	–	–
β-Caryophyllene	–	0.02	–	X	0.39	0.17	0.09
β-Copaene	–	–	–	X	–	–	–
trans-α-Bergamotene	–	–	–		0.49	0.24	–
Farnesene	–	–	–	X	–	–	–
β-Humulene	–	–	–	X	–	–	–
α-Humulene	–	–	–	X	–	–	–
n-Dodecanol	–	0.02	–	X	–	–	–
(*E*,*E*)-α-Farnesene	–	–	–		0.25	–	–
β-Bisabolene	–	–	–		0.37	0.41	–
δ-Cadinene	–	–	–		–	–	0.02
Elemol	–	–	–	X	–	–	–
Intermedeol	–	–	–	X	–	–	–
β-Sinensal	–	–	–	X	–	–	–

continued

TABLE 3.9 (continued)
Composition of Essence Oils

	Orange				Key Lime	Lemon	Grapefruit
	a	**b**	**c**	**d**	**e**	**f**	**g**
α-Sinensal	–	–	–	X	–	–	–
Valencene	1.70	0.70	0.4[i]	X	–	–	0.10
Nootkatene	–	–	–	X	–	–	0.04
epi-α-Selinene	–	–	–	X	–	–	–

Notes: tr: traces; [a] Johnson and Vora (1983). Relative percentage of peak areas; [b] Coleman et al. (1969). Valencia variety; relative percentage of peak areas; [c] Pino (1982). wt%; [d] Moshonas and Shaw (1979). X = present; [e] Haro-Guzmán (1985). Relative percentage of peak areas; [f] Ziegler (1998). Relative percentage of peak areas; [g] Coleman et al. (1972). Relative percentage of peak areas; [h] furfural, decanal, and linalool elute together; [i] α-terpineol and valencene elute together; [j] geranyl acetate and geranial elute together.

(Johnson and Vora, 1983). Its content is higher when compared with cold-pressed oil. It provides a notable difference between these two types of oil (Coleman et al., 1969).

3.5 CHEMICAL TRANSFORMATIONS OCCURRING DURING THE DISTILLATION

Because citrus oils are usually in aqueous acidic environments, the major mechanism by which oxygen is added to unsaturated terpenes is through acid-catalyzed hydration and not oxidation (Clark and Chamblee, 1992). Nevertheless, oxidation reactions do occur, particularly in other environments. Oxidation of orange and lemon oils typically produces limonene peroxides and carvone (Russel and Naim, 2000). Subject to a wide range of temperatures and acidity for long periods of time, ranging from seconds to 8 to 10 hours, many different chemical transformations can take place to different extents:

α- *and* β-*Pinene.* When treating β-pinene under conditions similar to those of a typical distillation, Haro-Guzmán and Huet (1970) obtained important proportions of 1,4-cineole, terpinolene, and α-terpineol, as well as lesser quantities of 1,8-cineole and *p*-cymene. Clark and Chamblee (1992) mention as products limonene, terpinolene, α-terpineol, borneol, isoborneol, 1,4- and 1,8-cineole, camphene, endo-fenchol, and fenchene. It was calculated that β-pinene reacts about 10 times faster than α-pinene, which itself reacts more quickly than limonene by a factor of approximately 16. McHale (1980) considers that under acidic conditions, β-pinene yields α-terpineol, fenchol, and borneol.

Sabinene and β-*thujene.* The major hydration products are terpinen-4-ol, α- and γ-terpinene, terpinolene, and sabinene hydrate. McHale (1980) mentions 1,4-cineole as a product of sabinene. Spontaneous ring opening and proton elimination yield monoterpene hydrocarbons γ-terpinene, terpinolene, and α-terpinene (Clark and Chamblee, 1992).

p-*Cymene.* This compound is stable under normal distillation conditions (Clark and Chamblee, 1992).

Limonene. Slater and Watkins (1964) have shown that limonene, which makes up around 50% of lime oil can, under distillation conditions, give out α-terpineol, terpinen-4-ol, 1,4 and 1,8 cineole, α- and γ-terpinene, and terpinolene. Shaw (1979b) wrote that terpinen-4-ol and α-terpineol levels might be expected to vary considerably in lemon oil because the strongly acidic juice can catalyse the hydration of limonene and other monoterpene hydrocarbons to these two alcohols if the oil comes in contact with the juice during processing. It is well known that limonene will produce *cis*- and *trans*-β-terpineol during acid-catalyzed hydrolysis (Chamblee and Clark, 1997).

γ-Terpinene. When submitted to conditions similar to those of lime oil distillation, γ-terpinene remained practically unchanged (Haro-Guzmán, 1984). Ikeda et al. (1961) showed that when lemon oil is exposed to air at room temperature, *p*-cymene is readily formed while γ-terpinene decreases to the same extent. The same behavior was found when distilled lime oil was subjected to similar conditions (Haro-Guzmán, 1988). Wiley et al. (1984) reported a turpentine-like off-odor in a cola soft drink stored up to 8 weeks at 20°C or 40°C under fluorescent or UV light. They suggested that the off-odor was due to the formation of *p*-cymene, which they found raised drastically with increasing storage time. They also suggested that *p*-cymene was produced from the catalytic dehydrogenation of γ-terpinene and limonene. The fact that butylhydroxytoluene (BHT) reduced the rate of *p*-cymene formation indicated that free radicals were also involved. Schieberle and Grosch (1989) examined the products of lemon oil photoxidation under UV light and an oxygen atmosphere. They reported that *p*-cymene did not produce a turpentine aroma. Under their GC-O conditions, they reported that *p*-cymene produced a solvent-like odor and that a mixture of several *p*-menthene hydroperoxides individually and collectively were responsible for the turpentine aroma observed in stored lemon oil. Table 3.3 shows abnormal amounts in distilled Key lime oil for some components as determined by Kovats (1963): α-terpinene was not found, γ-terpinene content is 0.63%, and *p*-cymene is 11.59%. This situation make us think he worked with a very deteriorated sample of oil. In fact, the content of *p*-cymene is used as an indicator of the degree of deterioration of lime oil. A maximum of 2.2% is considered acceptable.

Terpinolene. It was allowed to react in aqueous buffer, pH = 3. After 12 days of reaction at room temperature, the major products were α-terpineol, terpinen-4-ol, and γ-terpineol (Chamblee and Clark, 1997).

Citronellal. It has long been known to be unstable under acidic conditions. It cyclizes to form *cis*- and *trans-p*-menthan-3,8-diols as the major products, plus a small amount of the corresponding isopulegols (Clark and Chamblee, 1992).

Terpinen-4-ol. 1,4-Cineole is also a secondary product probably formed from terpinen-4-ol (Clark and Chamblee, 1992).

Citral. It readily cyclizes in acidic conditions yielding some *p*-cymene, *p*-cymenene, and a wide range of other products (Loori and Cover, 1964; McHale, 1980; Clark and Chamblee, 1992). Citral has been accepted by many people as the precursor of *p*-cymene in deteriorated lemon oil (Ikeda et al., 1961). In more advanced examples of deterioration, the *p*-cymene content of the lemon oil ran between 6% and 8% of the terpene hydrocarbons (lemon oils contain ~85% terpene hydrocarbons). Citral alone could not be the precursor of the *p*-cymene found in the deteriorated oil because citral itself seldom exceeds 3.5% in lemon oil (Ikeda et al., 1961). Additionally, it is believed that citral polymerizes to a large extent under the conditions of elevated temperature during the distillation process (Clark and Chamblee, 1992). There is evidence of citral cyclization during the distilled lime process; however, most of the citral still appears to be either polymerizing and/or forming Schiff bases with juice amino acids and is lost during the distillation (Chamblee and Clark, 1997). Citral does not appear to deteriorate when exposed to air in a neutral medium at room temperature (Ikeda et al., 1961).

Neryl and geranyl acetates. The major products of terpene ester hydrolysis are diols, along with linalol and geraniol, which will partially cyclize to α-terpineol and a small amount of Kovats ether "tetrahydropyran" (Clark and Chamblee, 1992).

Table 3.10 shows the kinetic data for the acid-catalyzed transformations of most of the major constituents found in lime oil. This allows a comparison of relative rates of transformation (Chamblee and Clark, 1997).

In Table 3.11, the composition of five different Key lime oils is enlisted with the purpose of verifying to what extent the composition changes according to the method of extraction (Haro-Guzmán, 1999). Cold-pressed type B oil after the rupture of the oil cells is in contact with water for several minutes at room temperature. In the case of cold-pressed type A oil, it is in contact

TABLE 3.10
Kinetic Data for Main Reactions

Compound	K_{abs} (moles/L-hr)
Citronellal	5,820
Sabinene	3,990
α-Thujene	129
β-Pinene	46
Citral	6.8
α-Pinene	4.6
Geranyl acetate	1.0
Germacrene B	0.8
Limonene	0.1

Source: Chamblee, T.S., and Clark, B.C., *J. Essent. Oil Res.* 9, 267–274, 1997.

TABLE 3.11
Composition (Relative Percentage of Peak Areas) of Key Lime Oils

	Cold-Pressed				
	Type B	**Type A**	**Essence Oil**	**Distilled**	**Distilled HO**
α-Thujene	0.36	0.41	0.35	0.02	0.01
α-Pinene	2.06	2.39	2.44	1.2	0.91
Camphene	0.09	0.11	0.13	0.52	0.48
Methylheptenone	0.02	0.01	0.02	–	–
Sabinene	3.19	3.09	0.77	–	–
β-pinene	19.20	20.30	21.39	1.92	0.01
Myrcene	1.23	1.35	1.27	1.35	1.31
α-Phellandrene	0.05	0.06	0.08	0.47	0.43
1.4-Cineole + δ-3-carene	0.01	0.01	0.03	2.11	2.29
α-Terpinene	0.17	0.24	0.45	2.61	2.61
p-Cymene	0.18	0.19	1.03	1.91	1.9
D-Limonene + 1,8-cineole	47.45	47.80	52.5	48.08	46.58
γ-Terpinene	8.48	8.11	8.42	10.94	10.53
Terpinolene	0.44	0.44	0.69	7.71	8.01
Linalool	0.20	0.20	0.35	0.16	0.23
Citronellal	0.05	0.04	0.01	–	–
Endo-fenchol	–	–	–	0.84	0.90
Terpinen-1-ol	–	–	–	0.93	1.13
β-Terpineol	–	–	–	0.67	0.84
Borneol	–	–	–	0.50	0.62
Terpinen-4-ol	0.06	0.29	1.63	0.91	0.95
α-Terpineol	0.35	0.26	1.26	6.51	7.99

TABLE 3.11 (continued)
Composition (Relative Percentage of Peak Areas) of Key Lime Oils

	Cold-Pressed				
	Type B	**Type A**	**Essence Oil**	**Distilled**	**Distilled HO**
γ-Terpineol	0.01	0.00	0.02	0.99	1.41
Decanal	0.27	0.26	0.18	–	–
Nerol	0.06	0.02	0.04	–	–
Neral	1.94	1.45	1.06	0.03	0.01
Geraniol	0.20	0.04	0.05	–	–
Geranial	3.07	2.33	1.8	0.04	0.01
Undecanal	0.03	0.03	0.01	–	–
δ-Elemene	0.62	0.59	0.12	0.07	0.06
Neryl acetate	0.10	0.09	–	0.04	0.03
Geranyl acetate	0.24	0.22	0.12	0.08	0.06
Dodecanal	0.11	0.04	0.04	0.09	0.05
β-Caryophyllene	1.14	1.08	0.31	0.66	0.69
trans-α-Bergamotene	1.15	1.26	0.43	0.83	0.85
(*E*,*E*)-α-Farnesene	1.661	1.37	0.36	0.95	1.09
β-Bisabolene	1.869	1.83	0.56	1.35	1.43

Source: Haro-Guzmán, L., 1999. Unpublished work.

with the acidic juice (pH 2.2–2.4) at room temperature for a few hours. The essence oil has been in contact with the juice for some minutes before the temperature goes up to some 85°C for several minutes. In the case of the distilled oil, the emulsion is brought to boiling temperature, an operation that takes 45 to 60 minutes. The distillation starts immediately and takes around 8 more hours to take all of the oil out (Haro-Guzmán, 1980). In the case of a "High Oxys" (HO) oil, after the heating period the emulsion is hold at 96°C to 98°C for about an hour and then distilled.

All these changes in the composition of citrus oils due to the influence of heat, acidity, and water must be kept in mind when doing research work (i.e., using the Clevenger method to isolate the oil from the peel), as the process will introduce more or less important changes depending on the conditions the distillation is carried out.

Seasonal and maturity factors pass to play a secondary role in front of distillation conditions in determining the quality of distilled lime oils and have a relative influence in the other distilled oils.

It can be seen that the production of a good quality distilled lime oil depends mostly on carrying the reactions to the necessary extent to obtain the correct balance of constituents.

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4 Concentrated Citrus Oils

Herta Ziegler

CONTENTS

4.1 INTRODUCTION

In today's modern nutrition, citrus fruits play an increasingly important role. All over the globe their pleasant, refreshing taste is closely associated with their nutritional value and healthy image. As a result, citrus products have developed into a major industrial factor within the last century (Swaine, 1988). Apart from the fresh fruit market, the production of juice and citrus by-products constitutes an important branch of the citrus industry. The downstream products of juice production and concentration, namely peel oils, essence oils, and aqueous essences, are valuable raw materials for the flavor industry. The most important commercially available sources of citrus oils, the so-called cold-pressed peel oils, are the products of cold pressing processes applied during juice production. This chapter on concentrated citrus oils will focus on peel oils, as this raw material is commercially available for all citrus varieties in substantial amounts and, therefore, constitutes the quantitatively most important source for the production of concentrates. While concentrates of recovery oils and waterphases have encountered increasing commercial interest in the course of the past two decades, the concentration of petitgrain oils (water vapor distillates of leaves and twigs) and neroli oils (distillates of blossoms) has been less common, as these oils are mainly used as single-fold products on an industrial scale (Priest, 1981).

As a result of their pleasant sensory properties, citrus oils are widely used for flavoring purposes. Their range of application extends from the food sector to household cleansers and fine perfumery (Mariani and Imes, 1987).

The flavor properties of the citrus oils are based on compounds like aldehydes, esters, ketones, and alcohols. Aldehydes may play one of the most important roles in citrus flavors. Although flavor quality is closely tied to the composition of these middle-polar, small constituents, they only play a minor role as far as quantity is concerned. The main constituents of citrus oils are nonpolar terpenes and sesquiterpenes, with limonene contributing the lion's share. Limonene, the main constituent in all citrus oils, is present in a range between 40% (i.e., bergamot) and 98% (i.e., orange), depending on citrus variety and geographic origin (see Chapter 1). As a result, the chemical and physical properties of citrus oils are closely tied to the characteristics of these nonpolar constituents (Ziegler and Feger, 2007).

This insight was the foundation of the modern citrus concentration industry, which based its business of producing concentrated oils on the prerequisite of liberating the higher valued flavor compounds from the nonpolar terpenic matrix, especially limonene. The roots of the citrus concentration industry can be dated back nearly one century. Until today, little knowledge and insight have been given about the citrus processing industry to the open public. While real facts on the numbers of boxes of fresh fruits, as well as data on the production of single-fold oils, are available in annual reports from growing areas all over the world, the actual amounts of citrus oil concentrates produced per annum could only be speculative due to the fact that most citrus producers and concentration companies do not publish production figures (Nonino, 1997). Additionally, literature on the methods used for citrus concentration purposes continues to be indirectly proportional to their industrial range of usage. Within the last two decades, most literature could be found on methods, applied—until now—on a more academic scale (Budich et al., 1999; Dugo et al., 1995; Fang et al., 2004). Publications on technologies being used for the production of concentrates on an industrial scale are only available to a limited extent (Lopes et al., 2003). This article will, therefore, be the attempt to establish correlations between the different citrus concentrates and their respective methods of concentration.

For this purpose, concentrates obtained by different production technologies will be analytically verified and depicted comparatively with regard to their properties, characteristics, functionality, and potential usage. This evaluation will center on the examples of orange and lemon oil concentrates; short data profiles of other citrus species will also be provided. In this context, it has to be kept in mind that the commercially available concentrates encompass an endlessly pluralistic variety of products. The spectrum of concentrates is markedly more complex than the underlying single-fold oils of different geographic origins, and it should be remembered that, across the market, identical product designations do not always have the same connotations for all market participants.

Single-fold oils, obtained during juice production, are subjected to a concentration process for a number of reasons. The exclusion of climatic fluctuations and supply shortages in single provenances, reduced transportation costs, as well as prolonged shelf life, solubility, stability, reproducibility, and enhanced flavor potential have to be considered in this context.

Especially the poor solubility of the terpene hydrocarbons as well as their thermal instability and sensitivity to oxidation exert a considerable influence on the properties of citrus oils. Oxidation, degradation, and other transformation products of terpene hydrocarbons, which can form under stress conditions, lead to odor-relevant off-compounds (Balton and Reineccius, 1992; Clark and Chamblee, 1992; Clark et al., 1981; Grosch and Schieberle, 1988; Nguyen et al., 2009; von Campe, 1990; Ziegler et al., 1991). These reactivities are able to disturb the citrus flavor associations resulting in a negative impact on the flavored products and their respective shelf life. So, today more than ever, attempts are made to preclude undesired side reactions of terpene hydrocarbons by minimizing their content in citrus oils.

Enhanced stability, shelf life, and improved solubility are features that concentrated citrus oil products possess as a result of their reduced amounts of terpene hydrocarbons (Fleisher et al., 1987).

Especially applications in beverages and foods require improved solubility and stability, which are of particular importance for flavoring soft drinks, one of the main foci of citrus flavorings in the food sector (Pfannhauser et al., 1987; Popken et al., 1999; Rouseff and Naim, 2000; Ruiz Perez-Cacho and Rouseff, 2008).

Additionally, in the case of citrus concentrates, pronounced emphasis is put on the guaranteed reproducibility of the flavoring material, resulting from the huge blended batches that are the educts of concentration. Standardizations, often obtained by incorporating harvests of oils of one species from different geographic origins, permit the exclusion of climatic fluctuations to a large extent. As a consequence, concentrates aim to ensure uniformity despite climatic variations and, if supplied by the same producer, offer a more or less unique, inimitable composition. On the other hand, raw material price changes are every year closely correlated with the annual harvest. The development of the prices for grapefruit oil constitutes a good example. A few years ago, as a result of hurricanes in Florida, raw material prices went up significantly, a difficult situation as far as the concentrates are concerned, as rising raw material costs caused proportionally higher price increases for the respective x-fold concentrate. Scientific analytical research, therefore, constitutes an indispensable tool for distinguishing between concentrates resulting from authentic, recently harvested raw materials as well as up-to-date manufacturing practices and those based on inferior resources with a main focus on price. The evaluation of various citrus concentrates with regard to their composition, their physical properties, and their reactivity toward oxidative stress constitutes the aim of the present contribution.

The sensory aspect will not be dealt with explicitly here, as an objective evaluation would exceed such a review (Burgard, 1995). Nevertheless, it should not be forgotten that the retention of genuine flavor profiles is a main focus of sophisticated concentration processes.

4.2 CONCENTRATION METHODS

Taking the above remarks concerning citrus oils and the essential properties of citrus concentrates into consideration, the interdependency between the quality of a citrus concentrate and the employed production method becomes obvious. Ideally, the selected concentration process not only takes the nature of the thermally sensitive citrus oils into consideration but also allows focusing on the level of quality required, the desired functional properties, as well as the intended application of the envisioned concentrate. A number of different techniques can be employed today to optimally concentrate citrus oils: distillative, extractive, and chromatographic methods are available; frequently combined multistep technologies are in use.

As pointed out, various parameters have to be considered for the selection of the appropriate concentration method. First of all, the undesired nonpolar constituents (mono and sesquiterpene hydrocarbons and waxes) have to be removed in order to equip the concentrated oil with improved solubility and enhanced stability. The selected procedure should additionally take the thermal liability of citrus oils as well as their complex flavor matrix into account. Moreover, the citrus oils' general sensitivity to oxidation has to be considered as the formation of peroxides and radicals causes subsequent destruction of the concentrate. The field of application of the concentrated product should, therefore, be directly correlated with the employed processing technology.

Naturally, the range of citrus flavors produced by concentration is enormously complex and the spectrum of available products encompasses a tremendous variety. The comparison of concentrates produced by cold versus heat-requiring concentration methods is challenging but highly interesting, as it permits an evaluation of the impact of the concentration process on their physical and sensory parameters as well as their beneficial effect on the final products flavored with the respective concentrate.

In the following, an attempt will be made to characterize the concentration processes and to evaluate the suitability of the various deterpenation methods currently employed for citrus oil concentration.

4.2.1 Distillative Separation Methods

Distillation is often employed on an industrial scale to produce concentrated, folded citrus oils (Tateo, 1990; Vora et al., 1983). Distillation is based on the evaporation of components and, therefore, heat has to be introduced to reach the boiling point of the liquid and to transfer the components into the gaseous phase. The liquid mixture is thermally separated by boiling the liquids, condensing the vapor, and collecting the components according to their boiling points. The fundamental principle of this separation is the fact that the liquid phase possesses a different composition than the corresponding vapor phase (Sattler, 1995; Stichlmair and Fair, 1998). However, as many citrus constituents are sensitive to heat and oxidation (Grosch and Schieberle, 1987), the application of gentle distillation methods is indispensable (Tateo, 1990). As a result of the thermal instability of the terpene hydrocarbons, distillative processes are carried out under vacuum to lower the boiling point. In order to increase separation efficiency, rectification is employed where the ascending vapor is in contact with the refluxing liquid in a column. The separation efficiency of a column is characterized by its height of a theoretical plate, where an equilibrium between the two phases, liquid and vapor, exists in each stage. During this intensive contact of the countercurrent phases of vapor and reflux, a mass and energy transfer occurs and the component with the lowest boiling point is enriched towards the top of the column. To reach high mass transfer rates in the column, various packings have found application in the citrus industry. Suitable packings are characterized by a high number of theoretical plates and throughput, as well as both negligible pressure loss per plate and low proneness to encrustations, and therefore have to be selected according to the respective application (Billet, 1992; Schultes, 1998).

Distillation can be carried out in discontinuous or continuous mode. While with the latter only one distillate fraction can be obtained per column, components can be separated successively according to their boiling points in discontinuous mode. Disadvantages of the discontinuous method are, however, high energy requirements and prolonged exposure to thermal stress.

Depending on the distillation requirements, different types of reboilers (Billet, 1981, 2000; Frank and Kutsche, 1969) are used in the citrus industry. Simpler constructions include vessels with double jackets or heat exchangers; the different evaporators are of more elaborate technical construction. Rotary evaporators are utilized for lower volumes; centrifugal evaporators are designed for large applications and are available with one or more stages. Centrifugal force creates a very thin and turbulent film resulting in the highest heat transfer coefficient with short residence times and, therefore, minimized exposure to thermal stress. Thin-film evaporators, on the other hand, use gravitational forces and a rotor with blades creates a thin film across the heated surface. For operation at very low vacuum, evaporator constructions with a condenser closely opposite to the evaporation surface result in almost molecular distillation and permit processing of sensitive, high molecular citrus constituents.

Falling-film and forced-circulation evaporators are employed for continuous distillation and allow gentle product handling for heat-sensitive materials as contact time is short and the liquid is not overheated during passage. For applications where volatiles are to be removed even from viscous and solid-containing liquids, columns with either fixed or rotating internals are available, which feature low pressure drop and high mass transfer efficiency due to the countercurrent flow of vapor and feed (Sykes et al., 1992).

Distillation permits a cost-effective separation of citrus constituents according to their boiling points. Removal of the monoterpene hydrocarbons results in concentrates, which not only show decreasing solubility as a result of an augmented content of the less volatile constituents on the one hand, but also exhibit losses in the volatile flavor compound range on the other. Thermal methods possess only limited suitability for the selective fractionation of desired valuable polar nonvolatiles (e.g., sesquiterpenoids); subsequent reduction of nonvolatiles in order to improve solubility may lead to a negative impact on stability (for further information see Section 4.4.3). The potential for radically or thermally induced transformation and crack processes can lead to the formation of

precursors which subsequently may cause aroma destruction and exert a negative impact on the aroma profile of the concentrated oils (Shen et al., 2002).

4.2.2 Extractive Methods

Extraction constitutes one of the oldest known techniques for the production of aromatic mixtures from plants with a solvent as an auxiliary agent (Arce et al., 2005; Fleisher et al., 1987; Fleisher, 1990, 1994; Moyler and Stephens, 1992; Ruys, 1957; Stahl, 1987; van Dijck and Ruys, 1937). In liquid–liquid extraction, a solvent is added to the liquid matrix to selectively remove transition components by the formation of two coexisting immiscible liquid phases. The selected solvent must be capable of preferentially dissolving the constituents (solutes) to be extracted and be either immiscible or only partly miscible with the carrier. The extract is the solvent-rich solution containing the extracted solute and the raffinate is the solvent-lean residual feed mixture. Extraction is, therefore, based on the different affinities of the constituents distributing between the two coexisting liquid phases and is characterized by the distribution coefficient (Hanson et al., 1983; Sorensen et al., 1981; Sorensen and Arlt, 1979, 1980).

The selection of a suitable solvent for the extraction process is of particular importance. The solvent must have a high extraction capacity, possess high selectivity and low solubility with the carrier and be permitted by the legislative guidelines. Additionally, the selected solvent should be easily removable after the extraction process and has to be recycled to ensure an economical as well as ecological mode of operation.

Extraction is usually carried out in discontinuous or continuous mode. In discontinuous cross-current extraction, the solvent is mixed with the feed and subsequently separated; the leaving raffinate is then again extracted with fresh solvent resulting in large solvent requirements.

Continuous extraction is performed in multistage countercurrent mode, where solvent and feed continuously move countercurrently towards each other in the extractor. The concentration of solute in the extract is much higher than in cross-current extraction and less solvent is necessary for the depletion of solute in raffinate. Depending on the technical construction of these extractors, two different types can be described: stagewise or differential contacting of the two countercurrently flowing phases.

The majority of extraction units are based on the transferal of the transition component from the continuous phase to the dispersed phase. To reach a high mass transfer coefficient, the selected extraction equipment has to ensure the generation of small droplets, a homogeneous droplet distribution, the prevention of axial back-mixing and the fast phase separation after the solute transfer. For the determination of extraction efficiency in these kinetic systems, the height of a transfer unit and number of a transfer unit (HTU-NTU) models were developed. The HTU term reflects the physical and fluid-dynamic parameter and the NTU term the number of theoretical stages as function of the solute concentration difference. These characteristic HTU values can be experimentally measured for a certain extractor type and are used for the projection of larger units. The NTU term characterizes the concentration profile and can be graphically determined by the loading diagram (Godfrey and Slater, 1994; Mueller, 1972; Teh et al., 1983). In a stagewise extractor (e.g., mixer-settler), the concentration profile changes in each stage, as the separated layer of extract and raffinate, is newly distributed in the subsequent unit. Two types of mixer-settlers are available, with phase separation either by centrifugal or gravitational forces and droplet generation in centrifugal chambers or by static blenders, respectively. For multistage liquid–liquid extraction, several units are set in series. Extraction towers with differential contacting have found application in the citrus industry. These extraction towers have in common that the internals are formed into compartments for agitation, achieved by rotating discs or impellers, on the one hand and calming on the other. The two phases enter at the opposite ends of the tower and gravitational forces are used for the phase flow. The internals of an extraction tower are constructed in such a way that high hold up and large

interface renewal for the dispersed phase are reached and all fluid elements have a narrow residence time distribution in the apparatus (Ziegler, 2007).

Extraction with membrane contactors constitutes a relatively new development, which is available as supported-liquid membrane (SLM) or as microporous membrane liquid-liquid extraction (MMLLE) (Ho and Sirkar, 1992; Schneider et al., 1988; Turner, 2006). In MMLLE, the carrier fills the pores of a microporous, hydrophobic membrane, which allows the subsequent diffusion of the transition compounds into a nonwetted acceptor phase. In general, MMLLE is offered as a microporous module and as a result of the membrane construction, the interface area for mass transfer by volume is very high compared to extraction towers. The advantage of these extractors is that neither density differences between the two phases are necessary, nor emulsions are formed. In SLM, the difference in solubility and diffusion into the polymer is the basis of a very selective separation (Auerbach, 1995).

For citrus oils, liquid–liquid extraction constitutes a valuable tool, as the matrix to be extracted contains compounds that are thermally labile, possess similar boiling points, and are present in low concentration and as complex mixtures. The extractive concentration can proceed at low temperatures, ensures minimal side reactions, and results in citrus concentrates with authentic flavor profiles. The process permits the simultaneous selective concentration of the flavor compounds and the valuable more polar nonvolatiles (sesquiterpenoids, coumarins, and flavones) of analogous polarity. This can result in complex products: even trace odor components or nonvolatiles, contributing to the stability of the flavor, can be selectively extracted.

The so-called washing of citrus oils constitutes a simple, traditional extraction method and is still widely employed in the beverage industry. Here, the citrus oils are extracted with aqueous ethanolic solutions and the more polar compounds are enriched in the aqueous ethanol depending on water content and oil/solvent ratio (Moyler, 2002; Owusu-Yaw et al., 1986). Due to the highly polar solvent system, the yield of flavor compounds is comparatively low and the formation of emulsions complicates phase separation (Moyler and Stephens, 1992) (see also Section 4.4.4).

4.2.3 Chromatographic Methods

Chromatographic separation processes provide a powerful tool for the separation of mixtures in which components have different adsorption affinities and, in thermodynamic equilibrium, are characterized by their respective adsorption isotherms (Guiochon and Golshan-Shirazi, 1994; Ruthven, 1984; Seidel-Morgenstern, 1995; Yang, 2003). The Multi-Langmuir equation can be employed for the mathematical description of the adsorption isotherms. In liquid–liquid chromatography, the movement of a mobile phase of solvent and solutes results in separation due the selective retention of the solutes on a stationary phase. The method originated from analytical laboratory techniques (Braverman and Solomiansky, 1957; Kirchner and Miller, 1952; Ziegler, 1971) and its application on various stationary phases is realized industrially today. Frequently, stationary phases (silica gel, aluminum oxide, anhydrous magnesium or calcium sulphate, celite, etc.) are employed where the constituents of a mixture are successively eluted as a result of increasing solvent polarity (Ferrer and Matthews, 1987; Tzamtzis et al., 1990). Moreover, a broad variety of stationary phases is available today on which liquids with various functional groups are immobilized on a support. In this partition chromatography, the solubility between the adsorbed components and the immobilized liquids dominates the separation process, resulting in a reversal of the elution order (reversed stationary phase). Additionally, porous hydrophobic adsorption polymers have encountered application for separation purposes (Liska et al., 1989).

In countercurrent chromatography (CCC), the liquid–liquid partition of solutes resembles extraction principles and separation is carried out without a solid stationary phase. One liquid phase is strongly retained in the chromatographic unit by centrifugal forces while the second immiscible phase flows through it. Different units are available, but so far only for applications on a smaller scale (Mandava and Ito, 1988).

Chromatography can be performed on two types of equipment: vertical and annular columns (Brozio and Bart, 2000). The separation efficiency on a column is influenced by its hydrodynamics (axial dispersion) as well as the kinetics of mass transfer (mass transfer resistance), and is expressed as height equivalent of a theoretical plate. In the case of a linear adsorption isotherm, the effects of mass transfer and the axial dispersion are cumulative and the relation of height equivalent of a theoretical plate to flow rate is expressed by the Van-Deemter equation. Based on these experimentally determined parameters, the chromatographic process has to be adapted to the respective matrix to be separated. For scale-up to industrial usage, it is necessary to optimize the flow profile of the mobile phase to reach the desired separation efficiency (Arlt and Deckert, 1994).

Adsorption chromatography allows the complete and selective separation of citrus oils into polar and middle-polar volatile flavor compounds, as well as polar and middle-polar nonvolatiles (e.g., sesquiterpenoids and heterocyclic aromatic compounds). Its powerful separation efficiency also allows the selective fractionation of compound classes by gradient elution. The solvents employed in the chromatographic process have to comply with the legislative guidelines and should be easily removable and recyclable. The resulting products possess the complete complex authentic profile of the citrus fruit and also retain the entire range of the nonvolatile, polar compounds with their stabilizing and emulsifying properties.

With partition chromatography, citrus oils can also be separated in aqueous alcoholic solutions as mobile phase on hydrophobic stationary phases (Fleisher, 1990). This technique is also used to recover flavor constituents from waterphases.

With the above described methods, the citrus industry has a broad spectrum of processing technologies at its disposal to offer its clients a comprehensive choice of products for all budgets and applications. Despite its natural raw material matrices, the industry is inherently a technology-driven one. Whether relatively simple traditional method or innovative and technologically elaborate concentration technique, the employed technological concept as well as know-how in process design and engineering have a decisive impact on the final product and its quality. In the following, a detailed analytical investigation of the relationship between processing method and concentrated product will be provided.

4.3 DEFINITIONS AND TERMINOLOGY ("X-FOLDS" VS. "TERPENE- AND SESQUITERPENE-FREE" VS. "HYDROCARBON-FREE AND HYDROCARBON-REDUCED")

Using the methods discussed under Section 4.2, the citrus concentration industry is able to produce nearly any level of concentration, as well as type of oil, equipped with the features the specific application requires and the client desires. As a result, the worldwide market offers such a multitude of concentrates that comparisons seem often difficult. Therefore, a few considerations on terminology are appropriate.

Concentrates are usually categorized as foldings of the single-fold oils they derive from. The correlation with the number indicating the folding level can be traced back to the earliest concentration processes. As these were solely of distillative nature, the folding level resulted numerically from the yield remaining in the flask residue (Auerbach, 1995). Example: if 80% of the volatile, terpene hydrocarbons containing part (*distillate*) was removed from the oil, a residue of 20% (*concentrate*) remained; thus a yield of 20% was obtained and the oil was characterized as 100/20 = 5-fold. Other feed/product ratios and, therefore, folding levels were calculated analogously (Lopes et al., 2003). This rule still applies to regular distillates and was subsequently transferred to the more recently developed cold concentration methods. It has to be kept in mind that for distillates, this strictly yield-based definition does not reflect the effective folding level of each individual flavor compound and is not only restricted to the enrichment of flavor constituents. In these processes, nonflavoring compounds of the higher boiling range are accumulated with increasing folding level, while flavor compounds of the low boiling range are lost. The folding, therefore, does not always provide a compound-specific insight on a molecular level.

If this model is transferred to cold concentration processes, the folding level is more closely correlated to the enhancement of the flavor constituents. In this case, a combination of yield and the values actually measured for the individual flavor constituents allows a more precise specification of the x-fold level.

Depending on the citrus variety, foldings are normally offered as 3-fold, 5-fold, 8-fold, 10-fold, and up to 20-fold, but all folding levels in between are also available on the market.

As the terms *terpeneless oils* and *sesquiterpeneless oils* are not unambiguously defined (Tzamtzis et al., 1990) for the multitude of commercially available concentrates, they will not be used within the scope of this article. Some suppliers—depending on the citrus variety—already employ these terms for 5-to-10-fold concentrates, while others use them only for concentrates 30-fold and higher. As a 100% terpeneless or sesquiterpeneless citrus oil does not exist anyway, this classification method will within this chapter be replaced with the more flexible but also precise term, x-fold, depending on the degree of concentration. Additionally, this will be complimented by the concentration method used and, where applicable, by [+nv] = "with nonvolatiles" or [–nv] = "reduced nonvolatiles" and/or "without nonvolatiles."

Both with producers and in the literature (Tzamtzis et al., 1990), the term *terpeneless oils* connotates a more or less comprehensive reduction of the terpene hydrocarbons, while the term *sesquiterpeneless oils* refers to the reduction of the sesquiterpenes as representatives for the entire nonvolatile fraction.

In the following, the term [–nv] will be used instead of *sesquiterpene-free* for products where these constituents—including nonpolar as well as middle-polar and polar compounds of the higher boiling range (e.g., sesquiterpene hydrocarbons, sesquiterpenoids, heterocyclic aromatic compounds [coumarins, psoralens = furocoumarins, and flavones]) as well as the nonpolar waxes and lipids—have additionally been reduced.

In the case of highly concentrated oils produced by extractive or chromatographic methods, the situation is somewhat more complex: ideally, these concentrates should be classified as *hydrocarbon-free* or *hydrocarbon-reduced*, as the nonpolar mono- and sesquiterpene hydrocarbons as well as all nonpolar higher compounds are minimized simultaneously, while all more polar sesquiterpenoids, heterocyclic aromatic compounds, and polar high molecular compounds are concurrently enriched by these methods (Ferrer, 1987). This is the result of processing the oils with methods that are not based on thermal differences but on varying polarity. These oils retain the important markers of the nonvolatile range—the heterocyclic aromatic compounds, visible in HPLC-measurements—while exhibiting good solubility. These products even contain enriched levels of heterocyclic aromatic compounds and sesquiterpenoids, valuable flavor constituents. Chromatographically produced high concentrates show, due to their highly enriched middle-polar and polar nonvolatiles, lower solubilities welcome for some applications (e.g., stabilized emulsions). For applications requiring an optimum of solubility, these products are additionally available with reduced nonvolatile fraction. Such products will further-on be called *x-fold, chrom.—nv* within the scope of the depicted examples.

This survey of the various citrus concentrates must remain restricted to examples, as the variety of products is immense and the user is normally not acquainted with the respective method of production. As the author is familiar with the parameters and production methods of the concentrates depicted in the present study, the respective x-fold concentrates can be assigned to the various processing technologies resulting in a detailed picture of the technique's potential.

4.4 PROPERTIES OF DIFFERENTLY PRODUCED CITRUS CONCENTRATES

4.4.1 Physical Properties

The physical properties (Table 4.1) of concentrated citrus oils differ from single-fold oils depending on their degree of concentration. While the latter vary mainly as a result of geographic and botanical origin and the harvest period of the fruits (Dugo et al., 2002; see also Chapter 1), the

TABLE 4.1
Physical Data of Different Citrus Oil Concentrates

Orange Oils	Aldehyde	Optical Rotation[a]	Refractive Index[b]	Density[c]	Solubility EtOH 93%[d]
1-fold	1.3–1.6%	98	1.473	0.846	1:9
12-fold, dist. +nv	8.2–9.2%	65	1.481	0.881	1:19
8-fold, dist. –nv	8–9%	84	1.470	0.848	1:1
5-fold, extr. +nv	5.8–6.3%	90	1.472	0.852	1:2
5-fold, extr. –nv	5.8–6.3%	92	1.469	0.845	1:2
40-fold, extr. +nv	35–40%	n.d.	1.483	0.928	1:1
30-fold, chrom. +nv	30–33%	n.d.	1.484	0.925	n.s.
50-fold, chrom. –nv	43–53%	18	1.462	0.883	1:1
Lemon Oils	**Aldehyde**	**Optical Rotation[a]**	**Refractive Index[b]**	**Density[c]**	**Solubility EtOH 90%[d]**
1-fold	3.2–3.6%	62	1.475	0.853	1:10
8-fold, dist. +nv	13–15%	n.d.	1.479	0.874	1:24
8-fold, dist. –nv	13–15%	51	1.475	0.859	1:2
5-fold, extr. +nv	9–11%	62	1.477	0.861	1:3
30-fold, extr. +nv	42–50%	–4	1.492	0.931	1:1
30-fold, extr. –nv	52–62%	–7	1.475	0.889	1:1
10-fold, chrom. +nv	19–23%	46	1.479	0.875	1:1
25-fold, chrom. +nv	34–46%	2	1.489	0.927	1:1
Grapefruit Oils	**Aldehyde**	**Optical Rotation[a]**	**Refractive Index[b]**	**Density[c]**	**Solubility EtOH 93%[d]**
12-fold, dist.	2.5–3.5 %	50	1.504	0.941	n.s.
6-fold, extr.	3.8–4.3%	88	1.481	0.875	1:1
12-fold, extr.	6.5–8 %	58	1.49	0.907	1:1
12-fold, chrom.	6.2–7%	54	1.494	0.916	1:1
Mandarin Oils	**Aldehyde**	**Optical Rotation[a]**	**Refractive Index[b]**	**Density[c]**	**Solubility EtOH 95%[d]**
1-fold	0.5–1.2%	74	1.475	0.866	1:3
4-fold, dist. +nv	2.5–3.5%	n.d.	1.480	0.867	1:10
4-fold, extr. +nv	3.9–4.8%	84	1.475	0.854	1:1
5-fold, chrom. +nv	3.7–4.7%	68	1.478	0.866	1:7
40-fold, chrom. +nv	19–21%	n.d.	1.491	0.941	1:1
Lime Oils cp	**Aldehyde**	**Optical Rotation[a]**	**Refractive Index[b]**	**Density[c]**	**Solubility EtOH 93%[d]**
1-fold	3.2–5.2%	50	1.479	0.863	1:5
6-fold, dist. +nv	14–15%	n.d.	1.483	0.889	1:15
5-fold, extr. +nv	13.5–14.5%	n.d.	1.497	0.921	1:1
5-fold, chrom. +nv	12.4–13.3%	n.d.	1.497	0.917	1:1
Lime Oils dist.	**Aldehyde**	**Optical Rotation[a]**	**Refractive Index[b]**	**Density[c]**	**Solubility EtOH 90%[d]**
1-fold	0.4–1%	35	1.479	0.860	1:5
5-fold, dist.	1.5–2.5%	–5	1.485	0.902	1:1
5-fold, extr.	0.5–1.5%	5	1.475	0.880	1:1

Notes: Nv, nonvolatiles; n.d., not detected/not measured due to oil darkness; n.s., not soluble; [a] range ~ ± 10; [b] range ~ ± 0.01; [c] range ~ ± 0.02; [d] part oil/part EtOH.

concentrates could supposedly be differentiated depending on the individual producer, the respective combination of employed raw material matrix as well as production method. As this would far exceed the scope of this article, commercially available concentrates, obtained according to good manufacturing practice (GMP), will be evaluated comparatively as an example.

The *aldehyde content* increases with the degree of concentration and serves in total as a marker for the augmentation of the flavor compounds. With the exception of prior nonvolatile reduction, *density* increases with concentration (Di Giacomo et al., 1999). In contrast (Pino et al., 1992), the values of *optical rotation* decrease with the progressing reduction of limonene. If a high content of nonvolatiles is present, optical rotation can occasionally only be determined imprecisely and will then be marked as "n.d." (not detected).

The changes in *solubility* are of particular importance with concentrates, as it has to be kept in mind that this property varies with the respective citrus oil. Lemon oils generally possess better solubility than the oils of orange and grapefruit, which can be more easily dissolved than mandarin oils. Table 4.1 clearly shows that the individual concentration method exerts a decisive influence on the product's solubility. The solubility of distilled oils decreases with increased concentration, while an additional process for the removal of the nonvolatiles leads to considerably improved solubility. However, in distilled concentrates of lemon oils this elimination of the nonvolatiles has a negative impact on the product's stability (see Figure 4.6, Aging of Orange Oils).

Improved solubility is of particular importance for the application potential of citrus oils, as the beverage sector constitutes one of their most important fields of usage. Higher concentration levels obtained via an optimized concentration process can ensure a significant increase in both solubility and the sensory flavor potential.

An equally important feature of concentration is the significant increase in the stability of citrus concentrates (vs. single-fold oils); stability will also be the focus of Section 4.4.3, where the employed model aging systems will be described in detail.

The usage of appropriate descriptors in radar charts constitutes a suitable graphical method for the visualization of the various production methods and is depicted for the example of orange oil concentrates (Figure 4.1). The comparison of medium concentrated extracted and distilled orange oils shows significant parameter profiles. These charts are based on a suitable combination of markers from GC and of physical properties and differ from each other and the underlying raw material. Reproducibly, distillates possess other net structures than extracts, highly concentrated oils vary clearly from medium concentration levels. While distillates exhibit octanal values in the centre, close to zero for the octanal axis (Tateo, 1990; Vora et al., 1983), the radar chart of 5-fold extracts is basically similar to the profile of single-fold oils with higher values for all flavor constituents and solubility as well as a decrease in the limonene axis. Additional thermal elimination of the nonvolatiles leads to a reduction of the nonvolatile marker β-sinensal as well as α-copaene.

Highly concentrated oils, obtained via extractive or chromatographic methods, always exhibit closely resembling patterns, which, however, differ considerably from single-fold oils. The most important factor in this context is the limonene value, which is nearly in the centre with far below 10%, while the flavor constituents approach the maximum in the selected depiction method.

Such simple diagrams, therefore, constitute a valuable tool for elucidating the respective concentration technique.

4.4.2 Analytical Data of Concentrated Citrus Oils

4.4.2.1 GC-Data

The gas chromatographic values of the various citrus oils depicted in Tables 4.2 through 4.6 show, apart from the different composition of the respective varieties, also the differences resulting from the particular processing method. The data approximately reflect the folding level on the one hand and the production method on the other. In general, it has to be kept in mind that the specified GC-values depict only the area percentage of the volatile part suitable for GC, a method of operation

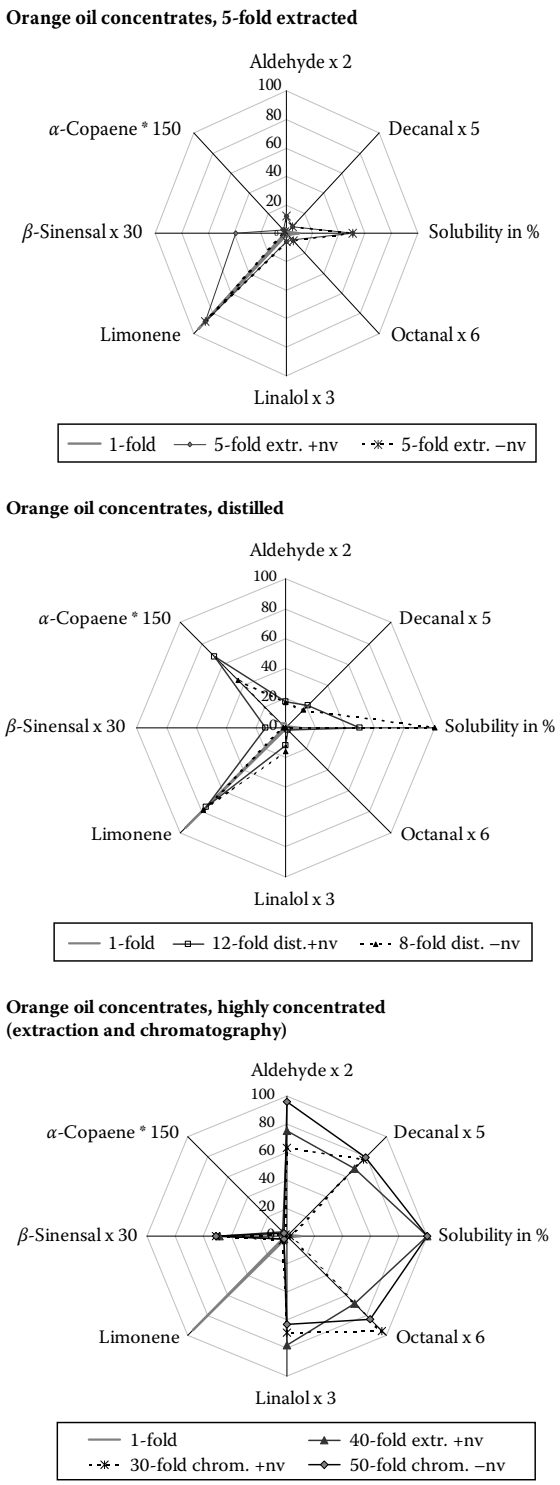

FIGURE 4.1 Differently produced orange oil concentrates.

TABLE 4.2
GC Composition of Differently Produced Lemon Oil Concentrates (Area %)

	1-Fold	5-Fold	30-Fold	10-Fold	25-Fold	8-Fold	8-Fold
Method	**Blend**	**Extr. +nv**	**Extr. +nv**	**Chrom. +nv**	**Chrom. +nv**	**Dist. +nv**	**Dist. –nv**
Nonvolatiles (%)	**2–4**	**6–10**	**34–40**	**19–23**	**38–43**	**~20**	**6–14**
α-Thujene	0.42	0.17	0.00	0.23	0.11	0.06	0.09
α-Pinene	1.86	0.71	0.01	0.96	0.34	0.28	0.43
Camphene	0.06	0.03	0.00	0.04	0.02	0.01	0.02
Sabinene	1.93	1.47	0.06	1.21	0.21	0.51	0.69
B-Pinene	11.68	7.35	0.15	7.03	2.21	2.84	4.47
Myrcene	1.51	1.54	0.17	1.06	0.08	0.66	0.76
Octanal	0.07	0.21	1.01	0.54	1.22	0.02	0.05
α-Phellandrene	0.04	0.03	0.00	0.03	0.01	0.02	0.02
δ-3-Carene	0.01	0.00	0.01	0.01	0.01	0.00	0.00
α-Terpinene	0.21	0.19	0.03	0.16	0.10	0.10	0.12
p-Cymene	0.11	0.33	0.12	0.26	0.03	0.12	0.23
Limonene	67.16	66.08	3.13	47.46	2.95	54.26	54.26
(*E*)-β-Ocimene	0.11	0.15	0.05	0.10	0.01	0.12	0.14
γ-Terpinene	8.80	9.18	0.63	7.09	0.32	11.86	12.06
Octanol	0.04	0.09	0.48	0.40	0.69	0.01	0.06
Terpinolene	0.39	0.38	0.06	0.31	0.05	1.13	0.80
Linalool	0.10	0.28	2.37	0.86	2.29	0.43	0.38
Nonanal	0.13	0.32	1.92	0.91	2.77	0.40	0.32
cis-1,2-Limonene oxide	0.00	0.01	0.02	0.04	0.02	0.03	0.02
trans-1,2-Limonene oxide + *p*-mentha-2-dien-1ol	0.01	0.01	0.09	0.08	0.06	0.03	0.03
Citronellal	0.09	0.26	1.53	0.48	1.04	0.34	0.31
Terpinene-4-ol	0.03	0.10	0.97	0.35	2.28	0.27	0.14
α-Terpineol	0.18	0.48	3.48	1.37	5.08	0.63	0.80
Decanal	0.05	0.12	0.83	0.29	0.95	0.15	0.19
Nerol	0.04	0.11	0.89	0.34	1.24	0.33	0.26
Neral	0.88	2.60	19.88	6.84	17.25	4.36	4.93
Carvone	0.01	0.02	0.13	0.06	0.17	0.03	0.02
Geraniol	0.03	0.06	0.54	0.18	0.55	0.10	0.18
Geranial	1.46	4.27	33.83	11.61	27.94	7.11	7.76
Perilla aldehyde	0.02	0.05	0.47	0.16	0.43	0.11	0.08
Undecanal	0.03	0.11	0.45	0.16	0.62	0.16	0.11
Citronellyl acetate	0.03	0.06	0.52	0.01	0.42	0.14	0.10
Neryl acetate	0.43	1.15	8.25	0.14	8.78	2.71	1.96
Geranyl acetate	0.30	0.78	5.53	1.88	5.25	1.15	1.42
Dodecanal	0.02	0.03	0.24	1.56	0.27	0.04	0.10
(*E*)-β-Caryophyllene	0.20	0.14	0.07	0.06	0.01	1.17	0.93
trans-α-Bergamotene	0.33	0.21	0.10	0.14	0.03	1.74	1.47
(*E*,*E*)-α-Farnesene	0.11	0.05	0.10	0.25	0.19	0.56	0.47
β-Bisabolene	0.52	0.31	0.21	0.04	0.09	2.65	2.13
Citropten	0.05	0.15	1.19	0.39	1.38	0.18	0.01

TABLE 4.2 (continued)
GC Composition of Differently Produced Lemon Oil Concentrates (Area %)

Method	1-Fold Blend	5-Fold Extr. +nv	30-Fold Extr. +nv	10-Fold Chrom. +nv	25-Fold Chrom. +nv	8-Fold Dist. +nv	8-Fold Dist. –nv
Hydrocarbons	95.4	88.3	4.9	66.4	6.8	78.1	79.1
Sesquiterpene hydrocarbons	1.2	0.7	0.5	0.5	0.3	6.1	5.0
Aldehydes	2.8	8.0	60.2	22.5	52.5	12.7	13.8
Alcohols and ketones	0.4	1.1	8.9	3.6	12.3	1.8	1.8
Acetates and esters	0.5	1.2	9.0	1.7	9.5	2.9	2.2

TABLE 4.3
GC Composition of Differently Produced Orange Oil Concentrates (Area %)

Method	1-Fold Blend	12-Fold Dist. +nv	8-Fold Dist. –nv	5-Fold Extr. +nv	5-Fold Extr. –nv	40-Fold Extr. +nv	30-Fold Chrom. +nv	50-Fold Chrom. –nv
Nonvolatiles (%)	**1–8[a]**	**24–33**	**3–7**	**4–10**	**2–5**	**>40**	**>50**	**25–35**
Ethyl butyrate	–	0.02	0.02	0.02	0.01	0.02	0.02	0.02
α-Pinene	0.54	0.12	0.20	0.17	0.22	0.01	0.06	0.03
Sabinene	0.33	0.12	0.10	0.21	0.26	0.04	0.05	0.03
β-Pinene	0.02	0.01	0.01	0.02	0.04	0.04	0.11	0.08
Myrcene	1.91	0.90	0.93	1.66	1.84	0.16	0.09	0.07
Octanal	0.29	0.22	0.05	1.19	1.30	11.42	16.28	13.86
δ-3-Carene	0.09	0.04	0.05	0.06	0.09	0.02	0.04	0.04
α-Terpinene	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.02
p-Cymene	0.002	0.00	0.01	0.00	0.00	0.012	0.012	0.008
Limonene	95.22	75.18	78.74	88.62	89.53	2.56	3.76	2.98
γ-Terpinene	0.01	0.01	0.02	0.01	0.04	0.03	0.05	0.05
Octanol	0.04	0.09	0.20	0.18	0.17	0.80	2.53	1.93
Terpinolene	0.02	0.05	0.15	0.01	0.02	0.02	0.04	0.02
Linalool	0.34	3.93	5.33	2.06	1.89	25.72	22.76	21.69
Nonanal	0.07	0.27	0.40	0.23	0.23	2.39	2.63	2.53
cis-1,2-Limonene oxide	0.00	0.06	0.28	0.04	0.03	0.07	0.04	0.13
trans-1,2-Limonene oxide + *p*-mentha-2-dien-1ol	0.01	0.20	0.40	0.08	0.09	0.65	0.70	0.78
Citronellal	0.05	0.75	0.71	0.25	0.25	2.69	2.38	2.61
Terpinene-4-ol	0.00	0.04	0.04	0.02	0.02	0.17	0.17	0.18
α-Terpineol	0.04	0.78	0.77	0.28	0.30	3.56	3.13	2.80
Decanal	0.24	4.38	3.43	1.34	1.34	13.74	15.19	15.94
Octyl acetate	0.03	0.01	0.01	0.05	0.00	0.23	0.29	0.36
Neral	0.05	0.82	0.61	0.26	0.26	3.71	2.99	2.91
Carvone	0.01	0.15	0.27	0.07	0.05	0.55	0.49	0.56
Geraniol	0.01	0.08	0.07	0.03	0.03	0.32	0.34	0.29
Geranial	0.08	1.46	1.09	0.43	0.41	6.14	5.11	5.35

continued

TABLE 4.3 (continued)
GC Composition of Differently Produced Orange Oil Concentrates (Area %)

Method	1-Fold Blend	12-Fold Dist. +nv	8-Fold Dist. –nv	5-Fold Extr. +nv	5-Fold Extr. –nv	40-Fold Extr. +nv	30-Fold Chrom. +nv	50-Fold Chrom. –nv
Perilla aldehyde	0.03	0.38	0.37	0.13	0.11	1.55	1.23	1.36
Undecanal	0.01	0.21	0.19	0.02	0.86	0.78	0.66	0.82
Neryl acetate	0.00	0.04	0.05	0.04	0.02	0.29	0.16	0.23
Geranyl acetate	0.00	0.07	0.04	0.01	0.01	0.12	0.10	0.16
α-Copaene	0.01	0.01	0.28	0.00	0.01	0.01	0.21	0.03
β-Cubebene	0.02	0.45	0.32	0.03	0.02	0.09	0.06	0.08
Dodecanal	0.05	0.88	0.51	0.27	0.24	2.27	2.91	3.36
(*E*)-β-Caryophyllene	0.01	0.29	0.21	0.02	0.01	0.03	0.02	0.01
β-Copaene	0.02	0.45	0.37	0.02	0.02	0.03	0.02	0.02
Germacrene D	0.01	0.32	0.16	0.01	0.01	0.01	–	0.01
Valencene	0.03	0.77	0.41	0.03	0.03	0.02	0.04	0.06
δ-Cadinene	0.02	0.36	0.18	0.03	0.02	0.19	0.05	0.19
Elemol	0.01	0.02	0.02	0.05	0.02	0.68	0.48	0.08
β-Sinensal	0.03	0.45	0.05	0.13	0.07	1.57	1.73	1.76
α-Sinensal	0.03	0.38	0.03	0.09	0.03	1.17	1.10	1.34
Nootkatone	0.02	0.23	0.01	0.08	0.02	0.69	0.74	0.91
Hydrocarbons	98.3	79.1	82.1	90.9	92.2	3.3	4.6	3.7
Sesquiterpene hydrocarbons	0.1	2.7	1.9	0.1	0.1	0.4	0.4	0.4
Aldehydes	0.9	10	7	4	5	47	52	52
Alcohols and ketones	0.5	5	7	3	3	33	31	29
Acetates and esters	0.03	0.14	0.11	0.11	0.04	0.67	0.56	0.77
Sesquiterpenoids	0.1	1.1	0.1	0.35	0.14	4.1	4.1	4.1

Notes: [a] High values are due to provenances, especially Florida.

TABLE 4.4
GC Composition of Differently Produced Grapefruit Oil Concentrates (Area %)

Method	1-Fold Blend	12-Fold Dist. +nv	6-Fold Extr. +nv	12-Fold Chrom. +nv	12-Fold Extr. +nv
Nonvolatiles (%)	**4–9**	**37–45**	**6–17**	**28–37**	**29–35**
α-Pinene	0.51	0.07	0.21	0.31	0.07
Sabinene	0.26	0.06	0.27	0.27	0.26
β-Pinene	0.02	0.01	0.05	0.06	0.36
Myrcene	1.83	0.42	1.70	1.41	1.57
Octanal	0.46	0.14	1.39	2.84	2.97
Limonene	93.74	65.75	87.70	75.49	74.92
(*E*)-β-Ocimene	0.09	0.22	0.13	0.09	0.14
γ-Terpinene	0.00	0.15	0.03	0.03	0.89
Octanol	0.03	0.19	0.19	0.67	0.31
Terpinolene	0.01	0.04	0.01	0.01	0.07

TABLE 4.4 (continued)
GC Composition of Differently Produced Grapefruit Oil Concentrates (Area %)

Method	1-Fold Blend	12-Fold Dist. +nv	6-Fold Extr. +nv	12-Fold Chrom. +nv	12-Fold Extr. +nv
Linalool	0.07	1.04	0.36	0.78	0.83
Nonanal	0.08	0.45	0.24	0.59	0.54
cis-1,2-Limonene oxide	0.00	0.06	0.05	0.02	0.06
trans-1,2-Limonene oxide + *p*-mentha-2-dien-1ol	0.01	0.19	0.09	0.16	0.16
Citronellal	0.08	0.72	0.22	0.49	0.33
Terpinene-4-ol	0.00	0.06	0.02	0.05	0.09
α-Terpineol	0.04	0.77	0.28	0.65	0.47
Decanal	0.41	4.35	1.45	2.89	2.76
Octyl acetate	0.05	0.45	0.11	0.40	0.27
Neral	0.04	0.49	0.14	0.24	0.43
Carvone	0.01	0.12	0.05	0.18	0.09
Geranial	0.07	1.00	0.26	0.45	0.79
Perilla aldehyde	0.01	0.21	0.06	0.11	0.13
Undecanal	0.02	0.21	0.08	0.15	0.13
Terpinyl acetate	0.02	0.22	0.06	0.22	0.10
Neryl acetate	0.01	0.13	0.04	0.08	0.09
Geranyl acetate	0.04	0.60	0.17	0.36	0.33
α-Copaene	0.10	1.20	0.07	0.08	0.05
β-Cubebene	0.11	1.29	0.09	0.08	0.07
Dodecanal	0.05	0.47	0.13	0.39	0.23
(*E*)-β-Caryophyllene	0.25	3.55	0.19	0.22	0.18
(*E*)-β-Farnesene	0.01	0.11	0.01	0.01	0.01
α-Humulene	0.04	0.49	0.03	0.03	0.03
Germacrene D	0.08	0.88	0.05	0.06	0.05
Valencene	0.00	0.45	0.03	0.06	0.08
δ-Cadinene + cubebol	0.12	1.39	0.08	0.20	0.14
α-Elemol	0.03	0.27	0.06	0.17	0.28
(*E*)-Nerolidol	0.01	0.13	0.03	0.07	0.09
Germacrene D 4-ol	0.01	0.20	0.04	0.05	0.09
Caryophyllene oxide	0.01	0.22	0.04	0.10	0.06
Intermedeol	0.02	0.14	0.04	0.09	0.09
β-Sinensal	0.01	0.16	0.05	0.09	0.13
α-Sinensal	0.01	0.03	0.01	0.03	0.02
Nootkatone	0.49	3.41	1.16	2.55	2.33
Hexadecanoic acid	0.03	0.42	0.15	0.23	0.33
Osthol	0.06	0.50	0.19	0.39	0.65
Isomeranzin	0.01	0.66	0.20	0.58	0.50
Meranzin	0.17	2.68	0.67	1.88	1.62
Hydrocarbons	96.7	77.0	90.9	79.2	79.4
Sesquiterpene hydrocarbons	0.8	10.3	0.8	1.5	1.1
Aldehydes	1.2	8	4	8.2	8.3
Alcohols and ketones	0.7	6.8	2.4	5.6	5
Acetates and esters	0.2	1.8	0.5	1.3	1.1
Sesquiterpenoids	0.8	7.3	1.6	3.5	3.4

TABLE 4.5
GC Composition of Differently Produced Mandarin Oil Concentrates (Area %)

Method	1-Fold Blend	1-Fold South America	1-Fold Italy	4-Fold Dist.	4-Fold Extr.	5-Fold Chrom.	40-Fold Chrom.
Nonvolatiles (%)	**0–3**	**2–5**	**1–3**	**4–8**	**1–8**	**~10**	**>50**
α-Thujene	0.67	0.16	0.49	0.04	0.05	0.39	0.01
α-Pinene	1.92	0.87	1.53	0.13	0.31	1.24	0.00
Sabinene	0.25	0.32	0.20	0.05	0.29	0.31	0.01
β-Pinene	1.37	0.37	0.94	0.30	0.36	1.57	0.04
Myrcene	1.72	1.90	1.80	0.73	2.11	1.51	0.20
Octanal	0.09	0.17	0.12	0.06	0.84	0.58	6.19
δ-3-Carene	0.00	0.12	0.00	0.00	0.06	0.02	0.03
α-Terpinene	0.34	0.08	0.24	0.25	0.08	0.21	0.01
p-Cymene	0.47	0.09	0.33	0.44	0.32	0.71	0.34
Limonene	75.03	90.64	80.94	62.73	85.76	74.46	5.56
γ-Terpinene	15.28	3.67	10.91	25.69	3.52	10.13	0.21
Octanol	0.01	0.01	0.01	0.00	0.05	0.06	0.74
trans-Sabinene hydrate	0.02	0.01	0.02	0.04	0.04	0.11	0.66
Terpinolene	0.72	0.19	0.51	1.64	0.18	0.46	0.11
Linalool	0.14	0.23	0.26	0.28	1.36	1.07	9.57
Nonanal	0.05	0.04	0.05	0.14	0.02	0.31	2.38
cis-1,2-Limonene oxide	0.00	0.00	0.01	0.00	0.01	0.01	0.32
trans-1,2-Limonene oxide + *p*-Mentha-2-dien-1ol	0.01	0.01	0.01	0.01	0.05	0.05	0.87
Citronellal	0.03	0.04	0.04	0.13	0.20	0.17	1.88
Terpinene-4-ol	0.07	0.01	0.02	0.14	0.06	0.14	1.79
α-Terpineol	0.15	0.05	0.12	0.51	0.29	0.58	5.87
Decanal	0.08	0.18	0.10	0.41	0.84	0.60	7.00
Citronellol	0.02	0.02	0.03	0.08	0.10	0.05	1.22
Thymol methyl ether	0.01	0.01	0.02	0.01	0.01	0.13	0.09
Neral	0.01	0.02	0.01	0.08	0.13	0.01	0.35
Perilla aldehyde	0.05	0.02	0.04	0.18	0.13	0.18	1.79
Thymol	0.05	0.02	0.06	0.26	0.10	0.21	1.80
Undecanal	0.01	0.01	0.02	0.05	0.05	0.07	0.67
Neryl acetate	0.01	0.02	0.01	0.01	0.12	0.06	1.08
Geranyl acetate	0.02	0.01	0.00	0.01	0.05	0.01	0.42
β-Cubebene	0.02	0.05	0.03	0.05	0.11	0.01	0.27
Methyl-*N*-methyl anthranilate + dodecanal	0.38	0.06	0.23	1.66	0.29	1.26	12.35
(*E*,*E*)-α-Farnesene	0.20	0.09	0.12	0.82	0.10	0.11	0.32
α-Sinensal	0.27	0.06	0.24	1.50	0.31	1.09	8.71
Hydrocarbons	98.0	98.6	98.0	92.9	93.2	91.1	7.1
Aldehydes	0.6	0.5	0.6	2.6	2.5	3.0	29.0
Alcohols and ketones	0.5	0.4	0.5	1.3	2.0	2.3	22.8
Acetates and esters	0.4	0.1	0.3	1.7	0.5	1.4	13.9

TABLE 4.6
GC Composition of Different Cold-Pressed Lime Oil Concentrates (Area %)

	1-Fold	6-Fold	5-Fold	5-Fold
Method	**Blend**	**Dist.**	**Extr.**	**Chrom.**
Nonvolatiles (%)	**5–15**	**27–35**	**25–33**	**25–33**
α-Thujene	0.50	0.00	0.12	0.28
α-Pinene	1.83	0.02	0.37	1.03
Sabinene	1.56	0.16	1.14	1.18
β-Pinene	9.29	1.18	4.96	6.68
Myrcene	1.46	0.36	1.38	1.12
Octanal	0.03	0.02	0.05	0.06
δ-3-Carene	0.01	tr	–	–
α-Terpinene	0.30	0.08	0.26	0.24
p-Cymene	0.09	0.45	0.35	0.26
Limonene	59.34	48.00	45.26	44.95
γ-Terpinene	12.82	13.17	12.24	10.45
Octanol	0.04	0.01	0.08	0.23
Terpinolene	0.60	0.50	0.60	0.54
Linalool	0.20	0.48	0.69	0.71
Nonanal	0.05	0.19	0.15	0.37
cis-1,2-Limonene oxide	–	0.03	–	0.01
trans-1,2-Limonene oxide + *p*-mentha-2-dien-1ol	0.01	0.04	0.01	0.09
Citronellal	0.05	0.18	0.19	0.17
Terpinene-4-ol	0.14	0.11	0.46	0.44
α-Terpineol	0.31	0.51	1.15	1.14
Decanal	0.10	0.16	0.23	0.21
Nerol	0.14	0.24	0.55	0.59
Neral	1.27	6.55	5.76	5.21
Geraniol	0.07	0.12	0.36	0.43
Geranial	2.00	11.54	9.13	8.46
Perilla aldehyde	0.04	0.07	0.12	0.11
Undecanal	0.02	0.09	0.06	0.01
δ-Elemene	–	0.11	0.11	0.11
Neryl acetate	0.94	3.56	3.49	2.79
Geranyl acetate	0.20	3.11	0.87	0.68
β-Elemene	0.10	0.15	0.11	0.10
Dodecanal	0.05	0.06	0.05	0.11
(*E*)-β-Caryophyllene	0.57	1.13	0.45	0.57
trans-α-Bergamotene	1.10	1.34	0.65	0.92
Germacrene D	0.15	0.14	0.10	0.14
(*E*,*E*)-α-Farnesene	0.46	0.53	0.38	0.49
β-Bisabolene	1.66	2.05	0.99	1.41
Germacrene B	0.12	0.05	0.08	0.11
Herniarin	0.28	0.17	1.03	1.05
Hexadecanal	0.11	0.09	0.19	0.25

continued

TABLE 4.6 (continued)
GC Composition of Different Cold-Pressed Lime Oil Concentrates (Area %)

Method	1-Fold Blend	6-Fold Dist.	5-Fold Extr.	5-Fold Chrom.
Citropten	0.19	0.23	1.06	1.00
Bergapten	0.11	0.07	0.55	0.60
Hydrocarbons	92.1	69.9	70.2	71.1
Aldehydes	3.7	18.9	15.9	15.0
Alcohols and ketones	0.9	1.5	3.3	3.6
Acetates and esters	1.1	6.7	4.4	3.5

Note: tr, traces.

routinely employed in the industry and, therefore, selected in this context (Bicchi et al., 2008; Chamblee et al., 1991; Lopes et al., 2003). It has to be kept in mind that in the case of concentrates with higher nonvolatile fraction, the actual values would show a downward deviation with increasing level of the respective nv-part; for purposes of orientation the present nonvolatile share (determined via GC-measurements with internal standard) is also given. Despite considerable residues, extractively manufactured concentrates nevertheless exhibit excellent solubility (Table 4.1) due to the fact that these residues constitute middle-polar and polar compounds.

In regular foldings, typically all constituents eluting before limonene in GC are disproportionately reduced. This includes, among others, the sensorially important aldehyde octanal that is completely eliminated with these methods (Tateo, 1990); thereby all the more volatile constituents contributing to the fruity citrus note are lost completely. Extractive and chromatographic concentration processes, where separation is not achieved thermally but based on differing solubilities or on the different adsorption properties of the valuable more polar flavor constituents, achieve a pronounced enrichment (see GC-profiles of chromatographically produced lemon concentrates [Figure 4.2] vs. distillatively and extractively produced orange oil concentrates [Figure 4.3]). Additionally, traditional distillative foldings show a distinctive increase in the range of the nonpolar sesquiterpene hydrocarbons, leading, together with the rise of the entire nonvolatile range (from nonpolar to polar compound classes), to a considerable reduction of solubility. A downstream removal of the nonvolatiles leads to a decrease of all compounds of the higher boiling range in a nonselective mode.

Generally, concentrates processed by cold concentration techniques maintain the authentic characteristics of the original oil in a more natural mode than vacuum-distilled deterpenated oils (Dugo et al., 1995).

4.4.2.2 HPLC-Data

In the following, the HPLC-data of the heterocyclic oxygenated compounds of orange and lemon will be analyzed as examples (Figures 4.4 and 4.5). For the analytical chemist, these compounds constitute important markers, suitable for the elucidation of the employed production method. For all citrus varieties (with the exception of distilled lime oils), the visualization of these compound classes, possessing an individual and significant composition for the every citrus oil (Verzera et al., 1999, see also Chapter 8), has turned out to be a useful tool for the evaluation of concentrates with regard to degree and modalities of concentration as well as varietal purity

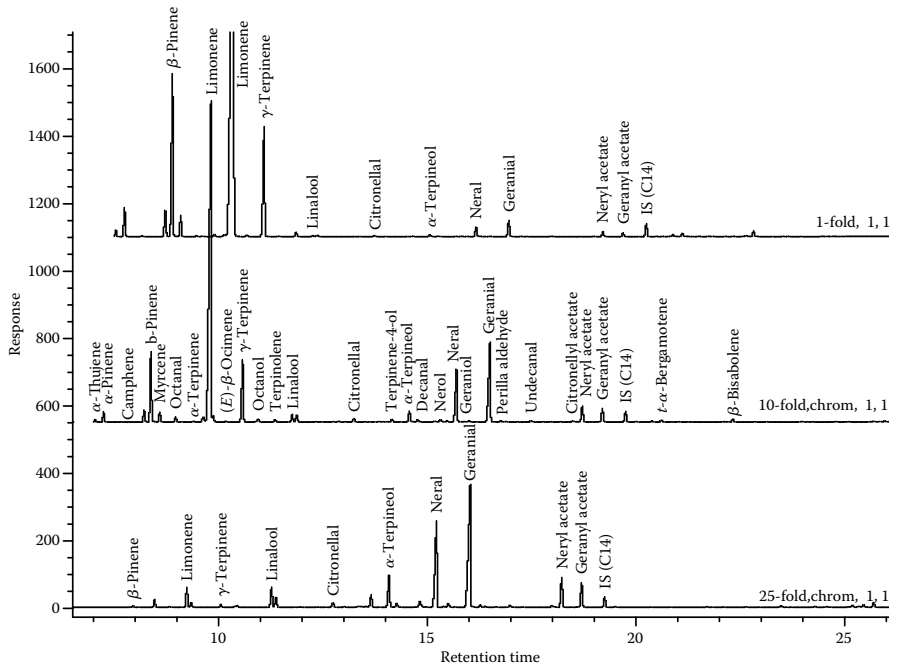

FIGURE 4.2 GC-profiles of lemon oils at different concentrations (1-fold; 10-fold chrom.; 25-fold chrom.).

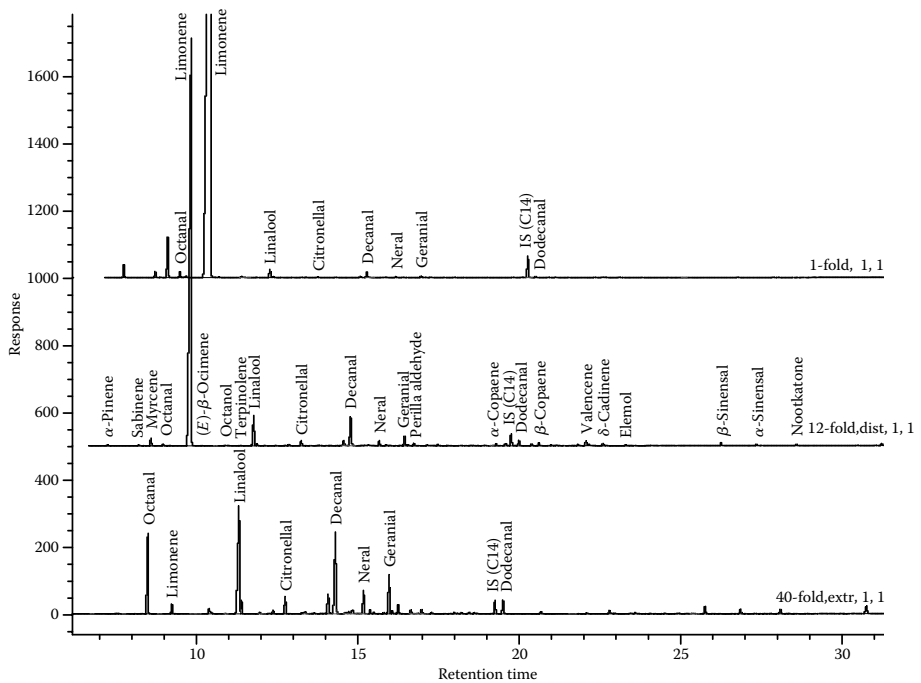

FIGURE 4.3 GC-profiles of orange oils at different concentrations (1-fold; 12-fold dist.; 40-fold extr.).

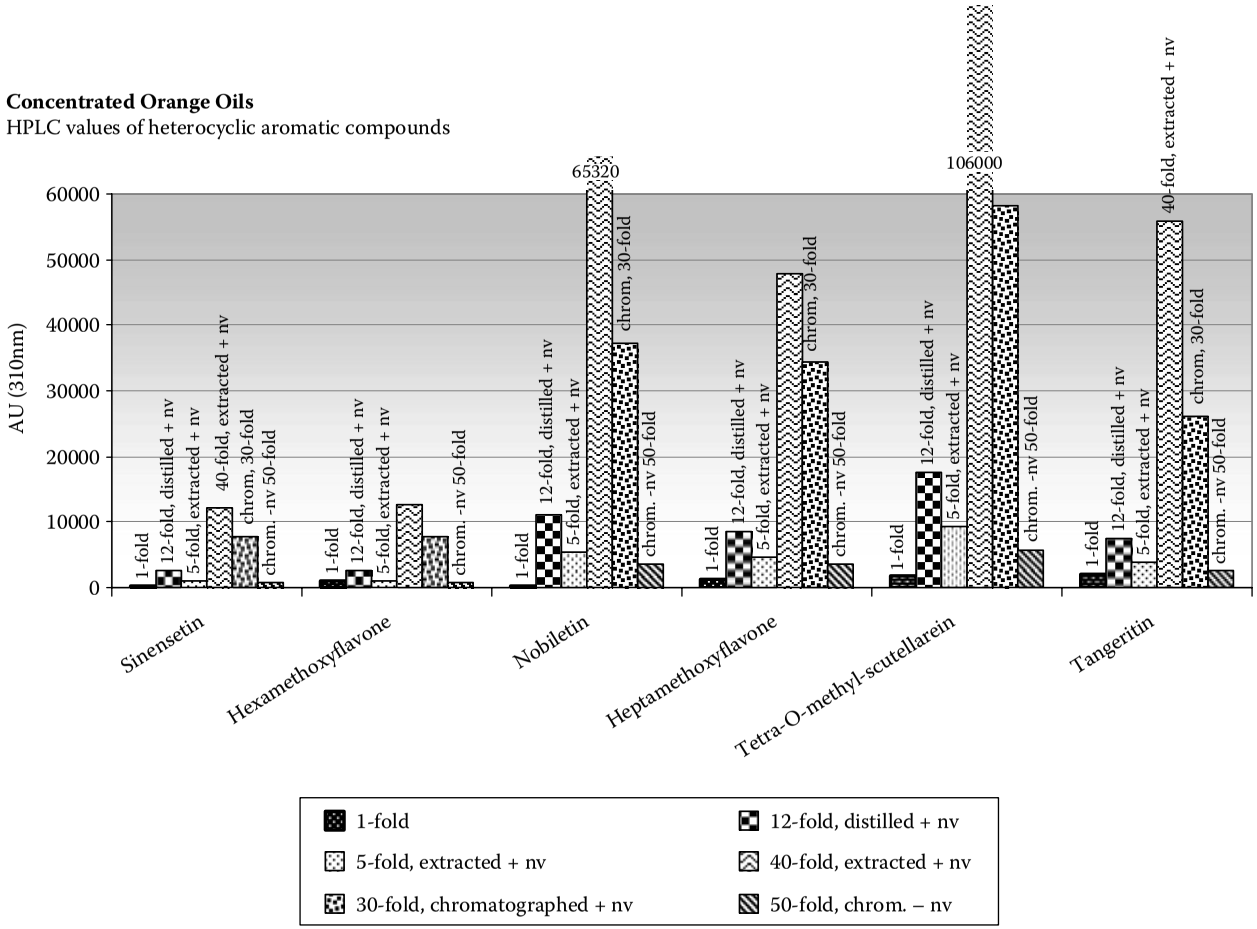

FIGURE 4.4 HPLC-data of differently produced orange oil concentrates.

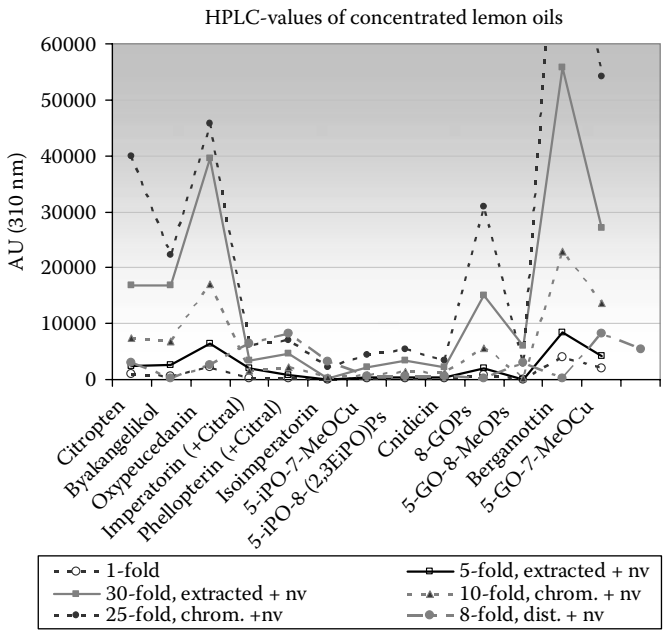

FIGURE 4.5 HPLC-data of differently produced lemon oil concentrates.

(Ziegler, H., 1992; Ziegler and Spiteller, 1992). For concentrates processed on differences in polarity, the individual constituents are selectively retained according to their polarity, resulting in different enrichment.

Schematic HPLC-data of the six most important heterocyclic aromatic compounds of orange show, depending on their concentration method, values that partly reflect their folding levels. This does not apply to the concentrates with secondary treatments to reduce the content of the nonvolatile fraction in order to achieve improved solubility, here called concentrates –nv (without nonvolatiles or, even better, reduced nonvolatiles; see Section 4.3). When compared to the analogous concentration levels, these products exhibit lower values in HPLC (e.g., orange 50-fold, chrom. –nv).

On the other hand, the heterocyclic oxygenated compounds detected by HPLC are not responsible for the reduced solubility, but only constitute generally suitable measurement markers for the content of all high-boiling nonvolatiles. This is corroborated by the fact that highly concentrated extracted oils show high values for the heterocyclic oxygenated compounds and possess excellent solubility (e.g., orange oil, 40-fold, extr. +nv). The negative impact on solubility derives from the nonpolar, nonvolatile compounds such as waxes, polymers, and sesquiterpenes, which are enriched with distillative methods but reduced together with the nonpolar limonene matrix in extractive technologies. HPLC, therefore, especially constitutes a valuable tool for ascertaining the extractive origin of soluble concentrates.

4.4.3 Stability of Citrus Oil Concentrates

4.4.3.1 Test Systems for Aging Models

While in the literature the assumption is often made that concentrates possess higher stability due to the reduction of the oxidatively instable terpenes (Grosch and Schieberle, 1988; Nagy and Rouseff, 1980; Nguyen et al., 2009; Rouseff and Naim, 2000), proof of this hypothesis was often missing for citrus concentrates. For this reason, the present discussion of concentrated citrus oils will include a

detailed study of their respective shelf life. In order to contrastingly evaluate the stability and shelf life of differently produced concentrated oils, two different model systems were used:

- System 1 called *Aging* in the following:
  The undiluted oils were stored at 70°C under air in the dark and opened once per week for sensory evaluation. Comparison with a reference sample, stored at 4°C in the dark, permitted a sensory evaluation of the degree of off-flavor formation. The experiment was conducted over a period of 44 weeks.
- System 2 called *Forced Aging* in the following:
  The undiluted oils were stored at 70°C in the dark after the injection of air and opened three times per week for the injection of air for 20 seconds. Sensory evaluations were performed once per week over a period of 22 or 26 weeks by comparison with a reference sample, which was stored at 4°C in the dark.

The values were obtained as follows: sensory comparison with the reference sample, which was stored under cold conditions, is performed by the test panel after thermal equilibration of all samples to room temperature. A scale from "0 to 10" was selected to describe the range and level of deterioration with "0" as starting point, as initially all oils were present in faultless sensory condition. In test system 1, extracts (with and without nonvolatiles) with low concentration levels are compared to highly concentrated oils. Forced aging model (test system 2) evaluated distillatively obtained concentrates in contrast to highly concentrated oils obtained by different production methods. In both test systems, sensory off-notes for the single-fold oils can already be detected after a short period of time. The diagrams (Figure 4.6) demonstrate that the highly concentrated orange oils of extractive or distillative origin possess excellent stability and in the end hardly showed a difference to the reference samples, which were kept in cold storage during the respective testing periods of 20 and 44 weeks. The final assessment "1" corresponds to a minimal loss of freshness.

The distillatively obtained concentrates are on the one hand more stable than the single-fold oils, but still reach the highest off-flavor valuation during testing. Interestingly, low to medium folding levels with downstream removal of the nonvolatiles also showed less optimal stability curves.

Highly concentrated oils, which were not of distillative origin, definitely remained stable also under the accelerated conditions of system 2, and received favorable assessments throughout the testing period.

The 5-fold extracted oil, part of both test series, demonstrated that the conditions of system 2 are clearly aggravated, as an off-flavor value of 3 to 4 was reached in system 1 more than 10 weeks later. Altogether, the model aging systems turned out to be very suitable for visualizing the increase in stability of orange oil concentrates obtained by different concentration processes.

An analogous aging method was employed for lemon oils. The comparison between concentrated and single-fold oils clearly shows that the latter always exhibit the highest off-flavor perception, reaching the maximum values significantly earlier than concentrates. In test system 1, high concentrates of extractive and chromatographic origin were compared with single-fold oils and an extracted low concentrate. Despite introduction of oxygen and exposure to high temperatures in the aging system, all concentrates remained completely stable over the long period of three months. Subsequently, the sensory off-flavors increased in the low concentrate, while after 44 weeks the high concentrates were still far from the maximum. The intensified testing conditions of the forced aging series permitted the distinction of the differently produced oils as depicted in Figure 4.7.

Single-fold oils and distilled concentrates with low residues showed the fastest aging process, while a 10-fold chromatographic concentrate (corresponding to a medium concentration level) turned out to be the most stable option under forced aging conditions.

Figure 4.8 depicts the profiles observed for grapefruit and mandarin under test system 2. For both oils, the simultaneous increase of stability with concentration level could again be confirmed for all investigated concentrates. The single-fold oils reached maximum off-values

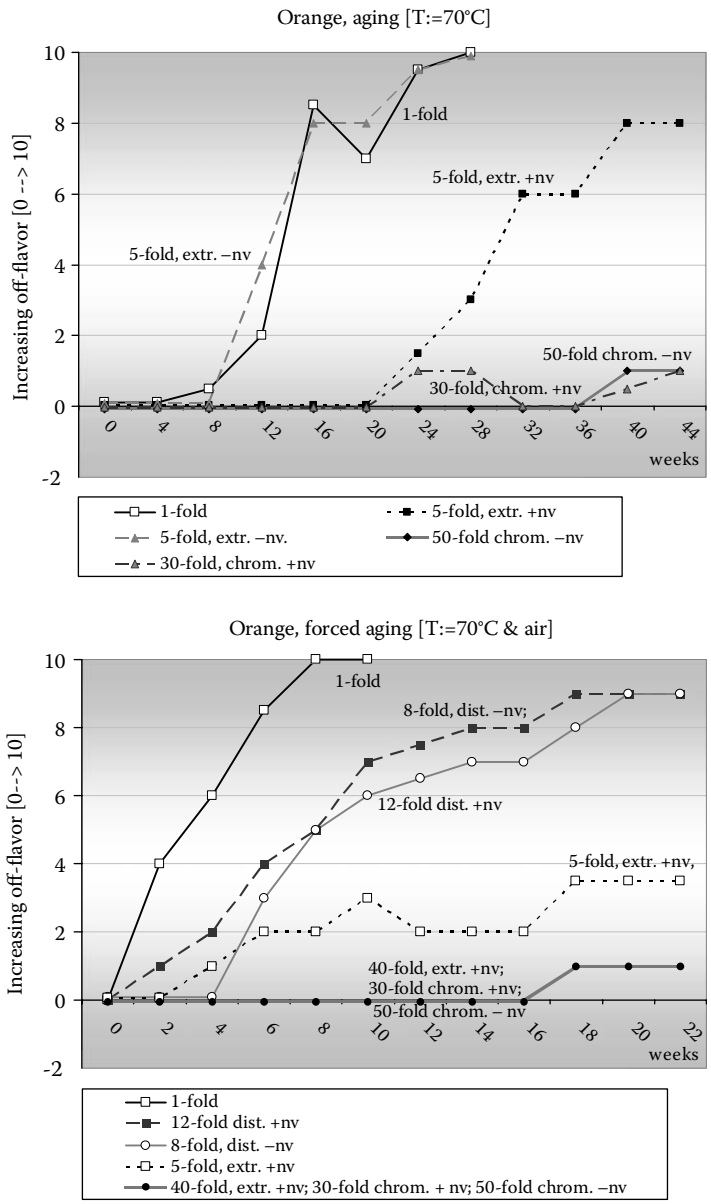

FIGURE 4.6 Aging of orange oils.

after 12 weeks for mandarin oils, while grapefruit oils were completely deteriorated after 20 weeks.

Distillative products could not match the properties of extractive and chromatographic concentrates of analogous folding levels; the latter two methods alternated as far as the highest stability was concerned.

4.4.3.2 GC-Monitoring of Compounds during Aging

Tracking the sensory changes in the course of aging by monitoring single compounds is of additional interest, as the transformations of the constituents significantly correlate with off-flavor detections. In the case of orange (Figure 4.9), GC and GC/MS-measurements were conducted during progressing sensory deterioration (System 1: 1-fold, after 12 weeks; 5-fold, extr. +nv, after

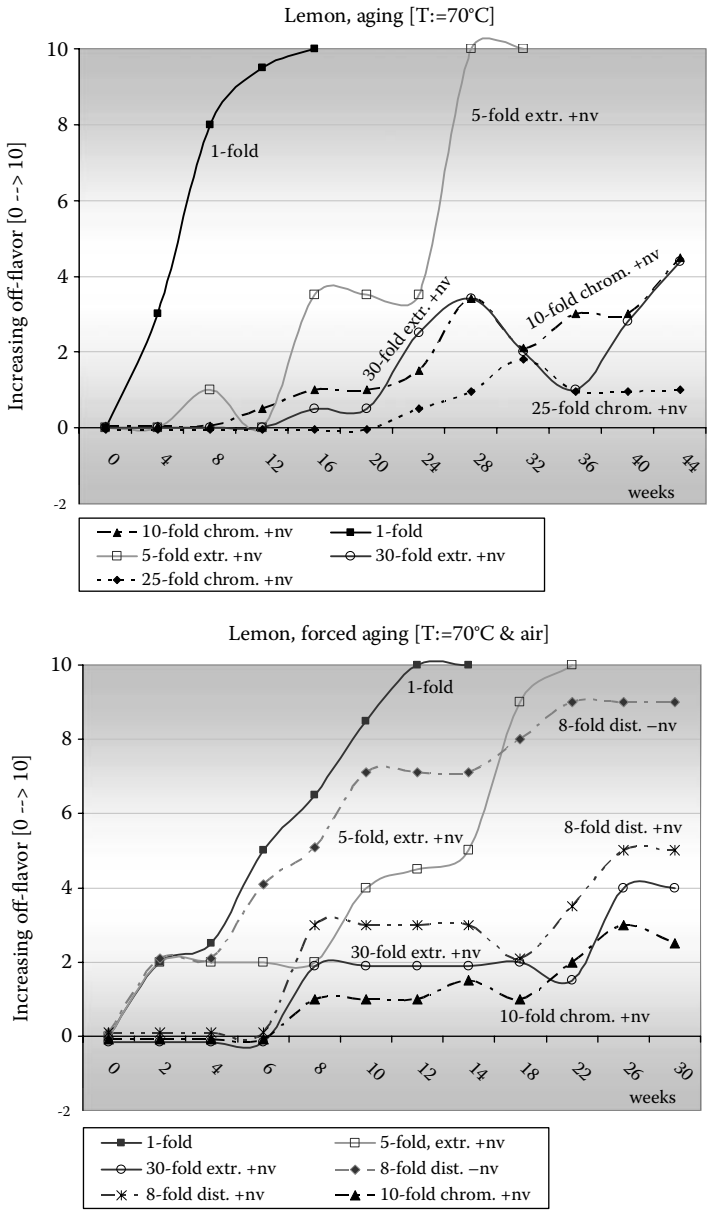

FIGURE 4.7 Aging of lemon oils.

34 weeks; 5-fold, extr. –nv, after 12 weeks). In this context, the main terpenes myrcene and limonene displayed significant percentage reductions. The aldehyde series also showed degenerations. Transformation and oxidation products, primarily arising from limonene, (e.g., the limonene oxides as well as the *p*-mentha dienols) could be detected (Nagy and Rouseff, 1980; Nguyen et al., 2009; Petersen et al., 1998; Rouseff et al., 2000; Ziegler M. et al., 1991). In addition, an increase in the acids and the off-flavor constituents, such as the carveols, could be established. The combination of sensory and chemical analytical evaluations, therefore, generated interesting insights into the changes of the chemical composition at the time of sensory off-flavor formation.

Also in the case of lemon oils, the sensory changes within the aging tests could be traced by compound monitoring (Grosch and Schieberle, 1987). The transformations of the constituents are

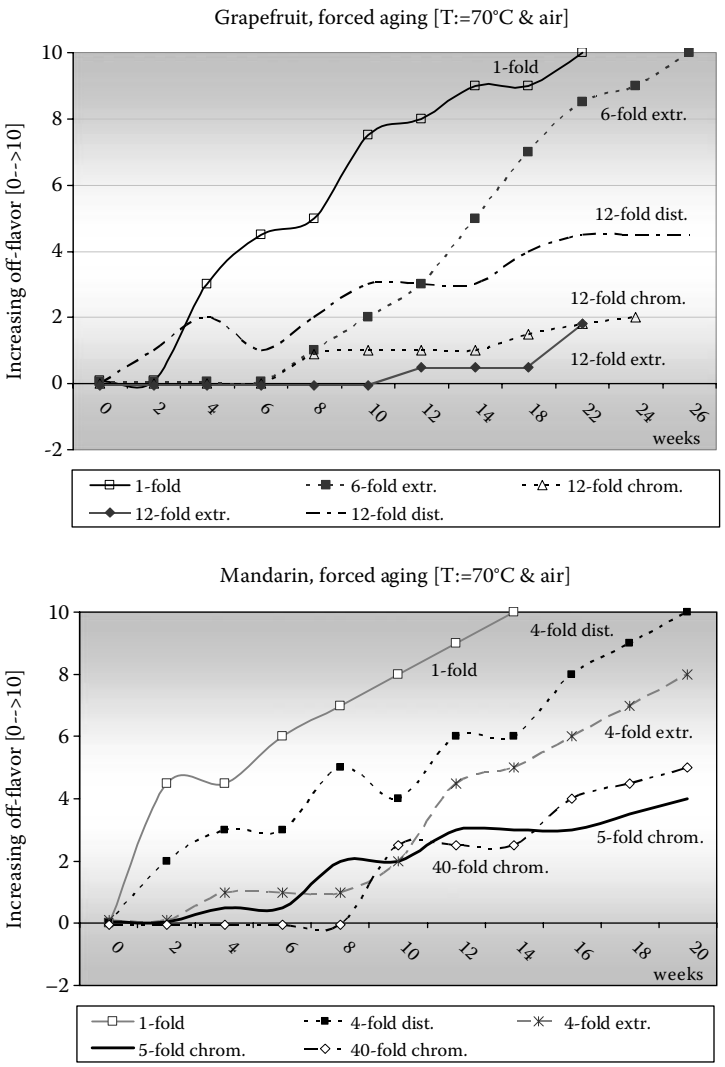

FIGURE 4.8 Forced aging of grapefruit and mandarin oils.

depicted in Figure 4.10. Again, samples of deteriorated oils (System 1: 1-fold, after 8 weeks; 5-fold, extr. +nv, after 25 weeks; System 2: 8-fold, dist. –nv, after 12 weeks) were submitted to GC and GC/MS. The main terpene hydrocarbons limonene and γ-terpinene as well as a number of relevant aldehydes once again declined in percentage points (Nguyen et al., 2009). The increase of the limonene oxides and the *p*-mentha dienols was just as significant as that for *p*-cymene and for the carveols (Choi and Sawamura, 2002). Sensory visualization combined with chemical analytical detection at the time of negative sensory impressions again elucidated the changes in chemical composition during forced, fast aging processes.

4.4.4 Washings of Citrus Oils

In the following, the topic of washings will be dealt with as many companies still employ this easiest of extractive processes (see Section 4.2.2) today—despite its relatively low efficiency and yield. For this method, aqueous alcohol is used for the extraction of citrus oils (Owusu-Yaw et al., 1986).

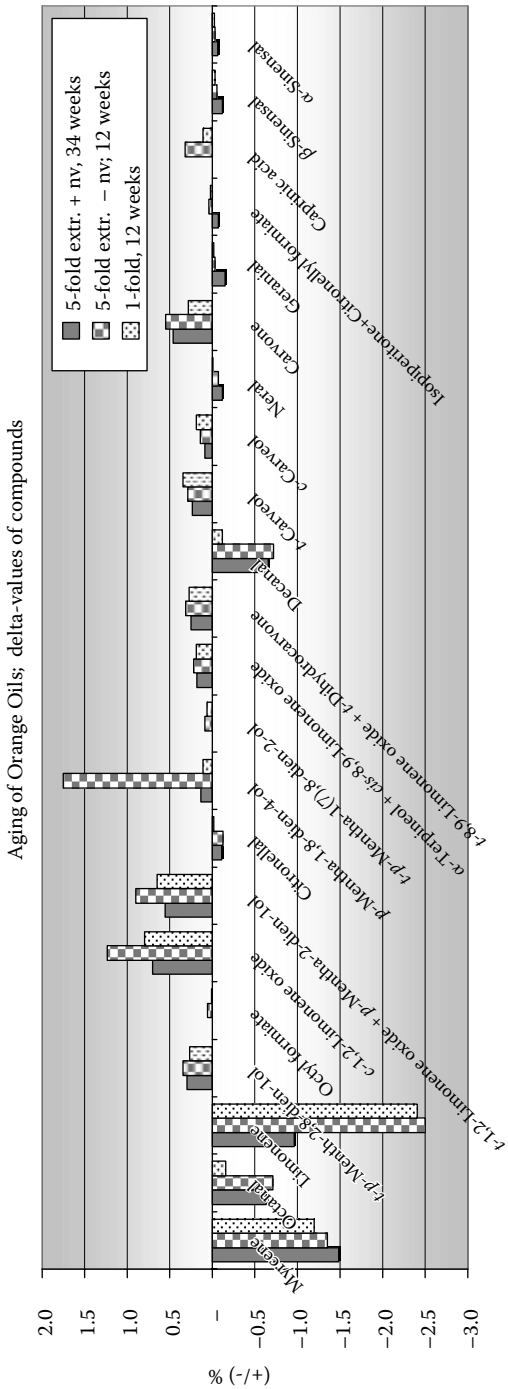

FIGURE 4.9 Compound monitoring of aged orange oils.

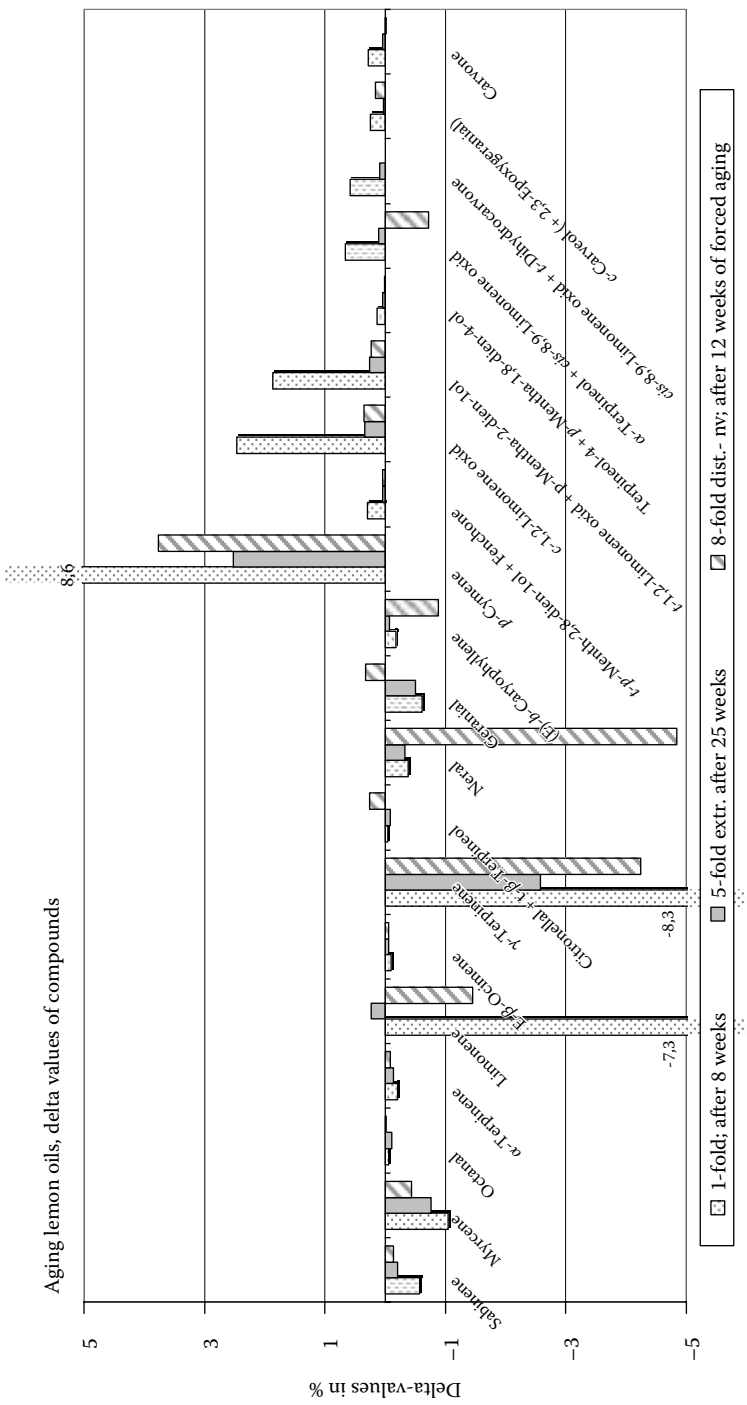

FIGURE 4.10 Compound monitoring of aged lemon oils.

One or more extraction steps result in the enrichment of the citrus flavor constituents in the polar medium. Oil and extractive medium are mixed in varying ratio of oil to aqueous ethanolic solution, leading frequently to stable emulsions, which can, for separation purposes, be subsequently submitted to cold temperatures, often a time- and energy-consuming process. The results are solutions that are often described as "deterpenized" concentrates in the literature. These extremely diluted alcoholic solutions (flavor content 2%–6%) should, therefore, more appropriately be described as dilutions of citrus flavors. Often the depiction of the GC-values results in the impression of highly concentrated flavors, as the solvent, which is present in a concentration of 94% to 98%, is not listed and the values for the aroma fraction resemble a high concentrate. In reality, the product constitutes an alcoholic solution with low flavor content. As a result of the relative simplicity, the extremely good solubility and the guarantee that no precipitations, separations, cloudiness, or similar effects are to be expected from these solutions, they are often used on an industrial scale. Table 4.7 depicts

TABLE 4.7
Citrus Oil Washings Derived from Various Concentrated Citrus Oils

	Lemon				Orange		Grapefruit	Mandarin
	1-Fold Oil	10-Fold, Dist. +nv	5-Fold, Extr. +nv	1-Fold Oil [3x(1:3)][a]	1-Fold Oil	5-Fold Extr. +nv	1-Fold Oil	1-Fold Oil
Oil content	**4.2%**	**5.6%**	**5.2%**	**2.9%**	**3.0%**	**4.5%**	**2.3%**	**3.8%**
α-Thujene	–	–	–	–	–	–	–	110
α-Pinene	625	205	235	440	130	65	55	305
Sabinene	825	375	605	570	125	85	75	340
β-Pinene	4635	2080	2820	3200	10	10	55	340
Myrcene	638	340	610	420	605	575	410	615
Octanal	112	80	360	110	500	2695	560	110
δ-3-Carene	5	5	5	10	10	20	5	10
α-Terpinene	45	35	75	50	15	–	–	70
p-Cymene	305	70	280	60	–	–	5	235
Limonene	25225	20660	25365	15990	26640	26735	19780	25880
γ-Terpinene	3150	4235	3135	2050	0	10	30	3930
Octanol	50	15	170	60	50	380	55	15
Terpinolene	110	255	130	80	5	10	5	170
Linalool	140	595	505	140	650	4105	150	180
Nonanal	145	575	505	170	80	455	65	55
trans-p-Mentha-2,8-dien-1ol	5	15	10	–	10	45	30	5
cis-Limonene oxide	30	20	50	10	5	55	70	–
trans-Limonene oxide	30	30	50	10	20	120	75	5
Citronellal	120	490	365	100	80	480	45	20
Terpinene-4-ol	60	230	165	40	5	40	5	40
α-Terpineol	230	1500	825	250	100	660	90	160
Decanal	55	300	155	50	400	2530	260	100
Octyl acetate	5	20	10	–	15	45	25	5
Nerol	60	450	195	70	–	–	–	–
Neral	1060	5890	3935	1200	100	550	40	5

TABLE 4.7 (continued)
Citrus Oil Washings Derived from Various Concentrated Citrus Oils

	Lemon				Orange		Grapefruit	Mandarin
	1-Fold Oil	10-Fold, Dist. +nv	5-Fold, Extr. +nv	1-Fold Oil [3x(1:3)][a]	1-Fold Oil	5-Fold Extr. +nv	1-Fold Oil	1-Fold Oil
Oil content	**4.2%**	**5.6%**	**5.2%**	**2.9%**	**3.0%**	**4.5%**	**2.3%**	**3.8%**
Carvone	10	50	35	10	15	125	50	5
Geraniol	30	250	120	50	–	–	–	–
Geranial	1830	9020	6470	1940	160	905	95	5
Perilla aldehyde	30	150	95	30	45	250	5	40
Thymol	–	–	–	–	–	–	–	–
Undecanal	60	210	125	30	20	115	80	25
Neryl acetate	355	2085	1155	310	5	75	60	40
Geranyl acetate	300	1430	795	210	–	15	65	10
Methyl *N*-methyl anthranilate + dodecanal	–	–	–	–	–	–	–	–
Dodecanal	10	30	10	–	60	405	20	–
(*E*)-β-Caryophyllene	20	200	25	20	0	0	20	190
trans-α-Bergamotene	30	240	30	20	–	–	–	–
(*E*,*E*)-α-Farnesene	10	125	30	10	–	–	–	–
β-Bisabolene	45	335	40	20	–	–	–	–
Valencene	–	–	–	–	5	10	–	–
Elemol	–	–	–	–	20	20	25	–
β-Sinensal	–	–	–	–	30	150	10	–
α-Sinensal	–	–	–	–	20	105	5	150
Nootkatone	–	–	–	–	–	170	165	–
Citropten	65	240	175	60	–	–	–	–
Hydrocarbons	35578	28915	33265	22880	27535	27500	20435	31920
Aldehydes	3422	16745	12020	3630	1495	8640	1185	810
Alcohols and ketones	645	3155	2125	640	895	5550	575	455
Acetates and esters	1305	6690	4085	1160	915	5685	725	1420

Notes: Oil content given in (%), constituents in (ppm); [a] Produced according to Owusu-Yaw, J., Matthews, R.F., and West, P.F. 1986. Alcohol deterpenation of orange oil**.** *J. Food. Sci.* 51:1180–1182.

typical compositions of washing flavors. The values for the aroma compounds are shown in *ppm* and have to be divided by 10,000 to obtain the percentage values common with concentrates. From an economic point of view, such a production would, as a result of the low yields, be of limited commercial interest. However, these solutions have found widespread application in clear beverages and the by-products are usually reprocessed and can then be employed for simple applications (Owusu-Yaw et al., 1986). The often prolonged contact with water and oxygen may induce aging processes in

these matrices that can have a negative impact on the remaining material. Usage as citrus terpenes, after distillative removal of the solvent residues, forms another field of commercialization.

Stability tests, submitted to the enforced test conditions for concentrated oils, have also been performed for these aqueous solutions, confirming the negative impact of watery conditions to be expected. A correlation between sensory alterations and chemical analytical values was established, indicating that, as a result of the aqueous alcoholic fraction, aging proceeds along different lines. This corresponds to differing sensory profiles exhibited by washings when compared to aged oils. While washings ultimately form the typical off-notes associated with deteriorated perfumes, the off-flavors of aged oils are evaluated as resiny, diesel type, carvony and terpeny-like.

The application of easily soluble, highly concentrated citrus oils, which are only rediluted to aqueous ethanolic solutions at the time of usage, constitutes a valuable alternative to the washing process. Concentrates available for such processes offer the decisive advantage of clearly prolonged stability (see Section 4.4.3) with decidedly improved yields.

4.5 CONCLUSIONS AND OUTLOOK

It has been the aim of this contribution on the industrially important concentrated citrus oils to establish analytical profiles of products that were obtained by physical processes. Particular emphasis has been put on elucidating why these concentrates possess properties that exert a positive influence on a flavored product palpable for both industry and consumer. Despite an immensely complex market, the commercially available products offer significant advantages as far as stability and solubility are concerned and, therefore, constitute valuable building blocks for a broad range of applications, including the creation of pleasant citrus notes in beverages. As shown, the various concentrates possess clearly different analytical profiles and characteristics—with helpful tools for the optimized product selection depending on application, envisioned sensory impact, stability requirements in the final application, and budget. Predefined product properties constitute one important criterion for the selection of a concentrate specific to the projected application. In addition, the interactions between the flavor concentrate and the matrix to be flavored have to be closely evaluated to ensure specific stability in the final product as well as harmonious synergies. The cold-pressed oils will certainly continue to be the focus of citrus oil concentrates, but the increasing importance of recovery oils (essence oils) and concentrated waterphases is already evident today and certainly constitutes another interesting topic within the range of concentrated citrus products.

The stabilization and usage of citrus oils in form of their more stable concentrates forms the basis of our industry. Continuous engineering efforts and the application of state-of-the-art production methods and technologies will be the driving force for development and progress with regard to envisioned product properties. Additionally, analytical progress will permit the continued collection of an increasingly detailed knowledge on the composition of these interesting natural products, providing those passionate about the topic citrus with new insights. Cultivation of the species citrus, which is of considerable importance for our health and well-being, will therefore constitute an interesting area of work for chemists, life scientists, and engineers in order to provide the market with sophisticated, natural, and stable citrus products.

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5 Composition of Petitgrain Oils

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CONTENTS

5.1 INTRODUCTION

Petitgrain oils are obtained by steam distillation of the leaves, buds, and small branches of the different citrus species.

Due to its odor properties, bitter orange petitgrain is the most important and the most appreciated among these oils. It is produced mainly in the Mediterranean countries and in Paraguay. The oils produced in the Mediterranean area present better odor properties if compared with those produced in Paraguay, which are usually obtained from an hybrid named "Apepu-Jhai" (Di Giacomo, 1974; Huet, 1991).

Lemon, mandarin, bergamot petitgrain oils are produced at small quantities, almost exclusively in Italy. Grapefruit petitgrain is rare, due to its flat odor properties (Peyron, 1965). However, small amounts of this oil are seldom found on the market (Lawrence, 1993). Sweet orange petitgrain, only occasionally produced industrially, is considered the least valuable among these oils, and sometimes it is used to adulterate the more valuable ones. Because of its low cost, the production of this oil lacks in attention, and scant selection of the raw material, along with the uncleanness of the production lines, compromises the purity of the product. It is common, therefore, to find sweet orange petitgrain oils industrially produced, contaminated by other citrus species, rendering difficult to find pure industrial samples (Dugo et al., 1996).

It has been noticed that the quality of the raw material available for distillation in different countries is a problem, compromising the production of the different petitgrain oils. The increased cost of agriculture specialized workers, the consequent use of occasional workers, and the contraction of some cultivated areas are the reasons why the raw material used for the industrial production is not homogeneous

Most of the components in citrus petitgrain oils are the same as those present in the volatile fraction of the correspondent citrus peel oil. Substantial qualitative and quantitative differences exist among the two types of oil. The qualitative differences are determined by the different matrix used for the extraction and by the different process used to obtain them. The extraction by distillation of petitgrain oils can determine some transformation of the components naturally present in the leaves and causes the absence of the non-volatile components present in the peel oils obtained by cold extraction. The quantitative differences are mainly due to the higher content of oxygenated compounds, highly represented in bitter orange and bergamot petitgrain oils if compared to the hydrocarbons content. Limonene, the main component of almost all the peel oils, is present at levels even lower than 1% in bitter orange petitgrain oil. This oil is characterized by linalyl acetate and linalol that together can exceed the 80% of the whole oil. In mandarin petitgrain oil, methyl *N*-methyl anthranilate is equal to about the 50% of the whole oil. Among the monoterpene hydrocarbons of this petitgrain oil, the most abundant is γ-terpinene. In sweet orange petitgrain oil, the main component is sabinene (about 40%) and among the oxygenated components linalol is predominant. In lemon petitgrain oil, the main component is limonene but with percentages greatly lower than the correspondent peel oil (about 30% vs. 65%).

Most of the papers on the composition of citrus petitgrain have been reviewed by Lawrence (1980, 1993, 2003), by Mondello et al. (1996b, 1997a,b,c), and by Dugo et al. (2002). The composition of industrially processed petitgrain oils has been the subject of few papers and grapefruit and bergamot oils have been scantly studied. In the last decade, information on industrial petitgrain is very limited. There is more abundant research on laboratory-extracted oils, obtained by distillation or solvent extraction. These last studies were focused on the biogenesis and the ontogenesis of the volatile flavoring components in citrus and on the characterization and classification of the different citrus species and hybrids.

Due to the complexity of petitgrain oils—but mostly due to the nature of the samples hitherto analyzed that differ for the geographic origin, the cultivar, the extraction technology used, and the age and freshness of the leaves—the composition of citrus petitgrain is not as well defined as the correspondent peel oils.

This chapter will describe the composition of bitter orange (*C. aurantium* L.), mandarin (*C. deliciosa* Ten.), tangerine (*C. tangerina* Hort. ex Tan.), clemetine (*C. clementina* Hort. ex Tan.), lemon (*C. limon* [L.] Burm), sweet orange (*C. sinensis* [L.] Osbeck), bergamot (*C. bergamia*) Key lime (*C. aurantifolia* [Christm.] Swing.), Persian lime (*C. latifolia* Tan.), and grapefruit (*C. paradisi* Macf.) petitgrain oils.

The literature results relative to industrial products and to laboratory-extracted oils will be summarized in tables and discussed in the text. The same criteria and symbols used in Chapter 1 will be here applied.

5.2 BITTER ORANGE PETITGRAIN OIL (*CITRUS AURANTIUM* L.)

Bitter orange petitgrain, often called bigarade petitgrain, presents the most appreciated odor character among all the citrus petitgrain.

The literature is full of information on its composition, but most of the published data is relative to oils extracted in laboratory by distillation or by solvent extraction.

5.2.1 Industrial Oils

Since 1965, studies by gas chromatographic techniques have been carried out to determine the composition of bitter orange petitgrain industrially processed oils, or at least so denominated. The determinations were qualitative or semiquantitative (Peyron, 1965, 1973; De Vottero et al., 1978) but quite numerously quantitative as well (Calvarano, 1968; Lawrence, 1980; Prager and Miskiewicz, 1981; Formáček and Kubeczka, 1982; Boelens and Sindreu, 1988; Haubruge et al., 1989; Boelens and Oporto, 1991; Mondello et al., 1996a,b; Dugo et al., 1996, 2010a).

The qualitative studies named above provided all together the identification of the following components: *p*-cymene, limonene, myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, α-pinene, β-pinene, γ-terpinene, octanal, geraniol, linalol, nerol, α-terpineol, citronellyl acetate, geranyl acetate, linalyl acetate, neryl acetate, and terpinyl acetate*. Results relative to quantitative studies are summarized in Table 5.1.

The data in Table 5.1 provide good information on the basic composition of bitter orange petitgrain oil. The oxygenated components are present at higher amounts than hydrocarbons and in some cases reach the 90% of the whole oil. Esters are the most represented fraction followed by alcohols. Aldehydes are present at very low levels and none of these components ever exceed 1%. The main components are linalyl acetate, almost never less than 40% and that reaches values of 71%, and linalol, which varies between 12% and 34%. The sesquiterpene hydrocarbons are present at low amounts (reasonably for distilled oil); the most abundant is β-caryophyllene, reported by almost all the authors of the papers cited in Table 5.1. In Italian (Dugo et al., 1996; Mondello et al., 1996a,b) and in Spanish oils (Boelens and Sindreu, 1988; Boelens and Oporto, 1991), monoterpene hydrocarbons, limonene, myrcene, (*E*)-β-ocimene, and β-pinene seldom exceed the value of 1%; limonene in Spanish oils is present at higher levels (4.0%–5.43%) than in Italian oils (0.44%–2.17%) and in Egyptian oil (1.91%). The chromatogram of this oil is reported in Figure 5.1. The value, reported by Calvarano (1968), of γ-terpinene (1.56%) for an Italian petitgrain oil, could be due, in our opinion, to a contamination with mandarin petitgrain oil, where this component is more than 20%. This explanation is also proved by the fact that Calvarano reported for the same oil the presence of 2.51% of methyl *N*-methyl anthranilate, present in mandarin at 40% to 50%. The values of γ-terpinene and methyl *N*-methyl anthranilate reported for this sample are compatible with a bitter orange petitgrain contaminated with about 5% of mandarin petitgrain oil.

Not reported in Table 5.1 are the results listed below, obtained by Vernin and Vernin (1966) on bitter orange petitgrain of different geographic origins:

	Spain	Tunisia	Morocco	Paraguay
Limonene	10%	3.5%	7%	3.5%
Linalol	28%	26%	30%	29%
Terpineol*	6.5%	3%	5%	7%
Linalyl acetate	32%	46%	30%	36%
Methyl *N*-methyl anthranilate	tr	0.15%	1%	0.5%

Urbeita-Rehnfeld and Jennings (1974) found that Paraguayan petitgrain oil has lower ester and higher alcohol content than French oil and determined the following main components in a redistilled oil: myrcene (5.36%), (*E*)-β-ocimene (3.32%), (*Z*)-β-ocimene (1.68%), geraniol (2.33%), linalol (27.95%), nerol (1.01%), α-terpineol (7.55%), geranyl acetate (2.61%), linalyl acetate (44.29%), neryl acetate (0.55%), and α-terpinyl acetate (2.29%).

Maurer and Hauser (1992), identified in the alkaline extract of a commercial sample of bitter orange petitgrain oil, trace amounts of (*Z*)-3-(but-1-enyl)pyridine.

TABLE 5.1
Percentage Composition of Industrial Bitter Orange Petitgrain Oils

	1	2	3	4	5	6	7	8,9	10	11
					Hydrocarbons					
Monoterpene										
Camphene	tr	–	–	0.11	–	–	0.01	tr–0.01	tr	0.01
δ-3-Carene	0.31	–	–	0.39	tr	–	0.03	0.21–0.67	1.15	0.71
p-Cymene	0.76	1.0–2.7	–	0.07	tr	tr	0.05	0.03–0.08	0.12	0.09
p-Cymenene	–	–	–	–	tr	–	–	0.01–0.08	–	–
Limonene	1.07	0.7–1.1	0.3–2.3	1.05	4.0	4.4	5.43	0.44–2.17	2.02	1.91
Myrcene	1.59	1.3–5,5	0–2.4	1.96	2.5	1.5	2.60	0.56–1.24	2.31	1.23
(*E*)-β-Ocimene	–	tr–3.3	0–2.7	1.29	2.5	–	2.44	0.57–1.76	3.64	1.61
(*Z*)-β-Ocimene	–	tr–1.1	0–0.8	0.52	0.1	–	0.84	0.20–0.44	0.89	0.42
α-Phellandrene	0.03	tr–0.2	–	–	–	–	0.01	tr–0.03	0.05	0.04[d]
β-Phellandrene	–	–	–	0.10	tr	–	–	0.03–0.04	–	–
α-Pinene	0.11	tr	–	0.22	0.2	0.2	0.19	0.03–0.30	0.17	0.08
β-Pinene	0.83	0.7–1.7	0.3–2.7	1.57	2.5	2.4	2.53	0.65–1.15	2.20	0.71
Sabinene	0.10	tr–0.4	0–1.4	0.30	0.4	0.4	0.40	0.13–0.23	0.45	0.17
α-Terpinene	0.02	tr	–	–	0.3	–	0.06	tr–0.02	0.03	0.01
γ-Terpinene	1.56	0.5–1.1	–	–	0.1	–	0.06	0.01–0.09	0.18	0.09
Terpinolene	0.46	tr–0.1	–	0.19	0.4	0.2	0.29	0.08–0.22	0.59	0.26
α-Thujene	tr	–	–	–	tr	–	0.02	tr–0.01	0.02	0.01
Tricyclene	–	–	–	–	–	–	–	tr	tr	–
Sesquiterpene										
trans-α-Bergamotene	–	–	–	–	–	–	–	tr–0.01	0.01	–
Bicyclogermacrene	–	–	–	–	–	–	–	0.04–0.30	0.28	0.16
δ-Cadinene	–	–	–	–	–	–	0.07	0.02–0.03	0.04	0.02
β-Caryophyllene	–	–	0.3–0.9[a]	0.67	1.6	–	1.77	0.48–0.61	0.71	0.51
α-Copaene	–	–	–	–	–	–	–	tr–0.01	0.01	–
α-Cubebene	–	–	–	–	–	–	–	tr–0.01	0.02	–

β-Elemene	–	–	–	–	–	–	0.03	tr–0.02	0.04	0.02
δ-Elemene	–	–	–	–	–	–	–	0.01	0.02	0.01
(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	0.01–0.06	0.05	0.02
(*E*)-β-Farnesene	–	–	–	–	–	tr	0.46	–	–	0.04
(*Z*)-β-Farnesene	–	–	–	–	–	–	0.08	0.04–0.07	0.07	–
Germacrene D	–	–	–	–	0.1	–	0.04	–	–	–
α-Humulene	–	–	–	–	0.2	0.1	0.18	0.04–0.06	0.07	0.05
Aldehydes										
Aliphatic										
Decanal	0.12	tr	–	–	–	–	0.02	–	–	tr
Nonanal	0.03	–	–	–	–	–	–	0.02–0.05	–	–
Octanal	0.02	–	–	–	–	–	0.01	–	–	–
Monoterpene										
Citronellal	0.03	–	–	–	–	–	0.05	0.01–0.04	0.05	0.04
Geranial	0.11	tr	–	–	tr	–	0.07	0.38–0.64	0.67	0.30
Neral	0.03	tr	–	–	tr	–	0.03	0.21–0.43	0.40	0.19
Ketones										
Aliphatic										
6-Methyl-5-hepten-2-one	–	–	–	–	–	–	–	0.01–0.10	0.08	0.06
Alcohols										
Monoterpene										
Citronellol	0.47	tr–0.02	–	–	–	–	–	–	–	–
Geraniol	0.34	2.0–3.5	0.5–3.0	2.24	1.8	2.3	3.00	0.71–0.95	–	e
Linalol	17.66	19.9–26.9	12.3–33.7	26.62	24.1	17.0	20.20	21.70–32.55	29.80	27.82
cis-p-Menth-2-en-1-ol	–	–	–	–	–	–	–	tr–0.01	–	0.01
Nerol	1.22	1.0–1.5	0.4–1.2	0.95	0.8	0.8	1.00	0.75–0.99	1.28	0.66
Terpinen-4-ol	0.74	0.5–0.8	0.3–0.9[a]	–	0.1	–	0.15	0.05–0.08	0.12	0.05
α-Terpineol	4.16	4.6–7.6	2.1–6.8	5.10	5.2	3.7	4.00	3.09–5.63	5.39	2.97

continued

TABLE 5.1 (continued)
Percentage Composition of Industrial Bitter Orange Petitgrain Oils

	1	2	3	4	5	6	7	8,9	10	11
Sesquiterpene										
Nerolidol	–	–	–	–	0.15*	0.1*	0.12*	0.05–0.08[b]	0.06[b]	0.07[f]
Spathulenol	–	–	–	–	–	–	–	0.03–0.13	0.03	0.02
Esters										
Monoterpene										
Citronellyl acetate	+	–	–	–	–	0.1	0.07	tr	0.11	–
Geranyl acetate	+	–	1.9–4.5	2.89	4.2	3.2	3.92	1.90–3.16	4.22	2.75
Linalyl acetate	+	46.3–55.0	38.4–71.0	50.81	45.5	56.8	48.85	50.68–62.57	39.75	54.64[e]
Linalyl propanate	–	–	–	–	–	–	–	0.02–0.04	–	0.04
Methyl geranate	–	–	–	–	–	–	–	tr–0.03	–	0.03
Neryl acetate	+	2.1–2.6	1.1–3.0	1.69	2.2	–	2.15	1.04–1.73	2.27	1.31
α-Terpinyl acetate	+*	0.2–2.2	–	–	0.1	–	0.10	0.08–0.16	0.06	0.06
Ethers and oxides										
Monoterpene										
1,8-Cineole	–	–	–	–	–	–	–	0.02–0.05	0.06	0.01
cis-Linalol oxide	–	–	–	–	0.2[c]	tr	0.06	0.03–0.09	0.05	0.04
trans-Linalol oxide	–	–	–	–	0.2[c]	tr	0.04	0.01–0.03	0.03	–
Perillene	–	–	–	–	tr	–	0.01	–	–	–
Sesquiterpene										
Caryophyllene oxide*	–	–	–	–	–	–	0.04	0.02–0.07	0.02	0.02
Others										
Methyl anthranilate	–	–	–	–	0.1	–	0.10	–	–	–
Methyl *N*-methyl anthranilate	2.51	–	–	–	tr	–	0.05	tr–0.14	0.17	0.31
Phenylethanol	–	–	–	–	0.2	–	0.20	–	–	–

Notes: tr, traces; *, correct isomer not characterized; +, present, not quantified; [a] β-caryophyllene + terpinen-4-ol; [b] (*E*)-nerolidol; [c] *cis*-linalol oxide + *trans*-linalol oxide; [d] α-phellandrene + (*Z*)-3-hexenyl acetate; [e] linalyl acetate + geraniol; [f] (*Z*)-nerolidol.

Appendix to Table 5.1

1. Calvarano I. (1968). Calabria, Italy; 5 samples; chemical and TLC fractionation; GC/FID on stainless steel capillary column (45 m × 0.5 mm) coated with UCON LB 550X; the original paper reports the composition of the fractions into which the oil (a mixture of the 5 samples) has been separated and the amount of each fraction; the relative percentages have been calculated on the basis of these values. Calvarano also found β-ocimene* (0.21%), heptanal (0.02%), and a total content of esters of 62.92%. The percentage of esters and methyl *N*-methyl anthranilate was obtained by conventional laboratory procedures.
2. Lawrence (1980). Several samples of commercial oils; GC/FID; IR. Lawrence also found trace amounts of (*Z*)-3-hexenol, thymol.
3. Prager and Miskiewicz (1981). 10 samples (5 from France, 4 from Paraguay, and 1 from Egypt); GC/FID and GC/MS on capillary column (33 m × 0.5 mm) coated with Carbowax 20M; relative percentage of peak areas. From the original author's results, one France sample with a high limonene level (8%), that can be considered adulterated, has not been included in the table.
4. Formáček and Kubeczka (1982). One sample; GC/FID on capillary column coated with WG-11; relative percentage of peak areas.
5. Boelens and Sindreu (1988). Spain; GC/FID on capillary columns coated with Carbowax 20M, UCON and SE-54; GC/MS; relative percentage of peak areas. Boelens and Sindreu also found farnesols* (0.05%), *cis*- + *trans*-dehydroxylinalol oxide (0.1%), and trace amounts of 2-methoxy-3-isobutylpirazine, indole.
6. Haubruge et al. (1989). One commercial sample; GC/FID and GC/MS on capillary column (50 m × 0.25 mm × 0.2 μm) coated with CP-TM-VAX 52 CB; relative percentage of peak areas. Haubruge et al. also found cyclofenchene (0.1%), *m*-cymene (0.5%), and trace amounts of *p*-menth-6-en-2-one.
7. Boelens and Oporto (1991). Spain. Boelens and Oporto also found valencene (0.03%), perilla aldehyde (0.01%), nootkatone (0.03%), δ-cadinol (0.01%), mentha-1,8(9)-dienyl acetate (0.01%), 2,2,6-trimethyl-6-vinyl-tetrahydropyran (0.01%).

8,9. Mondello et al. (1996b); Dugo et al. (1996). Sicily, Italy; 5 samples; GC/FID (quantitative analyses) on capillary column (30 m × 0.32 × 0.40–0.45 μm) coated with SE-52; GC/FID and GC/MS quadrupole on capillary columns (60 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52 and Carbowax 20M; coupled LC-GC/MS (ITD) on a LC column (10 cm × 2 mm) packed with Spherisorb 5 μm silica and GC capillary columns as for GC/MS (quadrupole) analyses; MS libraries: Adams, Flavour and Fragrances Natural and Synthetic Compounds (FFNSC) home-made; relative percentage of peak areas. These authors also found *o*-cymene (0%–0.01%), isoterpinolene (0.02%–0.06%), β-bisabolene (0%–0.01%), *trans*-*p*-menth-2-en-1-ol (tr–0.02%), *cis*-sabinene hydrate (tr–0.01%), and trace amounts of α-fenchene, hexanal.

10. Mondello et al. (1996a). Sicily, Italy; one sample; coupled LC-GC/MS (ITD) on a LC column (10 cm × 2 mm) packed with Spherisorb 5 μm silica and a GC capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/FID on the same capillary column; wt%.
11. Dugo et al. (2010a); Egypt; one sample; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found β-sesquiphellandrene (0.01%), *p*-cymen-8-ol (0.01%), (*E*)-2-hexenyl acetate (0.01%).

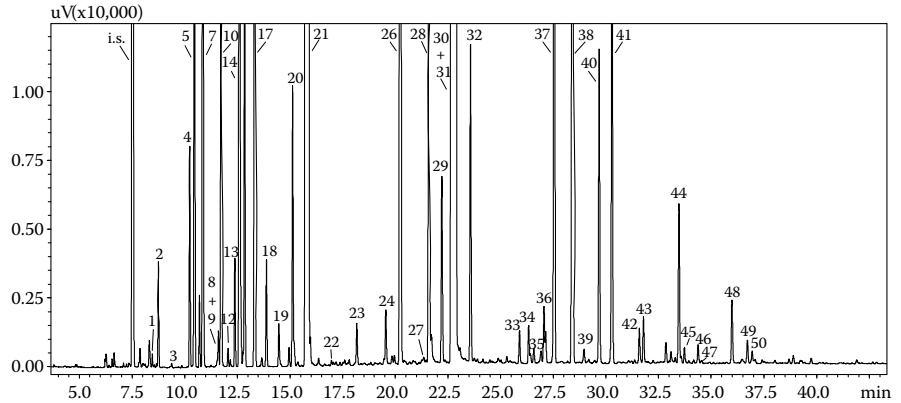

FIGURE 5.1 GC-FID chromatogram of a bitter orange petitgrain oil from Egypt. Peak identification: **i.s.** internal standard; **1** α-thujene, **2** α-pinene; **3** camphene; **4** sabinene; **5** β-pinene; **6** 6-methyl-5-hepten-2-one; **7** myrcene; **8** (*Z*)-3-hexenyl acetate; **9** α-phellandrene; **10** δ-3-carene; **11** (*E*)-2-hexenyl acetate; **12** α-terpinene; **13** *p*-cymene; **14** limonene; **15** 1,8-cineole; **16** (*Z*)-β-ocimene; **17** (*E*)-β-ocimene; **18** γ-terpinene; **19** *cis*-linalol oxide; **20** terpinolene; **21** linalol; **22** *cis*-*p*-menth-2-en-1-ol; **23** citronellal; **24** terpinen-4-ol; **25** *p*-cymen-8 -ol; **26** α-terpineol; **27** decanal; **28** nerol; **29** neral; **30** linalyl acetate; **31** geraniol; **32** geranial; **33** methyl geranate; **34** linalyl propanate; **35** δ-elemene; **36** α-terpinyl acetate; **37** neryl acetate; **38** geranyl acetate; **39** *cis*-β-elemene; **40** methyl *N*-methyl anthranilate; **41** β-caryophyllene; **42** (*E*)-β-farnesene; **43** α-humulene; **44** bicyclogermacrene; **45** (*E*,*E*)-α-farnesene; **46** γ-cadinene; **47** β-sesquiphellandrene; **48** (*Z*)-nerolidol; **49** spathulenol; **50** caryophyllene oxide*. (From Dugo, G., et al., *J. Essent. Oil Res.* 2010a. In press.)

5.2.2 Laboratory Oils

Attaway et al. (1966) in a steam-distilled oil identified, using GC, TLC and MS, the following components: *p*-cymene, limonene, myrcene, β-ocimene*, α-pinene, β-pinene, sabinene, terpinolene, geranial, geraniol, linalol, nerol, terpinen-4-ol, α-terpineol, linalyl acetate, and neryl acetate.

In Table 5.2, the quantitative results relative to papers on the composition of oils extracted from bitter orange leaves of different geographic origins by distillation or solvent extraction are reported.

The composition of the oils reported in Table 5.2 present some peculiar characteristics, more or less accentuated, if compared with the industrial samples described in Table 5.1. These differences are surely related to the botanical origin of the vegetable matrix used and also to the extraction process, storage conditions, the period of harvest of the leaves, and their age, and are affected by the analytical capabilities of the methods used.

The Egyptian oil analyzed by Karawya et al. (1970) was characterized by the high content of limonene (ca. 27%) and monoterpene aldehydes (ca. 5%), values that are similar to those of lemon petitgrain oil (Mondello et al., 1997c). This oil also presented a content of linalol (ca. 5%) and linalyl acetate (ca. 3.5%) extremely lower than those normally determined for bitter orange petitgrain oils, as well as a high content of methyl anthranilate (2.42%).

In the oil extracted with methylene chloride from leaves of *C. aurantium* L. of the Kabusu form (*Daidai*) cultivated in Japan, Kamiyama (1970) determined amounts of monoterpene hydrocarbons (ca. 23%) greatly higher than those usually determined in bitter orange leaf oil. Strangely, two years later, Kamiyama and Amaha (1972), analyzing one oil obtained from the same matrix, determined a total amount of monoterpene hydrocarbons of 8.2%.

The oils analyzed by Ortiz et al. (1978), obtained from numerous cultivars of bitter orange (see appendix to Table 5.2), were characterized by a high amount of α-phellandrene (0.76%–2.66%) and a slightly high amount of geranial (1.00%–2.10%), while the Algerian oil analyzed by Baaliouamer

TABLE 5.2
Percentage Composition of Laboratory-Extracted Bitter Orange Petitgrain Oils

	1	2	3	4	5	6	7	8	9a	9b	10	11
						Hydrocarbons						
Monoterpene												
Camphene	1.15	0.1	tr	–	–	tr	–	–	tr	tr	–	–
δ-3-Carene	–	–	tr	–	0.20	–	–	–	–	–	–	2.55
p-Cymene	1.50	4.1	0.2	0.20–0.35	0.16	–	–	–	–	–	–	1.50
p-Cymenene	–	0.3	tr	–	–	–	–	–	–	–	–	–
Limonene	26.79	0.9	0.4	0.14–0.58	1.25	0.78	0.97	–	0.60	0.68	6.82–11.13	2.32
Myrcene	0.92	1.6	1.0	0.51–1.24	1.82	1.02	0.04	tr	2.29	2.30	2.03–2.29	8.25
β-Ocimene*	1.84	–	–	1.01–2.56	1.92	0.30	–	–	–	–	–	–
(*E*)-β-Ocimene	–	–	1.5	–	–	–	–	tr	1.11	2.33	–	–
(*Z*)-β-Ocimene	–	–	–	–	–	–	–	tr	3.00	0.91	2.07–2.61	–
α-Phellandrene	–	–	–	0.76–2.66	–	–	–	0.12	–	–	–	–
β-Phellandrene	–	0.1	tr	–	0.01	–	–	–	0.04	0.03	–	–
α-Pinene	1.66	4.4[b]	0.5	–	0.19	0.25	–	–	0.14	0.20	–	1.65
β-Pinene	2.65	9.4[c]	3.0	tr–2.51	1.76	tr	0.40	–	2.15	1.26	1.71–2.25	2.98
Sabinene	0.95	9.4[c]	0.5	0.13–0.63	1.58	–	0.03	–	0.33	0.21	–	–
α-Terpinene	–	–	tr	tr	–	–	0.02	–	0.03	0.03	–	–
γ-Terpinene	–	2.7	1.0	tr	0.08	–	–	–	0.03	0.04	–	4.05
Terpinolene	–	–	tr	0.08–0.37	0.30	–	–	0.18	0.58	0.49	–	–
α-Thujene	–	4.4[b]	0.1	–	–	tr	–	–	–	–	–	–
Sesquiterpene												
δ-Cadinene	–	–	–	–	+	–	–	–	–	–	–	–
β-Caryophyllene	–	0.8	0.6	–	0.21	0.11	0.10	–	0.42	0.31	–	4.42
β-Elemene	–	0.1[d]	tr	–	+	–	–	–	–	–	–	–
(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–

continued

TABLE 5.2 (continued)
Percentage Composition of Laboratory-Extracted Bitter Orange Petitgrain Oils

	1	2	3	4	5	6	7	8	9a	9b	10	11
β-Farnesene*	–	–	tr	–	+	–	–	–	–	–	–	–
(*Z*)-β-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–
Humulene*	–	0.9[f]	tr	–	–	–	–	–	–	–	–	–
α-Humulene	–	–	–	–	+	1.35	–	–	–	–	–	–
Aldehydes												
Aliphatic												
Decanal	–	–	tr	tr–0.02	–	–	–	–	–	–	–	–
(*E*)-2-Hexenal	–	0.2	0.3	–	–	–	–	–	–	–	–	–
Nonanal	–	–	tr	–	–	0.45	–	–	–	–	–	–
Octanal	–	–	–	–	–	–	–	–	–	–	–	–
Undecanal	–	–	–	–	–	–	–	–	–	–	–	–
Monoterpene												
Citronellal	0.87	0.2	tr	–	+	–	–	–	–	–	–	3.52
Geranial	2.43	–	0.2	1.00–2.10[e]	+	tr	0.93	0.99	–	–	–	–
Neral	1.84	–	–	tr–2.21	0.12	tr	0.78	–	–	–	–	5.10
Alcohols												
Aliphatic												
(*Z*)-3-Hexenol	–	0.3	tr	0.80–2.34	–	–	–	tr	–	–	–	–
Octanol	–	–	–	–	–	–	–	0.34	–	–	–	–
(*Z*)-2-Pentenol	–	0.1	tr	–	–	–	–	–	–	–	–	–
Monoterpene												
Citronellol	–	–	–	1.00–2.10[e]	–	–	–	2.33	–	–	–	4.65
Geraniol	1.38	–	0.2	1.07–2.13	3.97	0.98	0.08	6.60	6.10	7.07	4.85–8.43	3.30
Isopulegol	–	–	tr	–	–	–	–	–	–	–	–	–
Linalol	4.95	36.0	38.4	59.23–71.04[g]	36.10	11.72	94.1	66.10	24.70	36.81	24.24–26.32	18.90
Nerol	6.72[a]	–	0.1	1.13–4.33	1.51	tr	0.36	–	2.20	2.44	1.70–1.93	3.30

cis-Sabinene hydrate	–	–	–	–	–	–	–	–	–	–	–	–
Terpinen-4-ol	–	0.1[d]	tr	0.17–0.61	0.42	tr	–	20.86	0.15	0.15	–	–
α-Terpineol	1.96	0.9[f]	1.0	3.02–5.87	6.80	1.26	0.38	0.30	8.30	11.67	7.11–7.90	16.80
Thymol	–	–	–	tr	–	–	–	–	–	–	–	0.45
Sesquiterpene												
Farnesol*	6.72[a]	–	–	–	–	0.23	0.16	–	–	–	–	–
Nerolidol*	–	–	–	–	+	–	–	–	0.05	0.02	–	–
(*E*)-Nerolidol	–	–	–	–	–	tr	–	–	–	–	–	–
Spathulenol	–	–	–	–	–	–	–	–	–	–	–	–
Esters												
Monoterpene												
Citronellyl acetate	–	–	–	–	–	–	–	–	–	–	–	–
Geranyl acetate	3.45	0.8	0.5	–	3.54	1.73	0.49	–	6.15	6.02	5.19–5.50	12.45
Geranyl formate	–	–	–	–	2.00	–	–	0.20	–	–	–	–
Linalyl acetate	3.45	35.4	49.8	13.59–20.51[g]	28.94	44.08	–	–	35.50	22.06	37.39–39.85	–
Methyl geranate	–	–	–	–	–	–	–	–	–	–	–	–
Neryl acetate	–	0.6	0.3	–	+	0.83	0.82	0.53	3.20	3.20	1.70–2.78	12.45
α-Terpinyl acetate	–	–	–	–	+	1.11	–	–	–	–	–	–
Ethers and oxides												
Monoterpene												
1,8-Cineole	–	–	–	–	–	–	–	–	–	–	–	–
cis-Linalol oxide	–	–	–	–	0.10	5.40	–	–	0.04[i]	0.02[i]	–	–
trans-Linalol oxide	–	–	–	–	0.07	7.13	–	–	0.02[i]	0.04[i]	–	–
Sesquiterpene												
Caryophyllene oxide*	–	–	–	–	–	–	–	–	–	–	–	–
Others												
Methyl anthranilate	2.42	–	–	–	–	3.49	0.11	–	–	–	–	–

continued

TABLE 5.2 (continued)
Percentage Composition of Laboratory-Extracted Bitter Orange Petitgrain Oils

	12a	12b	13a	13b	13c	13d	13e	13f	14a	14b	14c	15
						Hydrocarbons						
Monoterpene												
Camphene	–	–	tr–0.03	0.07	0.07	0.05	0.16	0.19	0–tr	0.2	0.1	tr
δ-3-Carene	–	–	tr–0.38	0.21	0.65	–	3.12	tr	0–tr	1.8	0.1	0.3
p-Cymene	–	–	0–0.03	0.10	0.13	8.62	5.24	0.04	0–tr	0.3	0.1	tr
p-Cymenene	–	–	–	–	–	1.49	–	–	–	–	–	–
Limonene	1.55	0.32	0.53–0.68	1.92	1.85	4.42	6.90	2.61	0.6–2.2	4.0	2.3	2.5
Myrcene	0.59	tr	2.06–2.65	3.06	3.04	1.08	1.18	0.48	2.0–2.8	0.7	3.5	1.8
β-Ocimene*	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-β-Ocimene	–	–	2.28–2.91	18.55	18.57	1.55	4.67	11.46	2.5–3.5	6.2	15.1	1.9
(*Z*)-β-Ocimene	–	–	0.79–1.03	0.59	0.53	0.08	0.17	0.30	0.9–1.1	0.2	0.5	0.3
α-Phellandrene	–	–	0–tr	0.11	0.12	0.07	0.09	–	0–tr	0.2	0.1	tr
β-Phellandrene	–	–	0.03–0.06	0.72	0.75	0.09	0.52	0.94	0–0.1[h]	–	0.8	tr
α-Pinene	1.21	1.13	0.10–1.08	2.08	2.15	4.04	2.14	3.04	0.1–0.4	2.2	1.9	0.1
β-Pinene	0.34	0.05	0.78–2.95	4.37	4.91	7.79	26.56	49.78	2.1–5.9	36.7	4.4	0.8
Sabinene	–	–	0.26–1.70	55.10	54.39	1.34	5.25	8.15	0.3–0.8	5.7	52.6	0.2
α-Terpinene	–	–	tr–0.06	0.73	0.62	0.42	0.35	0.07	0–0.1	0.3	2.0	tr
γ-Terpinene	–	–	0.03–0.10	1.31	1.15	39.90	6.57	0.18	0–0.1	7.7	3.2	0.1
Terpinolene	0.34	tr	0.36–0.52	0.33	0.34	1.77	0.67	0.08	0.4–0.6	0.7	0.8	0.2
α-Thujene	0.73	0.12	tr–0.02	0.48	0.45	1.82	0.43	0.14	0–tr	0.3	0.5	–
Sesquiterpene												
δ-Cadinene	–	–	0.02–0.04	0.09	0.07	0.04	0.04	0.49	–	–	–	0.1
β-Caryophyllene	–	–	0.14–0.53	0.84	0.76	0.22	0.45	2.80	0–0.3	0.2	0.2	0.7
β-Elemene	–	–	tr–0.04	0.03	0.03	0.02	0.05	0.75	–	–	–	tr
(*E*,*E*)-α-Farnesene	–	–	tr–0.03	0.04	0.11	–	–	1.24	–	–	–	tr
β-Farnesene*	–	–	–	–	–	–	–	–	–	–	–	–
(*Z*)-β-Farnesene	–	–	0–0.03	–	–	0.67	–	0.35	–	–	–	0.1

Humulene*	–	–	–	–	–	–	–	–	–	–	–	–
α-Humulene	–	–	0.02–0.07	0.11	0.10	0.06	0.15	0.68	0–tr	–	–	0.1
Aldehydes												
Aliphatic												
Decanal	–	–	–	0.06	0.06	tr	–	0.03	–	–	–	–
(*E*)-2-Hexenal	–	–	–	–	–	–	–	–	–	–	–	–
Nonanal	–	–	0.03–0.14	0.23	0.18	–	0.08	tr	0–tr	–	–	tr
Octanal	–	–	–	–	–	–	–	–	0–0.1	tr	–	–
Undecanal	–	–	tr–0.08	–	–	0.04	–	0.07	–	–	–	–
Monoterpene												
Citronellal	–	–	0.03–0.30	0.06	0.09	0.60	4.15	0.89	0–tr	1.0	–	tr
Geranial	–	–	0.05–0.34	0.04	0.04	0.14	3.50	tr	0–0.2	1.0	–	0.6
Neral	–	–	0.04–0.31	0.02	0.02	0.05	2.37	tr	0–0.1	0.7	tr	0.3
Alcohols												
Aliphatic												
(*Z*)-3-Hexenol	–	–	–	–	–	–	–	–	–	–	–	–
Octanol	–	–	0–tr	0.03	0.03	0.03	0.03	0.04	–	–	–	–
(*Z*)-2-Pentenol	–	–	–	–	–	–	–	–	–	–	–	–
Monoterpene												
Citronellol	–	–	tr–0.09	0.04	0.03	0.20	0.87	0.41	–	–	–	–
Geraniol	–	–	4.00–6.09	0.05	0.06	0.05	0.68	tr	5.2–6.7	0.2	–	1.2
Isopulegol	–	–	tr–0.02	0.07	0.06	0.39	–	–	–	–	–	–
Linalol	37.38	4.04	31.93–39.75	0.42	0.42	9.75	10.70	1.52	27.7–37.7	22.6	1.0	24.8
Nerol	–	–	1.52–1.86	0.06	0.07	0.14	0.59	0.08	1.8–2.3	0.1	tr	0.6
cis-Sabinene hydrate	–	–	0.02–0.04	0.43	0.34	tr	0.04	tr	–	–	–	tr
Terpinen-4-ol	–	–	0.08–0.28	2.33	1.94	0.15	0.41	0.29	0–0.2	0.8	7.0	0.1
α-Terpineol	6.34	tr	7.42–10.39	0.15	0.10	0.08	0.85	0.08	9.1–11.8	1.3	0.3	6.2
Thymol	–	–	tr	tr	tr	8.13	–	tr	–	–	–	–

continued

TABLE 5.2 (continued)
Percentage Composition of Laboratory-Extracted Bitter Orange Petitgrain Oils

	12a	12b	13a	13b	13c	13d	13e	13f	14a	14b	14c	15
Sesquiterpene												
Farnesol*	–	–	–	–	–	–	–	–	–	–	–	–
Nerolidol*	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-Nerolidol	–	–	0.07–0.16	0.02	0.03	0.28	0.39	3.98	0.1	0.4	–	0.1
Spathulenol	–	–	0.02–0.06	0.26	0.33	0.26	0.41	0.81	–	–	0.1	0.1
Esters												
Monoterpene												
Citronellyl acetate	–	–	0.03–0.09	0.04	0.07	0.13	0.66	0.45	0–tr	–	tr	–
Geranyl acetate	3.77	tr	4.36–5.64	0.06	0.07	0.03	2.64	–	4.3–5.5	0.5	0.1	3.4
Linalyl acetate	40.28	87.87	24.18–34.74	–	–	–	–	–	21.7–36.8	–	–	50.1
Methyl geranate	–	–	tr–0.04	–	–	–	–	–	–	–	–	tr
Neryl acetate	1.87	0.02	2.33–3.10	0.54	0.61	0.23	0.95	0.72	2.3–2.9	0.1	0.2	1.9
α-Terpinyl acetate	–	–	0.08–0.11	–	–	–	0.18	–	0–0.2	–	–	0.2
Ethers and oxides												
Monoterpene												
1,8-Cineole	–	–	0–tr	–	–	–	1.13	–	0–0.1[h]	2.4	–	tr
cis-Linalol oxide	–	–	0.03–0.12	0.03	0.03	tr	0.05	0.06	0–0.1	0.2	tr	0.1
trans-Linalol oxide	–	–	–	–	–	–	–	–	0–0.1	0.1	–	tr
Sesquiterpene												
Caryophyllene oxide*	–	–	0.03–0.13	0.38	0.43	0.10	0.19	0.78	–	–	–	tr
Others												
Methyl anthranilate	–	–	–	–	–	–	–	–	–	–	–	–

Notes: tr, traces; *, correct isomer not characterized; +, present, not quantified; [a] nerol + farnesol* + unknown; [b] α-pinene + α-thujene; [c] β-pinene + sabinene; [d] β-elemene + terpinen-4-ol; [e] geranial + citronellol; [f] humulene* + α-terpineol; [g] the highest value of linalol and the lowest value of linalyl acetate were determined in the cv. Paraguay; [h] β-phellandrene + 1,8-cineole; [i] furanoid or pyranoid form.

Appendix to Table 5.2

1. Karawya et al. (1970). Giza, Egypt; one sample steam distilled; column chromatography; TLC; GC/FID on stainless steel capillary column (90 m × 0.25 mm) coated with Nujol; wt%. Karawya et al. also found terpinene* (2.53%).
2. Kamiyama (1970). Japan; one sample of Daidai bitter orange leaf oil; extraction with methylene chloride, concentration of extract, steam distillation of the residue and extraction with methylene chloride; column chromatography on silica gel; analytical and semipreparative GC on packed columns of PEG 20 M, Apiezon M, β, β'-oxydipropionitrile; IR; relative percentage of peak areas.
3. Kamiyama and Amaha (1972). Japan; one sample of Daidai bitter orange leaf oil extracted as reported by Kamiyama (1970); GC/FID on packed columns of PEG 20 M, Apiezon M, β, β'-oxydipropionitrile, UCON 50HB 280X, LAC-3R-728; relative percentage of peak areas. Kamiyama and Amaha also found trace amounts of β-selinene, (*E*)-2-hexenol.
4. Ortiz et al. (1978). USA; one sample each from the cvs.: Bittersweet, Bouquet de fleurs, Daidai, Granitos, Myrtifolia, Paraguay, Salicifolia, Seville, Sour, Standard, Stow n. 15; steam distillation; GC on packed column of LAC 446; relative percentage of peak areas.
5. Baaliouamer and Meklati (1986). Algeria; one sample steam distilled; GC/FID and GC/MS on capillary column coated with FFAP; GC/MS analysis was also performed on capillary column coated with CP-SIL-5; relative percentage of peak areas. Baaliouamer and Meklati also found cyclofenchene (0.57%), bicycloelemene (0.20%), farnesyl acetate* (0.09%), phytol (0.23%) and the following components not determined quantitatively: aromadendrene, γ-patchoulene, terpinen-2-ol, neryl formate.
6. Lin et al. (1986). China; one sample; column chromatography on silica gel; GC/FID on capillary column (36 m × 0.32 mm) coated with SP-2305; GC/MS on capillary column (30 m × 0.52 mm) coated with SE-30; LRI on SE-30 are reported; IR; relative percentage of peak areas. Lin et al. also found γ-muurolene (0.45%), *p*-mentha-1,4-dien-7-ol (0.07%), *cis*-linalol oxide (pyranoid) (0.17%), *trans*-linalol oxide (pyranoid) (0.60%), and trace amounts of camphor, *trans*-pinocarveol.
7. Germanà et al. (1990). Sicily, Italy; one sample hydrodistilled; GC on packed column of WEAS; relative percentage of peak areas. Germanà et al. also found benzaldeyde (0.07%).
8. Gurib-Fakim and Demarne (1995). Mapou, Mauritius; one sample steam distilled; GC/FID on capillary column (50 m × 0.2 mm × 0.32 μm) coated with HP-101; GC/MS on capillary column (50 m × 0.32 mm × 0.3 μm) coated with HP-101; LRI on HP-101 are reported; relative percentage of peak areas. Gurib-Fakim and Demarne also found β-copaene (0.23%), *cis*-carveol (0.98%).
9. Boussaada (1995) (from Lawrence 2003). One sample each hydrodistilled from (a) Creta, Greece, (b) Tunisia; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5; GC/FID analysis was also performed on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP Innowax; Wiley 275 MS library; relative percentage of peak areas. Boussaada also found farnesene* (0.02% in sample a and 0.11% in sample b). The results relative to the Tunisian oil were reported also in a different paper (Boussaada and Chemli, 2006).
10. Protopapadakis and Papanikolau (1998). Creta, Greece; four samples obtained by hydrodistillation from leaves of the cvs.: Chania, Brazilian, Keen, Bittersweet; GC/FID; GC/MS.
11. Haggag et al. (1999). Giza, Egypt; one sample steam distilled; GC/FID and GC/MS. Haggag et al. also found *m*-cymene (4.57%), α-caryophyllene (4.65%), carvacrol (0.90%), eugenol (1.20%), apiole (4.05%).
12. Adami et al. (2000). Italy; (a) one sample hydrodistilled, (b) one sample solvent (diethyl ether) extracted; GC/FID on capillary column (25 m × 0.33 mm) coated with DB-5; GC/MS on capillary column (30 m × 0.32 mm × 0.5 μm) coated with DB-5; relative percentage of peak areas. This paper also reports the results relative to the supercritical CO_2 extraction: the composition of the oil extracted with CO_2 is very similar to that extracted with solvent. Adami et al. also found caryophyllene* (2.18% in sample a and 2.17% in sample b), bisabolene* (0.96% in sample a and 1.25% in sample b).

continued

TABLE 5.2 (continued)
Percentage Composition of Laboratory-Extracted Bitter Orange Petitgrain Oils

13. Huang et al. (2000). China; steam distillation; one sample each from the following cvs.: (a) African, Brazilian, Spanish, Morocco, Italian, Banggan, Daidai, Xingshan, Bittersweet, Anti-blight, Rubidoux, (b) Australian, (c) Guangpi, (d) Zhulan, (e) Gou tou, (f) Jiangjin; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas; Huang et al. also found δ-elemene (0.89% in sample f), germacrene B (0.06%–0.21%, 0.34%, 0.29%, 0.12%, 0.24%, and 1.17% in samples a, b, c, d, e, and f respectively), valencene (0.52% in sample b and 0.49% in sample c), elemol (0.08% in sample d and 1.91% in sample f).
14. Lota et al. (2001a). Corsica, France; hydrodistillation; one sample each from the following cvs.: (a) Apepu, Brazilian, Doux amer, Menton, Alibert de Corse, Commune de Tuléar, Sae Algérie, Bouquetier de Nice a fruits plats, A fleurs ferrando, Granito, Bouquetier de Nice, Doux, Sans épine, Luisi, Santucci, De Floride, Algerian, Alibert hybride 12, Corsigliese, Tunisian, Bouquet de fleurs, Maroc, Petit Pierre, Espagne, (b) Gou tou, (c) Australian; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; NIST and Wiley MS libraries; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found allo-ocimene (0–tr in samples a and traces in samples b and c), (*Z*)-α-bisabolene (0%–0.3% in samples a and 0.2% in sample c), *cis*-*p*-mentha-2-en-1-ol (0–tr in samples a and 0.4% in sample c), *trans*-sabinene hydrate (0–tr, 0.2%, and 0.6% in samples a, b, and c, respectively). The results relative to the cvs.: Bouquetier de Nice, De Floride, Gou tou and Australian were also reported in a different paper (de Rocca Serra et al., 1998).
15. Kirbaslar and Kirbaslar (2004). Antalya, Turkey; one sample steam distilled; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-5; Wiley and NIST MS libraries; relative percentage of peak areas. Kirbaslar and Kirbaslar also found trace amounts of isoterpinolene, tricyclene, *trans*-α-bergamotene, β-bisabolene, 6-methyl-5-hepten-2-one, *trans*-*p*-mentha-2-en-1-ol, linalyl propanate, methyl *N*-methyl anthranilate.

and Meklati (1986) contained trace amount of components unusual in bitter orange petitgrain oils, such as sesquiterpene hydrocarbons (bicycloelemene and γ-patchoulene) and esters (geranyl and neryl formate) unusual in bitter orange petitgrain oil.

The Chinese oil analyzed by Lin et al. (1986) showed a high content of α-humulene (1.35%), usually present in bitter orange petitgrain oil at levels lower than 0.2%, γ-muurolene (0.45%), a high content (3.49%) of methyl anthranilate and, most strangely, a total content of *cis*- and *trans*-linalol oxides, both in the furanoid and pyranoid forms, of ca 13%, indicating inappropriate storage conditions.

In the Italian oil analyzed by Germanà et al. (1990), the linalyl acetate was absent, while linalol was present at a very high level (94.1%). Linalyl acetate was also absent in the oil from Mauritius, analyzed by Gurib-Fakim and Demarne (1995), and in the Egyptian oil analyzed by Haggag et al. (1999). The oil from Mauritius was also characterized by the presence of high content of monoterpene alcohols and by the absence of limonene. The oil from Egypt was characterized by the presence of high amounts of geranyl and neryl acetate.

The oils from Crete (Protopapadakis and Papanikolau, 1998) showed high content of limonene (6.82%–11.13%).

The oils analyzed by Adami et al. (2000), extracted from the same matrix, one by distillation and one by diethyl ether, presented important differences among alcohols and esters. The distilled oil was characterized by high linalol value (37.38%), while the solvent-extracted sample had a very low value of linalol (4.04%) and an high content of linalyl acetate (87.87%).

Most of the cultivars grown in China studied by Huang et al. (2000), whose results are reported as ranges of variability in Table 5.2, present a composition very similar to that of genuine industrial bitter orange petitgrain oils. Some cultivars present peculiarities in their composition, such as high values of sabinene (cvs. Australian, Guangpi), similar to sweet orange petitgrain; high values of γ-terpinene (cv. Zhulan), similar to mandarin petitgrain; values of β-pinene (cvs. Gou tou and Jiangjin) higher than every other citrus petitgrain; and the absence of linalyl acetate in all of these cultivars, which are singularly described in the table.

Most of the cvs. cultivated in Corsica analyzed by Lota et al. (2001a), with the exception of high values of geraniol and α-terpineol, present a composition very close to that of genuine industrial oils. Although, cvs. Gou tou and Australian present compositional anomalies very similar to those encountered in the same cultivars by Huang et al. (2000).

The Turkish oil analyzed by Kirbaslar and Kirbaslar (2004) presents a composition very similar to Italian industrial oils analyzed by Mondello et al. (1996a,b).

In Table 5.2, the results reported by Lin and Hua (1992) are not included. The composition of this oil was: limonene (6.7%), myrcene (42.6%), γ-terpinene (6.5%), geranial and neral (3.9%), linalol (13.1%), and *cis*- and *trans*-linalol oxide (12.1%). The main anomalies were mostly due to the high value of myrcene and the absence of linalyl acetate. Moreover, the results are very different from those previously reported by the same research team (Lin et al., 1986) included in Table 5.2.

5.3 LEMON PETITGRAIN OIL (*CITRUS LIMON* [L.] BURM.)

5.3.1 Industrial Oils

The number of papers found in literature on the composition of lemon petitgrain oils industrially produced is small: there is one paper by Peyron (1965) on a semi quantitative determination; one on the dosage of very few components (Vernin and Vernin, 1966); one on the qualitative analysis on Argentinean oils (De Vottero et al., 1978); four papers on the quantitative determinations on Italian industrial production (Calvarano, 1967; Di Giacomo et al., 1985; Goretti et al., 1986; Mondello et al., 1997c); one paper on Turkish oils (Verdi et al., 1993) and one paper by Cartoni et al. (1987), where lemon petitgrain oil was used with other essential oils to prove two columns in series, one apolar and one polar, for the GC analysis of essential oils.

Peyron (1965) identified in lemon petitgrain oils the following components, listed in decreasing amount order: limonene, β-pinene, geranyl acetate + neryl acetate, geranial, neral + nerol, linalol, γ-terpinene, and α-pinene. De Vottero et al. (1978), during a study on GC analysis on packed columns with Carbowax 20M, identified in lemon petitgrain oil the following components in addition to those reported by Peyron: *p*-cymene, myrcene, citronellal, citronellol, geraniol, and α-terpineol. Cartoni et al. (1987) also identified camphene, α- and β-phellandrene, sabinene, terpinolene, α-thujene, bergamotene*, β-bisabolene, β-caryophyllene, humulene*, decanal, octanal, 6-methyl-5-hepten-2-one, terpinen-4-ol, citronellyl acetate, linalyl acetate, and 1,8-cineole. The GC system used by Cartoni et al. permitted to separate some critical pairs otherwise coeluted using two columns—one of SE-54 and one of Carbowax 20M—in series.

Vernin and Vernin (1966) determined in lemon petitgrain oil, by GC and TLC, the following composition: limonene (30%), geranial + neral (7%–10%), linalol (24%), geraniol + nerol + geranyl acetate + neryl acetate (15%–20%), terpineol* (0.1%), linalyl acetate (tr), methyl anthranilate (tr), and methyl *N*-methyl anthranilate (0.05%).

The results relative to the quantitative studies on industrial petitgrain oils are reported in Table 5.3.

TABLE 5.3
Percentage Composition of Industrial Lemon Petitgrain Oils

	1	2	3	4a	4b	5	6
			Hydrocarbons				
Monoterpene							
Camphene	0.09	0.08–0.11	0.12	0.12	0.06	0.05	0.05–0.07
δ-3-Carene	0.63	0.11–0.15	–	0.44	0.68	0.75	0.63–1.08
o-Cymene	–	0.04–0.06	–	–	–	0.01	tr–0.01
p-Cymene	1.28	0.67–0.82	0.91	0.02	0.23	0.51	0.04–0.51
α-Fenchene	–	–	–	–	–	0.01	0–tr
Limonene	30.67	30.18–35.57	30.71	30.22	37.20	30.66	28.41–34.82
Myrcene	1.01	1.02–1.80	1.49	1.17	0.79	0.83	0.79–1.60
(*E*)-β-Ocimene	–	–	–	2.30	1.25	1.50	1.50–2.43
(*Z*)-β-Ocimene	–	–	–	0.43	0.26	0.30	0.30–0.44
α-Phellandrene	0.08	–	0.16	–	–	0.03	0.03–0.09
α-Pinene	2.29	1.12–1.48	1.21[a]	1.36	0.42	0.87	0.84–1.13
β-Pinene	11.53	15.63–19.86	13.59	19.42	6.31	12.57	11.96–16.03
Sabinene	2.85	1.05–1.88	3.56	4.22	1.93	3.01	2.99–3.81
α-Terpinene	0.12	–	–	–	–	0.04	0.04–0.11
γ-Terpinene	1.80	2.16–3.19	2.32	0.17	0.01	0.42	0.34–0.70
Terpinolene	0.30	0.18–0.22	0.31	0.13	0.10	0.19	0.19–0.31
α-Thujene	0.01	–	1.21[a]	–	–	0.07	0.06–0.09
Tricyclene	–	–	–	–	–	tr	tr–0.01
Sesquiterpene							
trans-α-Bergamotene	–	–	–	–	–	0.06	0.03–0.20
Bicyclogermacrene	–	–	–	–	–	0.17	0.06–0.23
Bisabolene	–	0.09–0.10*	–	–	–	–	0.07–0.33[e]
δ-Cadinene	–	–	–	–	–	0.02	0.02–0.05
Caryophyllene	–	3.19–6.20*	0.95[f]	–	–	0.96[f]	0.60–1.54[f]
β-Elemene	–	–	–	–	–	0.03	tr–0.03
(*E*,*E*)-α-Farnesene	–	–	–	–	–	0.14	0.03–0.14

TABLE 5.3 (continued)
Percentage Composition of Industrial Lemon Petitgrain Oils

	1	2	3	4a	4b	5	6
(*Z*)-β-Farnesene	–	–	–	–	–	0.02	tr–0.03
α-Humulene	–	–	–	–	–	0.09	0.06–0.13
Aldehydes							
Aliphatic							
Decanal	0.09	0.01–0.05	0.06	0.04	0.05	–	0.03–0.09
Nonanal	0.16	0.29–0.35	–	0.27	0.19	0.08	0.08–0.22
Octanal	0.41	–	–	–	–	–	0.01–0.04
Undecanal	–	–	–	–	–	0.04	0.02–0.08
Monoterpene							
Citronellal	0.34	1.11–1.89	1.48	1.18	1.71	0.78	0.61–1.41
Geranial	10.30	10.91–17.27	10.93	16.66[d]	18.41[d]	11.67	9.87–14.07
Neral	5.80	8.30–12.63	6.48	11.27	12.01	8.13	6.64–10.78
Ketones							
Monoterpene							
6-Methyl-5-hepten-2-one	–	0.33–0.51	0.26	0.61	1.94	1.06	0.67–1.61
Alcohols							
Aliphatic							
Octanol	–	–	–	–	–	0.01	tr–0.01
Monoterpene							
Citronellol	0.84	0.02–0.19[b]	0.22	0.19	0.40	–	tr
Geraniol	1.95	0.43–1.05	0.51	0.30	0.78	5.44[g]	0.87–6.25
Isopulegol	–	–	–	–	–	tr	tr–0.03
Linalol	6.30	0.87–1.24	1.77	0.96	1.45	3.09	0.88–3.87
cis-p-Menth-2-en-1-ol	–	–	–	–	–	0.02	0.01–0.03
Nerol	2.20	2.66–3.10	1.30	0.80	2.20	2.66	1.90–3.14
cis-Sabinene hydrate	–	–	–	–	–	0.03	0.02–0.06
Terpinen-4-ol	0.91	0.15–0.20	0.24	–	–	0.51	0.25–0.59
α-Terpineol	2.65	0.38–0.45	0.37	0.41	0.63	0.96	0.53–1.00
Sesquiterpene							
α-Bisabolol	–	–	–	–	–	0.01	0.01–0.03
Campherenol	–	–	–	–	–	0.01	0.01–0.02
(*E*)-Nerolidol	–	–	–	–	–	0.01	0.01–0.03
Norbornanol[h]	–	–	–	–	–	0.02	0.02–0.06
Spathulenol	–	–	–	–	–	0.04	0.01–0.09
Esters							
Aliphatic							
Octyl acetate	10.12[c]	tr	0.04	–	–	–	–
Monoterpene							
Citronellyl acetate	–	–	0.25	0.07	0.18	0.21	0.13–0.23
Geranyl acetate	10.12[c]	0.19–0.88	2.91	1.33	2.33	2.92	2.17–2.92

continued

TABLE 5.3 (continued)
Percentage Composition of Industrial Lemon Petitgrain Oils

	1	2	3	4a	4b	5	6
Linalyl acetate	10.12[c]	0.02–0.19[b]	6.50	0.05	0.09	5.44[g]	0.29–0.42
Neryl acetate	10.12[c]	–	7.44	–	–	5.89	3.75–6.74
Terpinyl acetate*	10.12[c]	1.77–2.23	–	–	–	–	–
Ethers and oxides							
Monoterpene							
1,8-Cineole	–	–	–	2.07	2.68	1.34	1.12–2.13
cis-Limonene oxide	–	–	–	0.02	0.35	tr	0.01–0.05
trans-Limonene oxide	–	–	–	0.01	0.17	–	0.01–0.06
cis-Linalol oxide	–	–	–	–	–	0.01	tr–0.01
Sesquiterpene							
Caryophyllene oxide*	–	–	–	–	–	0.05	0.04–0.14
Others							
Methyl *N*-methyl anthranilate	+	0.03–0.07	0.78	–	–	0.24	tr–0.39

Notes: tr, traces; *, correct isomer not characterized; +, present, not quantified; [a] α-pinene + α-thujene; [b] citronellol + linalyl acetate; [c] terpinyl acetate* + geranyl acetate + linalyl acetate + neryl acetate + octyl acetate (listed in decreasing concentration order); [d] geranial + 2,7-dimethyl-2,6-octadien-1-ol; [e] β-bisabolene; [f] β-caryophyllene; [g] geraniol + linalyl acetate; [h] 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol.

Appendix to Table 5.3

1. Calvarano (1967). Calabria, Italy; 9 samples; conventional laboratory procedures; GC on stainless steel capillary column (45 m × 0.5 mm) coated with UCON LB 550X; the original paper reports the composition of the fractions into which the oil (a mixture of the 9 samples) has been separated and the amount of each fraction; the relative percentages have been calculated on the basis of these values. Calvarano also found β-ocimene (1.37%), heptanal (0.09%).
2. Di Giacomo et al. (1985). Calabria, Italy; 3 samples; GC/FID on capillary column (45 m × 0.5 mm) coated with UCON LB 550X; relative percentage of peak areas. The original paper reports the composition and yield of single fractions collected at different time intervals during the distillation; the composition reported in the table relatively to the three samples was determined from the composition of each fraction considering the relative yields.
3. Goretti et al. (1986). Calabria, Italy; one sample; GC/FID on capillary column (50 m × 0.30 mm) coated with FFAP; relative percentage of peak areas.
4. Verdi et al. (1993). Turkey; (a) one sample from Silifke area, (b) one sample from Mersin area; GC/FID and GC/MS; Verdi et al. also found, in sample (a): 1-methyl-3(1-methylethyl) ciclopentane (0.01%), 3,3,5-trimethyl-1,4-hexadiene (0.01%), piperitone (0.04%), *trans*-carveol (0.01%), *trans*-sabinene hydrate (0.08%); in sample (b): 1-methyl-3(1-methylethyl) ciclopentane (0.05%), 3,3,5-trimethyl-1,4-hexadiene (0.01%), piperitone (0.12%), *trans*-carveol (0.06%), *trans*-pinocarveol (0.05%), *trans*-sabinene hydrate (0.09%).
5. Mondello et al. (1996a). Sicily, Italy; one sample; coupled LC-GC/MS (ITD) on a LC column (10 cm × 2 mm) packed with Spherisorb 5 μm silica and a GC capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/FID on the same capillary column; wt%.
6. Dugo et al. (1996), Mondello et al. (1997c), Sicily, Italy; 6 samples; GC/FID (quantitative analyses) on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/FID and GC/MS on capillary columns (60 m × 0.32 mm × 0.40 - 0.45 μm) coated with SE-52 and Carbowax 20M; LC-GC/MS on LC column (10 cm × 2 mm) packed with Spherisorb 5 μm silica and GC capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; MS libraries: Adams, NIST, Wiley and FFNSC home-made; relative percentage of peak areas. These authors also found isoterpinolene (0.01%–0.03%), β-phellandrene (2.22%–2.60%), geranyl propanate (0.03%–0.04%), and trace amounts of *cis*-α-bergamotene and iso-(iso)pulegol.

The data relative to Di Giacomo et al. (1985), reported in Table 5.3, were extrapolated from the results found on the original paper, relative to the composition of fractions obtained by distillation at different times (from 30 min to 3 h) and considering the yield of each fraction. It is noteworthy that the fractions collected during the first hour of distillation were much richer in aldehydes—in particular neral and geranial—and esters, while the monoterpene hydrocarbons were not present at high levels, especially limonene and β-pinene. In the later fractions, collected after 90 to 180 minutes, a decrease of the aldehydes and esters content was observed, as was the increase of monoterpene hydrocarbons. The authors explained this behavior as chemical transformations of natural components occurring during the hot distillation, which was more or less evident in function of the time. In the same paper, an aliquot of the same leaves used for distillation was solvent extracted at 40°C. The composition determined for this oil, considered by the authors as representative of the natural oil composition, resulted very similarly to that observed in the fractions distilled in the first hour. The authors, therefore, concluded that the distillation prolonged for a long time produced higher yields of oil but also caused visible changes in the composition, while shorter distillation would avoid the formation of such evident alterations.

The composition of the Italian lemon petitgrain oils reported in Table 5.3, although relative to samples analyzed in a range of time of about 30 years, are homogeneous enough. For some components, variability ranges are quite wide. These variations are probably due to different lengths of the distillation process, causing the presence of lower or higher amounts of the least volatile components. It should, however, be highlighted that the percentage values of terpinyl acetate* were sensibly high in reports by Calvarano (1967) and by Di Giacomo et al. (1985). It could be possible that this component was misidentified. In fact, among the numerous papers on the composition of lemon petitgrain extracted in laboratory (see Table 5.4), only those by Scora et al. (1969) and by Melenderas et al. (1984) reported the presence of terpinyl acetate*. The latter authors determined, in a leaf oil of *C. limon* distilled in laboratory, only trace amounts of this component; the former found in the samples analyzed about 2% to 3% of terpinyl acetate*.

Turkish oils, analyzed by Verdi et al. (1993), are very similar to Italian oils, with the exception of few minor components.

5.3.2 Laboratory Oils

Numerous studies have been carried out on the laboratory-extracted oils, and the relative results are summarized in Table 5.4.

In the appendix of Table 5.4, detailed information is reported on the different botanical and geographic origins of the oils analyzed by the different authors, either obtained by solvent extraction or by distillation. Each vegetable material used for extraction by the different authors probably differed for the age of the tree and the period of harvest. These variables could explain the diversity of the composition among the oils that are described in Table 5.4. All of them are, however, characterized by high content of limonene and, with the exception of those analyzed by Ayedoun et al. (1996) and by Haggag et al. (1998), by high amounts of neral and geranial. These values are in agreement with what previously determined for the oils industrially processed. It should be, however, mentioned that in some of these oils (Baaliouamer et al., 1985; Zollo Amvam et al., 1998; Fujita et al., 2008) numerous minor components were identified that surely need further confirmation.

The results obtained by Lin and Hua (1992) are not included in Table 5.4 since they reported the following improbable composition relative to a leaf oil of Eureka lemon: limonene (21.8%), neral and geranial (24.7%), 6-methyl-5-hepten-2-one (24.4%), carvone (9.7%), and linalyl acetate (5.7%).

Not included in Table 5.4 are the results relative to the leaf oil of lemon Barum, obtained by de Rocca Serra et al. (1998) and by Lota et al. (2002), and those relative to the cv. Ponderosa obtained by Huang et al. (2000). The leaf oil of lemon Barum is characterized by low content of limonene (3.2%) and by high contents of linalol (38.9%), α-terpineol (11.4%), and linalyl acetate (18.5%). The leaf oil of lemon Ponderosa (Huang et al., 2002) is characterized by a low content of limonene

TABLE 5.4
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

	1	2	3	4	5	6	7a	7b	8	9	10	11	12	13
						Hydrocarbons								
Monoterpene														
Camphene	0.1	0–0.1	–	0.08	tr–0.16	0.08	0.10	0.06	–	tr	–	–	0.1	–
δ-3-Carene	–	–	–	1.08	0.57–0.98	0.39	–	–	–	–	–	–	–	–
p-Cymene	0.5	0.3–0.6	–	0.04	tr	0.12	0.12	0.11	–	0.25	–	–	0.1	0.29
p-Cymenene	–	–	–	–	–	–	–	–	–	–	–	0.50	–	–
α-Fenchene	–	–	–	–	–	0.01	–	–	–	–	–	–	–	–
Limonene	23.1	22.2–28.9	13.00–14.70	38.63	25.84–34.55	25.90	26.07	22.71	12.05–17.47	9.84	28.39	38.20	40.8	20.05
Myrcene	1.7	1.6–2.2	4.77–7.36	1.61	1.15–1.64	0.94[a]	1.03	0.98	–	0.66	1.04	1.20	1.5	–
β-Ocimene*	–	–	–	2.90	2.05–2.57	1.99	1.96	1.67	–	–	–	–	–	–
(*E*)-β-Ocimene	–	–	–	–	–	–	–	–	–	0.75	–	0.15	3.1	–
(*Z*)-β-Ocimene	–	–	–	–	–	–	–	–	–	–	–	0.10	0.8	–
α-Phellandrene	–	–	–	–	–	0.94[a]	–	–	–	2.04	–	0.19[b]	–	–
β-Phellandrene	–	–	–	0.55	–	–	–	–	–	9.33	–	0.35	–	–
α-Pinene	3.4[c]	2.1–3.6[c]	0.19–0.27	1.58	0.83–2.15[c]	1.38[c]	1.35	0.84	1.63–2.23	0.58	0.29	0.16	1.5	3.95
β-Pinene	12.4[d]	12.1–14.4[d]	–	18.75	9.79–26.86	17.11	18.43	13.32	3.44–6.85	0.02	6.72	0.10	18.5	9.72
Sabinene	12.4[d]	12.1–14.4[d]	–	2.06	2.24–3.96	4.53	–	–	–	0.41	0.58	0.32	3.8	–
α-Terpinene	tr	–	–	0.08	tr–0.28	0.05	–	–	–	–	3.41	0.19[b]	tr	–
γ-Terpinene	3.3	2.1–3.5	2.39–2.49	0.22	0.85–1.24	0.26	0.28	0.21	0.39–2.51	0.06	–	0.50	0.8	–
Terpinolene	–	–	–	0.29	0.25–0.31	0.16	–	–	–	0.04	–	0.16	0.1	–
α-Thujene	3.4[c]	2.1–3.6[c]	–	–	0.83–2.15[c]	1.38[c]	–	–	–	0.01	–	–	0.1	–
Sesquiterpene														
α-Bergamotene*	–	–	–	–	–	0.79	–	–	–	0.06	–	–	–	–
cis-α-Bergamotene	–	–	–	–	–	–	–	–	–	–	–	–	–	–
trans-α-Bergamotene	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–

Bicyclogermacrene	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bisabolene*	–	–	–	–	–	–	0.30	0.53	–	–	–	–	–	–
β-Bisabolene	–	–	–	–	–	2.78	–	–	–	–	–	–	0.3	–
δ-Cadinene	–	–	–	–	–	0.03	–	–	–	–	–	–	tr	–
Caryophyllene*	0.2	0.2–2.3	–	–	–	–	–	–	0.24–1.44	0.25	0.22	–	–	–
β-Caryophyllene	–	–	–	0.66	0.57–1.44	0.79	–	–	–	–	–	–	0.8	–
β-Elemene	0.4[f]	0.4–0.7[f]	–	–	–	–	–		–	–	–	–	–	–
(*E*,*E*)-α-Farnesene	–	–	–	–	–	0.03	–	–	–	–	–	–	0.2	–
(*Z*)-β-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Germacrene D	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
α-Humulene	–	–	–	0.21	–	9.38[h]	–	–	–	0.05	–	–	0.1	–
β-Selinene	1.9	1.4–2.0	–	1.03	–	–	–	–	–	–	–	–	–	–
Aldehydes														
Aliphatic														
(*E*)-2-Butenal	–	–	–	–	–	0.23	0.07	0.15	–	–	–	–	–	–
Decanal	–	–	–	–	tr–0.11	0.02	0.07	0.10	–	0.01	–	–	0.4	–
2,6-Dimethylhept-5-en-1-al	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
Dodecanal	–	–	–	–	–	–	–	–	–	0.03	–	–	tr	–
Hexanal	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-2-Hexenal	2.0	–	–	–	–	–	0.39	0.52	–	–	–	–	–	–
Nonanal	–	–	–	–	0.21–0.35	0.14	–	–	–	0.02	–	–	0.2	–
Octanal	–	–	–	–	tr	–	–	–	–	0.04	–	–	0.1	–
Undecanal	–	–	–	–	tr–0.12	–	–	–	–	0.05	–	–	0.1	–
Monoterpene														
Citronellal	–	–	2.03–3.12	0.91	1.06–1.90	1.07	–	–	–	2.63	–	0.06	16.5	2.16
Geranial	24.3	24.2–29.6	27.06–31.77	9.73	9.40–15.19	11.11	12.71	19.17	23.93–30.54	21.31	19.27	15.20	0.3	–
Neral	16.4	16.0–18.2	18.14–22.54	5.97	7.60–12.10	1.56[i]	12.03	14.79	21.48–25.32	13.96	12.22	10.20	0.3	–

continued

TABLE 5.4 (continued)
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

	1	2	3	4	5	6	7a	7b	8	9	10	11	12	13
Ketones														
Aliphatic														
6-Methyl-5-hepten-2-one	–	–	–	2.30	0.57–0.80	1.06	–	–	–	1.72	–	3.20	–	–
Monoterpene														
Camphor	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–
Alcohols														
Aliphatic														
Ethanol	–	–	–	–	–	0.29	–	–	–	–	–	–	–	–
Hexanol	–	–	–	–	–	–	0.09	0.02	–	–	–	–	–	–
(*E*)-2-Hexenol	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
(*Z*)-3-Hexenol	0.9	–	–	–	–	0.03	0.20	0.12	–	–	–	–	–	–
Nonanol	–	–	1.17–2.39	–	–	–	–	–	–	0.92	–	–	–	–
Octanol	–	–	–	–	–	–	0.02	0.01	–	–	–	–	–	–
Monoterpene														
Borneol	–	–	–	0.02	0.10–0.30	–	–	–	–	–	–	–	–	–
Carvacrol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12
trans-Carveol	–	–	–	–	–	0.02	–	–	–	–	–	–	–	–
Citronellol	–	–	–	0.02	0.27–0.39	0.02	–	–	–	–	–	1.40	2.3	22.95
p-Cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Geraniol	2.8	1.3–2.8	–	1.63	0.99–1.63	1.49	–	–	11.40–15.78	0.17	–	3.00	tr	3.20
Isopulegol	–	–	–	–	–	0.02	–	–	–	0.52	–	–	0.3	–
Linalol	3.1	1.7–3.2	1.75–2.11	1.20	1.24–1.46	1.60	1.03	0.89	–	17.26	1.64	8.00	0.9	1.50
cis-p-Menth-2-en-1-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Nerol	2.0	1.7–2.2	6.58–7.39	2.18	1.66–2.13	1.54	–	–	–	4.47	0.07	3.50	–	1.24
Sabinene hydrate*	–	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-Sabinene hydrate	–	–	–	–	–	–	–	–	–	–	–	–	–	–
trans-Sabinene hydrate	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Terpinen-4-ol	0.4[f]	0.4–0.7[f]	–	0.21	0.43–0.91	0.15	0.31	0.27	–	0.28	–	0.80	0.6	–
α-Terpineol	–	–	–	0.30	0.63–1.00	9.38[h]	0.27	0.31	–	0.07	4.59	3.00	0.6	18.93
β-Terpineol	–	–	–	–	–	0.10[g]	–		–	–	–	0.10*	–	–
Thymol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.23
Sesquiterpene														
Farnesol*	–	–	–	–	–	0.01	0.04	0.11	–	–	–	–	–	+
Nerolidol*	–	–	–	–	–	0.05	0.02	0.03	–	–	–	–	–	–
(*E*)-Nerolidol	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
Spathulenol	–	–	–	–	–	0.04	–	–	–	–	–	–	–	–
Esters														
Aliphatic														
Octyl acetate	–	–	–	–	–	0.01[e]	–	–	–	0.01	–	–	–	–
Monoterpene														
Citronellyl acetate	–	–	–	0.11	tr	9.38[h]	0.02	0.02	–	0.37	–	–	0.7	–
Geranyl acetate	–	–	5.38–7.98	2.58	2.30–3.31	1.75	0.26	0.59	2.71–4.03	1.66	16.37	–	0.3	–
Geranyl formate	–	–	2.57–3.32	–	–	0.21	–	–	–	–	–	–	–	–
Geranyl propanate	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–
Linalyl acetate	–	–	–	0.21	–	0.23	–	–	–	2.22	–	–	–	–
Methyl geranate	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Neryl acetate	–	–	1.32–1.75	–	4.12–8.18	1.56[i]	0.18	0.39	3.48–5.57	2.82	4.46	–	0.2	–
Neryl propanoate	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-Terpinyl acetate	–	–	2.48–2.97	–	tr	–	–	–	–	–	–	–	–	–

continued

TABLE 5.4 (continued)
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

	1	2	3	4	5	6	7a	7b	8	9	10	11	12	13
Ethers and oxides														
Monoterpene														
1,8-Cineole	–	–	–	0.70	–	4.36	–	–	–	–	–	–	2.9	–
cis-Limonene oxide	–	–	–	–	–	0.01[e]	–	–	–	–	–	–	–	–
trans-Limonene oxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-Linalol oxide	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–
β-Pinene oxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sesquiterpene														
Caryophyllene oxide*	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
trans-Caryophyllene oxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Others														
Geranic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Methyl *N*-methyl anthranilate	–	–	–	–	0.14–0.19	–	–	–	–	–	–	–	–	–
Toluene	–	–	–	–	–	0.01	0.02	0.02	–	–	–	–	–	–

	14	15a	15b	16	17,18	19	20	21	22a	22b	23	24	25	26a	26b
							Hydrocarbons								
Monoterpene															
Camphene	–	0.08	0.08	0.04–0.10	tr	0.04–0.08	0–0.11	0.1	–	–	tr	0.10	0.08	0.02	0.03
δ-3-Carene	0.7	0.75	1.06	0.17–1.46	0.1	0.33–0.67	0.60–1.42	0.1–0.7	1.45	4.26	0.6	0.15	0.44	0.51	0.81
p-Cymene	–	0.24	0.30	0.05–0.16	0.1	0.01–0.09	–	0–0.1	–	0.07	tr	0.11	–	0.18	0.05
p-Cymenene	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	tr
α-Fenchene	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	tr
Limonene	27.9	42.43	38.77	20.52–25.75	28.2	28.79–38.19	22.69–24.15	17.8–28.9	32.16	29.57	44.2	14.08	20.05	16.66	19.33
Myrcene	1.1	1.64	1.49	0.97–1.13	12.6[j]	0.96–1.31	1.42–2.36	0.8–1.1	1.64	1.92	1.8	0.70	1.05	0.60	0.91
β-Ocimene*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-β-Ocimene	2.0	1.99	1.88	0.90–1.67	2.5	1.63–2.72	2.45–3.93	1.2–2.2	1.77	1.17	1.7	1.65	1.75	0.82	1.37
(*Z*)-β-Ocimene	–	–	–	0.20–0.32	0.7	0.37–0.58	0.51–0.80	0.2–0.4	0.34	0.24	0.4	0.34	0.38	0.19	0.30
α-Phellandrene	–	0.07	0.07	tr–0.08	tr	0.02–0.14	–	tr–0.1	–	–	0.1	–	0.08	–	–
β-Phellandrene	–	–	–	0.25–0.32	–	–	–	0–0.8[m]	0.38	0.17	1.3	+	0.44	+	+
α-Pinene	0.9	1.32	1.15	0.64–1.16	0.8	0.71–1.28	0.75–1.47	0.7–1.7	0.66	0.29	0.6	1.02	1.07	0.40	0.54
β-Pinene	13.9	13.14	14.85	8.21–12.11	12.6[j]	8.77–18.16	10.96–16.13	10.5–25.1	6.61	0.57	10.6	10.70	13.66	4.01	6.86
Sabinene	2.2	3.38	2.60	1.70–3.24	1.8	3.70–5.68	–	1.9–5.1	2.04	0.63	2.4	4.57	2.87	1.16	1.59
α-Terpinene	–	0.15	0.10	tr–0.04	tr	0.04–0.36	0.10–0.21	tr–0.2	–	–	0.1	tr	–	+	0.03
γ-Terpinene	–	0.51	0.26	0.10–0.11	0.4	0.20–0.50	0.27–0.50	0.2–0.6	0.01	0.01	0.3	0.23	0.02	0.09	0.16
Terpinolene	–	0.25	0.26	0.04–0.25	0.2[t]	–	0.17–0.35	0.1–0.2	0.23	0.26	0.1	tr	0.12	0.08	0.18
α-Thujene	–	0.12	0.08	0.05–0.07	tr	0.05–0.08	0–0.11	0–0.1	0.05	0.03	tr	0.03	0.05	–	–
Sesquiterpene															
α-Bergamotene*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

continued

TABLE 5.4 (continued)
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

	14	15a	15b	16	17,18	19	20	21	22a	22b	23	24	25	26a	26b
cis-α-Bergamotene	–	–	–	0.10–0.17	–	–	–	–	–	–	–	–	–	0.08	0.03
trans-α-Bergamotene	–	0.06	–	–	–	0.31–0.58	–	0–tr	0.40	0.93	tr	–	1.02	0.48	0.31
Bicyclogermacrene	–	0.19	0.15	–	–	0.26–0.92	–	–	0.46	0.24	–	–	1.30	0.10	0.01
Bisabolene*	–	–	–	–	–	–	–	0–0.01[1]	0.53[1]	1.53[1]	–	–	–	–	–
β-Bisabolene	–	0.08	–	0.19–0.32	tr	0.44–0.83	–	0–tr	–	–	0.1	–	–	0.08	0.54
δ-Cadinene	–	–	–	0.02–0.06	tr	0.04–0.08	–	–	–	–	tr	–	–	0.08	0.06
Caryophyllene*	–	–	–	–	–	–	0.42–1.10	–	–	–	–	–	–	–	–
β-Caryophyllene	–	0.65	0.35	0.67–1.10	0.6	2.49–4.09	–	0.1–0.4	5.32	6.19	0.5	0.10	4.35	2.14	1.41
β-Elemene	–	0.11	0.17	tr–0.03	tr	–	–	–	–	–	tr	–	–	–	–
(*E*,*E*)-α-Farnesene	–	0.08	–	0.05–0.06	–	0.03–0.07	–	0–0.1	–	–	tr	–	–	0.02	0.04
(*Z*)-β-Farnesene	–	–	–	0.03–0.06	–	0.03–0.06	–	–	–	–	tr	–	–	–	–
Germacrene D	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	0.05
α-Humulene	–	0.11	0.10	0.08–0.14	tr	0.22–0.42	–	0–tr	0.40	0.46	0.1	–	0.34	0.24	0.16
β-Selinene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aldehydes															
Aliphatic															
(*E*)-2-Butenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Decanal	–	–	–	0.10–0.15	0.3	0.02–0.05	0.06–0.17	0–0.1	0.02	0.06	tr	0.15	0.05	–	–
2,6-Dimethylhept-5-en-1-al	–	–	–	–	–	–	–	–	–	–	–	tr	–	–	–
Dodecanal	–	–	–	tr	0.2	–	–	–	–	–	–	–	–	0.11	+
Hexanal	–	–	–	–	–	tr–0.08	–	–	0.21	–	–	–	–	+	0.01
(*E*)-2-Hexenal	–	–	–	0–0.08	–	0.09–1.04	–	–	0.36	–	–	–	–	0.08	0.10
Nonanal	–	–	–	0.17–0.25	0.8	0.01–0.25	0.18–0.43	0–0.4	0.02	–	0.1	1.1	0.17	0.13	0.14
Octanal	–	–	–	–	–	–	–	tr–0.1	–	–	tr	0.62	–	0.03	0.02
Undecanal	–	–	–	0.10–0.13	–	0.02–0.11	0.06–0.37	–	–	–	–	–	–	0.10	0.06

Monoterpene															
Citronellal	1.7	–	–	1.51–1.92	2.8	0.30–1.80	1.05–2.58	0.5–1.8	2.51	6.32	0.3	3.85	0.90	0.52	1.02
Geranial	16.4	9.36	9.49	15.88–20.00	14.7	10.45–13.54	14.93–20.17	14.3–22.6	19.24	16.93	14.1	17.31	26.18	25.52	22.80
Neral	10.9	2.71	4.60	12.22–15.56	9.6	4.62–9.96	16.23–18.77	10.4–16.1	12.24	7.85	6.2	14.31	16.54	21.84	17.55
Ketones															
Aliphatic															
6-Methyl-5-hepten-2-one	–	2.76	2.93	0.35–1.01	tr	–	–	0.7–3.2	0.01	–	0.3	2.37	–	0.39	0.55
Monoterpene															
Camphor	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	0.02
Alcohols															
Aliphatic															
Ethanol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.01
Hexanol	–	–	–	–	–	0.02–0.16	–	–	–	–	–	–	–	0.01	0.03
(*E*)-2-Hexenol	–	–	–	–	–	0.02–0.19	–	–	–	–	–	–	–	0.04	0.06
(*Z*)-3-Hexenol	–	–	–	0–0.02	–	0.07–0.55	–	–	–	–	–	–	–	0.11	0.07
Nonanol	–	–	–	–	tr	–	–	–	–	–	–	0.12	–	–	–
Octanol	–	–	–	tr–0.03	–	–	–	–	–	–	–	0.17	–	–	–
Monoterpene															
Borneol	–	0.39	0.28	–	–	–	–	–	–	–	–	–	–	–	–
Carvacrol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	0.06
trans-Carveol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	0.02
Citronellol	1.0	–	–	0.33–1.35	0.1	–	–	0.3–1.0	0.18	1.46	–	1.39	0.12	0.82	1.17
p-Cymen-8-ol	–	–	0.06	–	–	–	–	–	–	–	–	–	–	tr	–
Geraniol	2.4	1.82	3.43	1.34–3.76	0.8	0.77–5.97[k]	–	0.8–2.4	1.61	3.42	1.8	1.39	0.85	6.28	6.80
Isopulegol	–	0.65	0.61	0.05–0.13	–	tr–0.05	–	–	–	–	–	–	–	–	–
Linalol	1.9	1.81	1.88	0.94–1.75	2.8	1.25–1.68	1.17–3.03	1.4–2.1	0.88	0.70	0.7	4.04	1.05	1.13	1.32

continued

TABLE 5.4 (continued)
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

	14	15a	15b	16	17,18	19	20	21	22a	22b	23	24	25	26a	26b
cis-p-Menth-2-en-1-ol	–	–	–	–	–	–	–	0–0.1	–	–	tr	–	–	0.02	0.01
Nerol	3.4	7.85	7.79	2.09–6.38	3.1	1.41–13.29	–	1.2–3.4	1.62	2.16	3.6	3.57	0.65	4.29	4.97
Sabinene hydrate*	–	0.08	–	–	–	–	–	–	–	–	–	0.10	–	–	–
cis-Sabinene hydrate	–	–	–	0.05–0.07	–	–	–	–	–	–	tr	–	–	0.01	+
trans-Sabinene hydrate	–	–	–	–	–	–	–	tr–0.1	0.08	0.10	–	–	0.12	0.06	0.04
Terpinen-4-ol	0.7	0.84	1.06	0.16–0.23	1.0	0.77–1.25	0.61–0.99	0.2–1.0	–	–	0.2	0.41	–	0.15	0.13
α-Terpineol	0.7	1.29	0.77	0.35–0.70	1.5	0.44–0.94	0.76–1.49	0.4–1.8	0.20	0.11	0.7	1.47	0.37	0.78	0.59
β-Terpineol	–	0.06*	–	–	–	–	–	–	–	–	–	–	–	–	–
Thymol	–	0.06	–	0–tr	1.3	–	–	–	–	–	–	–	–	0.01	0.13
Sesquiterpene															
Farnesol*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nerolidol*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(*E*)-Nerolidol	–	–	–	tr–0.05	–	0.01–0.04	–	0–tr	0.05	0.19	tr	–	0.32	0.03	0.03
Spathulenol	–	0.20	0.12	0.06–0.20	tr	–	–	–	–	–	tr	–	–	0.15	0.06
Esters															
Aliphatic															
Octyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Monoterpene															
Citronellyl acetate	–	–	–	0.17–0.41	0.2	0.04–0.30	0.20–0.73	0–0.2	0.19	3.36	0.1	0.14	–	0.20	0.21
Geranyl acetate	2.0	1.02	1.14	2.79–4.53	2.3	0.47–1.30	3.38–5.11	0.8–3.2	2.49	7.94	2.3	1.40	1.51	1.68	1.78
Geranyl formate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Geranyl propanate	–	–	–	–	–	–	0.09–0.42	–	–	–	tr	–	–	0.07	0.06
Linalyl acetate	–	–	–	–	–	0.77–5.97[k]	–	–	–	–	0.3	–	–	–	–
Methyl geranate	–	–	–	0.06–0.24	–	–	–	–	–	0.53	–	–	–	–	–

Neryl acetate	3.0	–	–	5.62–6.85	7.3	0.28–2.28	4.83–7.60	0.7–5.1	2.79	–	3.1	3.36	1.06	0.45	0.33
Neryl propanoate	–	–	–	–	–	–	0.09–0.17	–	–	–	–	–	–	0.02	0.01
α-Terpinyl acetate	–	0.79	1.22	–	–	0.01–0.26	–	–	–	–	–	–	–	–	–
Ethers and oxides															
1,8-Cineole	0.9	–	–	0.26–1.59	1.2	–	–	0–5.2[n]	0.37	0.10	1.1	6.84	0.31	1.54	1.23
cis-Limonene oxide	–	tr	0.09	–	–	–	–	0–0.1	0.09	0.07	tr	0.11	–	–	–
trans-Limonene oxide	–	tr	–	–	–	–	–	0–tr	0.06	0.07	–	0.31	–	0.03	0.02
cis-Linalol oxide	–	–	–	0–0.05	–	–	–	–	–	–	tr	–	–	0.02	0.01
β-Pinene oxide	–	–	–	–	0.3	0.31–0.46	–	–	–	–	–	–	–	–	–
Sesquiterpene															
Caryophyllene oxide*	–	–	–	0.14–0.24	tr	–	0–0.14	0–0.1	–	–	tr	–	–	0.40	0.06
trans-Caryophyllene oxide	–	–	–	–	–	–	–	–	0.19	–	–	–	0.31	–	–
Others															
Geranic acid	–	–	–	–	–	–	–	–	–	–	–	–	0.43	0.46	0.35
Methyl *N*-methyl anthranilate	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
Toluene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; + identified but not quantitatively determined; [a] myrcene + α-phellandrene; [b] α-phellandrene + α-terpinene; [c] α-pinene + α-thujene; [d] β-pinene + sabinene; [e] octyl acetate + *cis*-limonene oxide; [f] β-elemene + terpinen-4-ol; [g] *trans*-β-terpineol; [h] α-humulene + α-terpineol + citronellyl acetate; [i] neral + neryl acetate; [j] myrcene + β-pinene; [k] geraniol + linalyl acetate; [l] α-bisabolene*; [m] β-phellandrene is present only in the cv. Santa Teresa; [n] 1,8-cineole is absent only in cv. Santa Teresa, in the other cvs. the lowest value is 0.8%.

Appendix to Table 5.4

1. Kamiyama (1967). Japan; one sample; extraction with methylene chloride, steam distillation of the extract and further extraction with methylene chloride; TLC; IR; GC/FID on packed columns of Carbowax 20M, Reoplex 400, Apiezon M, Succinate polyester and β,β'-oxydipropionitrile; relative percentage of peak areas.

continued

TABLE 5.4 (continued)
Percentage Composition of Laboratory-Extracted Lemon Petitgrain Oils

2. Kamiyama (1968). Japan; four samples from leaves collected from June 1966 to January 1967; extraction as in above reference; GC/FID on packed column of Carbowax 20 M; relative percentage of peak areas.
3. Scora et al. (1969) (from Lawrence, 1993). USA; samples extracted from the cvs.: Eureka, Lisbon, San Fernando. Scora et al. also found neryl formate (0.55%–2.39%).
4. Cheng and Lee (1981) (from Lawrence, 1993). Taiwan; Prefractionation techniques; GC; Cheng and Lee also found thymol methyl ether (0.03%).
5. Melendreras et al.(1984) (from Lawrence, 1993). Spain; samples extracted from the cvs.: Verna, Fino, Eureka, Villafranca, Lisbon.
6. Baaliouamer et al. (1985). Algeria; one sample extracted by hydrodistillation from the cv. Eureka; preparative GC on packed column of FFAP; analytical GC/FID and GC/MS on capillary column (50 m × 0.2 mm) coated with FFAP; relative percentage of peak areas. Baaliouamer et al. also found methylcyclopentane (0.01%), δ-4-carene (0.01%), cyclofenchene (0.33%), tricyclene (0.01%), bicycloelemene (0.62%), α-farnesene* (0.03%), γ-gurjunene (0.03%), perilla aldehyde (0.01%), 2,3-dihydro farnesal (0.01%), epicamphor (0.03%), *p*-menth-3-en-1-one (0.01%), pulegone (0.44%), 2-butyloctanol (0.04%), isogeraniol (0.03%), cedrenol (0.04%), epiglobulol (0.03%), (Z)-3-hexenyl acetate (0.01%), farnesyl acetate* (0.03%), methyl salicilate (0.01%), phytol (0.22%), *p*-tolyl aldehyde (0.05%), 3-tert-butylphenol (0.05%), 3-(4-methylpent-3-enyl) furan (0.01%), and trace amounts of cedrol, citronellyl propanate, 2,2,3-trimethyl butane, (tetrahydro-2H-pyran-2-yl) methanol.
7. Adeishvili and Karebava (1987). Ex URSS; two samples extracted by distillation from the cvs.: (a) Georgian and (b) Monachello; GC/FID on capillary column (50 m × 0.5 mm) coated with OV-101; relative percentage of peak areas. Adeishvili and Karebava also found in sample (a) and (b) respectively dibutyl phthalate (0.08%, 0.09%, probably a contaminant), eneicosane (0.20%, 0.19%), and trace amounts of furfural and methyl anthranilate.
8. Crescimanno et al. (1988). Sicily, Italy; oils extracted by petroleum ether from the cvs.: Femminello, San Giuseppe Larena, Femminello Favazzina, Monachello non reflowering, Eureka Frost; four samples from November to September for each cultivar; GC on packed column of WEAS; relative percentage of peak areas. The authors observed the highest citral content in the winter samples and in Eureka Frost and Monachello cvs.
9. Wen et al. (1989). China; one sample of steam distilled lemon leaf oil; GC/FID and GC/MS on capillary columns coated with OV-101 and PEG-20M; LRI on OV-101 are reported. Wen et al. also found carvone (2.02%), *trans*-linalol oxide (0.17%), and trace amount of 1,4-cineole.
10. Germanà et al. (1990). Sicily, Italy; one sample extracted by hydrodistillation; GC on packed column of WEAS; relative percentage of peak areas. Germanà et al. also found benzaldehyde (0.50%).
11. Kumar et al. (1992). India; one sample of hydrodistilled Pant lemon-1 leaf oil; GC/FID and GC/MS on capillary column (85 m × 0.5 mm) coated with FFAP; relative percentage of peak areas. Kumar et al. also found linalol oxide* (1.80%).
12. Ayedoun et al. (1996) Benin; one sample extracted by steam distillation; GC/FID and GC/MS on capillary columns coated with HP-1 and DB-5; relative percentage of peak areas.
13. Haggag et al. (1998) Egypt; one sample steam distilled; TLC, GC; relative percentage of peak areas. Haggag et al. also found eugenol (0.21%).
14. de Rocca Serra et al. (1998). Corsica, France; one sample hydrodistilled from the cv. Eureka; GC on polar and apolar capillary columns; ^{13}C-NMR; relative percentage of peak areas.
15. Zollo Amvam et al. (1998) Yaoundè, Cameroon; one sample each hydrodistilled from the cvs.: (a) Lisbon, (b) Eureka; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 and Carbowax 20M; GC/MS on capillary column (25 m × 0.22 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found α-copaene (0.06%), δ-elemene (0.06%), menthone (0.27%), terpinen-1-ol (0.12%), γ-terpineol (0.06%), terpinyl acetate* (0.79%), α-methyl naftalene (0.08%) in sample (a) and δ-elemene (0.09%), menthone (0.16%), terpinen-1-ol (0.14%), γ-terpineol (0.05%) in sample (b).
16. Huang et al. (2000). China; steam distillation; one sample each from the cvs.: Eureka, Lisbon, Verna, Botswana; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas. Huang et al. also found germacrene B (0.07%–0.18%).

17,18. Alonzo et al. (2000a,b). Sicily, Italy; steam distillation; one sample extracted from the cv. Femminello; GC/FID and GC/MS on capillary column (30 m × 0.2 mm × 0.25 μm) coated with HP-5; MS libraries: NIST and Flavour and Fragrance Natural and Synthetic Compounds (FFNSC); LRI on HP-5 are reported; relative percentage of peak areas. Alonzo et al. also found chrysanthenol (0.3%) and trace amounts of *o*-cymene, *m*-acetyl anisole.

19. Ahmed et al. (2001). California, USA; steam distillation; range of composition of samples extracted from April 1993 to April 1994 from the Lupa strain of the cv. Lisbon; GC/FID and GC/MS on capillary column (30 m × 0.32 mm × 1.0 μm) coated with DB-1; Adams and home made MS libraries; relative percentage of peak areas; Ahmed et al. also found neo-iso(iso) pulegol (tr–0.04%), (*Z*,*E*)-farnesyl acetate (tr–0.07%).

20. Vekiari et al. (2002). Creta, Greece; hydrodistillation; range of composition of samples extracted from December 1996 to April 1998 from the cv. Zambetakis; GC/FID on capillary column (30 m × 0.32 mm × 0.25 μm) coated with SE-52; GC/MS on capillary column (25 m × 0.2 mm × 0.2 μm) coated with HP-1; the results of this paper are expressed as milligrams per Kilogram of essential oil, the percentage composition was calculated based on the original data. Vekiari et al. also found bergamotene* (0%–0.15%), farnesene* (0%–0.16%), humulene* (0.09%–0.25%), (*Z*)-isocitral (0.31%–0.69%), (*E*)-isocitral (0.35%–0.56%), dodecanal (0%–0.10%).

21. Lota et al. (2002). Corsica, France; hydrodistillation; one sample each extracted from the cvs.: Eureka, Fino, Limoneira, Berna, Santa Teresa, Lisbon, Corpaci, Lapithou, Menton, Panachè; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found allo-ocimene (0–tr), (E)-β-farnesene (0%–0.1%).

22. Gancel et al. (2003). Corsica, France; (a) one sample from the cv. Eureka and (b) one sample from the cv. LAC (Lemon Apireno Cantinella) extracted with a mixture of pentane and diethyl ether (1:1); GC/FID on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax and DB-1; LRI on DB-Wax and DB-1 are reported; the results of this paper are expressed as micrograms per gram of dry leaves, the percentage composition was calculated based on the original data; Gancel et al. also found α-bisabolene* (0.53%), (*Z*)-2-pentenol (0.05%), *cis*-caryophyllene oxide (0.06%) in sample (a) and α-bisabolene* (1.56%), epoxyterpinolene (0.04%), acetic acid (0.03%) in sample (b). The composition of sample (b) is also reported by Gancel et al. (2005).

23. Kirbaslar and Kirbaslar (2004). Antalya, Turkey; one sample steam distilled; GC/FID and GC/MS on capillary column (60 m × 0.25 mm × 0.25 μm) coated with DB-5; Wiley and NIST MS libraries; relative percentage of peak areas. Kirbaslar and Kirbslar also found trace amount of isoterpinolene.

24. Smadja et al. (2005). Reunion Island, France; one sample hydrodistilled from the cv. Eureka; GC/FID and GC/MS on capillary columns coated with Supelcowax (60 m × 0.20 mm × 0.20 μm) and HP-5 (30 m × 0.20 mm × 0.25 μm); LRI on Supelcowax and HP-5 are reported; relative percentage of peak areas.

25. Fanciullino et al. (2005). Corsica, France; one sample extracted from the cv. Eureka with pentane/diethyl ether (1:1); GC/FID and GC/MS; experimental conditions and results expression are reported as in Gancel et al. (2003).

26. Fujita et al. (2008). Japan; one sample each hydrodistilled from the cvs.: (a) Lisbon, (b) Eureka; GC/FID on capillary column (30 m × 0.25 mm) coated with DB-Wax; relative percentage of peak areas. Fujita et al. also found in sample (a) and (b) respectively germacrene A (0.01%, 0.03%), β-santalene (0.03%, 0), (*E*)-photocitral (0.10%, 0.03%), 2-methyl-3-buten-2-ol (0.40%, 0.54%), undecanol (0.01%, tr), *cis*-carveol (0.01%, tr), (*E*)-isogeraniol (0.09%, 0.05%), (*Z*)-isogeraniol (0.05%, 0.12%), γ-isogeraniol (0.02%, 0.03%), *trans*-*p*-mentha-2,8-dien-1-ol (0.10%, 0.02%), *trans*-*p*-menth-2-en-1-ol (0.03%, 0.01%), myrtenol (0.04%, tr), *cis*-verbenol (0.20%, 0.22%), *trans*-verbenol (0.24%, 0.41%), α-bisabolol (0.08%; 0.06%), α-cadinol (0.12%, 0.01%), germacrene-D-4-ol (0.01%, 0.05%), γ-muurolol (0.03%, 0.02%), bornyl acetate (0.01%, 0.02%), 2,3-dihydro-1,8-cineole (0.01%, 0.01%), isocaryophyllene oxide (0.09%, 0.01%), myristic acid (0.02%, tr), palmitic acid (0.11%, 0.60%), neric acid (0.10%, 0.05%), indole (0.03%, 0), phytol (0.33%, 0.30%), vinyl guaiacol (0.02%, 0.01%), and trace amounts in one or both the analyzed samples of α-copaene, α-curcumene, heptanal, nootkatone, perilla alcohol, limonene-10-ol.

(5.12%) and high contents of (*E*)-β-ocimene (6.48%) and sabinene (32.11%). Other petitgrain oils of Ponderosa lemon, analyzed by Fujita et al. (2006), presented, on the contrary, higher values of limonene (13%–17%), very high values of neral and geranial (42%–49% in total), and low values of sabinene (0.58%–0.79%).

5.4 MANDARIN PETITGRAIN OIL (*CITRUS DELICIOSA* TEN.)

This section will consider petitgrain oils that present high content of methyl *N*-methyl anthranilate obtained from leaves of Mediterranean plants of mandarin, which botanical name is *Citrus deliciosa* Ten., although in the original papers it may be indicated by a different botanical name. These oils are also characterized by levels of γ-terpinene higher than 10%.

5.4.1 Industrial Oils

The information on industrially processed mandarin petitgrain oils is quite scant. The data found in literature are relative to the papers by Peyron (1965, 1966) and by Vernin and Vernin (1966) on the main components of this oil, one qualitative study by De Vottero et al. (1978) on oils produced in Argentina, and two quantitative studies on Italian oils: the first by Calvarano (1967) and the second, more recent and extensive, by Mondello et al. (1997a). Recently Sciarrone (2009, personal communication) also analyzed an Egyptian oil. In his first paper, Peyron (1965) identified in mandarin petitgrain oil the following components, listed in decreasing amount order: limonene, linalol, methyl *N*-methyl anthranilate, α-pinene, linalyl acetate, β-pinene, γ-terpinene. In his second paper, Peyron (1966) also identified small amounts of *p*-cymene, and considered the high content of γ-terpinene peculiar of mandarin petitgrain oil. In this paper, Peyron also mentioned the existence of mandarin petitgrain oils with methyl *N*-methyl anthranilate that ranged between 15% and 22%, differing from what previously had been determined for mandarin petitgrain oils obtained from the market, which contained 45% to 65% of methyl *N*-methyl anthranilate. Peyron explained such great differences by the long lasting periods of distillation, by the lack of homogeneity of the vegetable material used for distillation (presence of clementine and tangerine foliage that did not contain methyl *N*-methyl anthranilate), or by fraudulent preparation of reconstituted oils from different citrus products or from synthetic compounds.

Vernin and Vernin (1966) found in mandarin petitgrain oil limonene (35%), linalol (2%), terpineol* (1%), linalyl acetate (5%), methyl anthranilate (tr), and methyl *N*-methyl anthranilate (10%–15%).

De Vottero et al. (1978), in addition to all the compounds reported by the above mentioned authors, identified myrcene, citronellol, α-terpineol, and geranyl acetate.

The results of Calvarano (1967) and the more recent data of Mondello et al. (1997a) on the industrial produced mandarin petitgrain oil are summarized in Table 5.5.

The study of Calvarano (1967) was carried out on 11 samples of mandarin petitgrain oils using classical methods by fractionation, then gas chromatographic analysis of whole oil and of the fractions of a mixture of the 11 samples.

The research by Mondello et al. (1997a) was carried out on five samples produced in Sicily (Italy) by GC and GC/MS on capillary SE-52 and Carbowax 20M columns, and by an online HPLC-HRGC/MS fully automated system that allowed the fractionation of the oils into the hydrocarbons and oxygenated compounds, and the direct GC/MS analysis of the fractions.

The results obtained by Mondello et al. (1997a) on Italian mandarin petitgrain industrially produced differed from the former results obtained by Calvarano (1967), not only for the extension of the investigation but also for the quantitative values of some of the components reported in both papers, such as those relative to methyl anthranilate. The value reported by Calvarano (1967) of methyl anthranilate (1.66%) is not compatible with the composition of genuine mandarin petitgrain oil and is likely due to the inadequate analytical techniques used at that time.

TABLE 5.5
Percentage Composition of Industrial and Laboratory-Extracted Mandarin Petitgrain Oils

	Industrial			Laboratory							
	1	**2**	**3**	**4**	**5**	**6,7**	**8**	**9**	**10**	**11**	**12**
						Hydrocarbons					
Monoterpene											
Camphene	0.02	0.01–0.02	0.01	–	–	–	–	–	–	–	tr
δ-3-Carene	0.11	0.01–0.10 (0.29)[a]	tr	–	–	–	–	0–tr	–	–	tr
p-Cymene	3.43	2.96–5.19	4.81	4	–	3.10	tr	2.2–3.9	0.85	1.51	2.9
p-Cymenene	–	0.10–0.18	0.10	–	–	–	–	0.1	–	–	0.1
Limonene	8.22	7.18–12.59	8.65	6	24.24	4.91	14.52	4.6–8.9	8.11	5.77	4.9
Myrcene	0.88	0.62–0.82	0.55	1	8.03	0.31	–	0.5–0.7	0.28	0.37	0.3
(*E*)-β-Ocimene	–	0.42–0.92	0.35	–	–	–	–	0.5–0.6	0.28	0.33	0.3
(*Z*)-β-Ocimene	–	0.15–0.20	0.14	–	–	0.04	–	0.1–0.2	0.14	0.12	0.2
α-Phellandrene	0.12	0.03–0.06	0.02	12	–	0.05	12.61	–	–	–	tr
β-Phellandrene	–	0.03–0.05	–	–	–	0.03	–	tr–0.1	–	–	tr
α-Pinene	2.16	1.75–2.30	2.04	3	0.96	1.34	3.22	1.3–2.3	0.67	1.00	0.9
β-Pinene	1.86	1.90–2.45	2.01	2	0.96	0.72	3.02	1.3–2.5	0.65	1.10	1.2
Sabinene	tr	0.22–0.90(2.33)[a]	0.22	–	0.54	–	–	0.1–0.9	0.09	0.14	0.1
α-Terpinene	0.17	0.19–0.33	0.13	–	4.07	0.15	0.64	0.2–0.3	0.05	0.09	0.1
γ-Terpinene	13.74	23.94–28.48	25.14	–	–	12.60	–	16.5–28.6	7.61	12.53	12.8
Terpinolene	0.59	0.71–0.88	0.53	–	–	0.53	–	0.5–0.8	0.20	0.38	0.3
α-Thujene	0.01	0.78–1.04	0.84	–	–	–	–	0.6–2.8	0.33	0.76	0.5
Sesquiterpene											
Bicyclogermacrene	–	0.03–0.13	–	–	–	–	–	–	0.09	0.10	–
β-Caryophyllene	–	0.92–1.40	0.54	–	–	0.14	–	0.1–0.4	1.53	1.75	0.1
β-Elemene	–	tr–0.01	tr	–	–	–	–	0–0.1	–	–	–
α-Humulene	–	0.07–0.13	0.05	–	–	0.08	–	0–tr	0.12	0.13	–
α-Selinene	–	tr–0.02	0.02	–	–	–	–	–	0.05	–	–

continued

TABLE 5.5 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Mandarin Petitgrain Oils

	Industrial			Laboratory							
	1	**2**	**3**	**4**	**5**	**6,7**	**8**	**9**	**10**	**11**	**12**
					Aldehydes						
Aliphatic											
Decanal	0.01	0.01–0.02	0.01	–	–	–	–	0–tr	–	–	–
Nonanal	tr	tr–0.01	0.01	–	–	–	–	–	0.01	–	–
Octanal	0.01	tr	–	–	–	–	–	–	0.01	–	–
Monoterpene											
Citronellal	0.13	0.02–0.08	–	–	–	–	0.39	–	–	–	–
Geranial	0.01	tr–0.10	–		1.99	–	–	–	–	–	–
Neral	tr	tr–0.06	–	–	0.03	–	–	0–0.1	–	–	tr
					Alcohols						
Aliphatic											
(*Z*)-2-Pentenol	–	–	–	–	–	tr	–	–	0.03	–	–
Monoterpene											
Carvacrol	–	0.01	0.01	–	–	–	–	–	–	–	–
p-Cymen-8-ol	–	0.01–0.02	0.01	–	–	0.10	–	–	–	–	–
Citronellol	1.14	tr	–	–	–	–	–	–	–	–	–
Geraniol	0.10	tr	–	–	1.11	–	–	–	–	0.07	–
Linalol	7.92	0.27–1.10	0.10	–	4.37	0.11	–	0.2–0.9	0.08	0.24	0.3
cis-p-Menth-2-en-1-ol	–	tr	tr	–	–	–	–	0–tr	–	–	–
Nerol	0.04	0.01–0.03	–	–	1.27	–	–	0–tr	–	0.04	–
cis-Sabinene hydrate	–	0.01–0.05	0.02	–	–	–	–	–	–	–	–
trans-Sabinene hydrate	–	–	0.01	–	–	0.01	0.33	0–tr	0.03	0.07	tr
Terpinen-4-ol	0.09	0.20–0.42	0.07	–	–	1.63	–	0.1–0.2	–	–	0.1
α-Terpineol	0.01	0.16–0.26	0.07	–	–	0.24	–	0.1–0.2	0.13	0.27	0.1
Thymol	–	0.11–0.17	0.12	–	–	0.12	–	0.1–0.3	0.09	0.08	0.1

Sesquiterpene											
Spathulenol	–	tr–0.02	0.02	–	–	–	–	–	–	–	–
Esters											
Monoiterpene											
Linalyl acetate	+	0.02–0.10(0.96)[b]	0.05	–	–	–	–	–	–	0.02	–
Geranyl acetate	+	tr–0.02	–	–	0.78	–	–	–	–	–	–
Neryl acetate	+	tr–0.05	tr	–	0.17	–	–	–	–	–	–
Terpinyl acetate	+*	tr[c]	–	–	–	–	–	–	–	–	–
Ethers and oxides											
Monoterpene											
Thymol methyl ether	–	0.10–0.16	0.09	–	–	–	–	0–0.1	0.02	0.03	–
Sesquiterpene											
Caryophyllene oxide*		0.01–0.08	0.06	–	–	tr	–	–	–	–	–
Others											
Methyl anthranilate	1.66	tr–0.03	0.02	3	–	–	–	–	0.11	0.04	–
Methyl *N*-methyl anthranilate	55.19	41.61–51.93	52.57	60	50.10	65.71	63.68	48.0–66.1	78.15	72.89	74.3

Notes: tr, traces; *, correct isomer not characterized; + present, not quantified; [a] the value in parentheses indicates the percentage of δ-3-carene and of sabinene determined in the same sample obtained from foliage that probably contained 3% of sweet orange leaves; [b] the value in parentheses indicates the percentage of linalyl acetate determined in a sample obtained from foliage that probably contained 2% of bitter orange leaves; [c] α-terpinyl acetate.

Appendix to Table 5.5

1. Calvarano (1967). Calabria, Italy; 11 samples; chemical and TLC fractionation; GC/FID on stainless steel capillary column (45 m × 0.5 mm) coated with UCON LB 550X; relative percentage of peak areas. Calvarano also found β-ocimene* (0.68%) and trace amounts of heptanal and octyl acetate. The original paper reports the composition of the fractions into which the oil (a mixture of the 11 samples) has been separated and the amount of each fraction; the relative percentages have been calculated on the basis of these values; percentage of methyl *N*-methyl anthranilate was obtained by conventional laboratory procedures.

continued

TABLE 5.5 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Mandarin Petitgrain Oils

2. Mondello et al. (1997a); Dugo et al. (1996). Sicily, Italy; 5 samples; GC/FID (quantitative analyses) on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/FID and GC/MS quadrupole on capillary columns (60 m × 0.32 mm × 0.40–0.45μm) coated with SE-52 and Carbowax 20M; coupled LC-GC/MS (ITD) on a LC column (10 cm × 2 mm) packed with Spherisorb 5 μm silica and GC capillary columns as for GC/MS (quadrupole) analyses; relative percentage of peak areas. These authors also found α-fenchene (tr–0.01%), *p*-mentha-1,3,8-triene (tr–0.02%), β-bisabolene (tr–0.02%), (E,E)-α-farnesene (tr–0.03%), 6-methyl-5-hepten-2-one (tr–0.01%), *trans-p*-menth-2-en-1-ol (0.02%–0.03%), 1,8-cineole (0.01%–0.02%), (*Z*)-3 hexenylbenzoate (tr–0.02%), methyl *N*-dimethyl anthranilate (tr–0.04%), and trace amounts of tricyclene, *o*-cymene, δ-cadinene, octanol, *cis*-linalol oxide.
3. Sciarrone (2009, personal communication). Egypt; one sample; for analytical methods see point 11 of appendix to Table 5.1. Sciarrone also found thuja-2,4(10)-dien (0.01%), *trans*-limonene oxide (0.01%), ethyl *N*-dimethyl anthranilate (0.01%), 4-vinyl guaiacol (0.16%), and trace amounts of 4,8-dimethyl-1,3(*E*),7-nonatriene, γ-cadinene.
4. Karawya and Hifnawy (1979). Egypt; one sample steam distilled from the cv. Balady; TLC; GC on packed column of XE 60; GC/MS on packed column of Carbowax 20M; relative percentage of peak areas.
5. Germanà et al. (1990). Sicily, Italy; one sample hydrodistilled from the cv. Tardivo di Ciaculli; GC/FID on packed column of WEAS; relative percentage of peak areas. Germanà et al. also found caryophyllene* (0.06%), terpineol* (0.71%), farnesol* (0.40%), benzaldehyde (0.11%).

6,7. Fleisher and Fleisher (1990b,1991). Israel; one sample hydrodistilled from the cv. Balady; GC/FID and GC/MS on capillary columns; relative percentage of peak areas. Fleisher and Fleisher also found valencene (0.05%), (*Z*)-3-hexenol (0.06%), *cis*-carveol (0.04%), *trans*-carveol (0.04%), terpinen-1-ol (0.01%), (*Z*)-3-hexenyl formate (0.11%), phytol (0.14%), methyl salicilate (1.00%), ethyl esters of the acids decanoic (0.03%), heptadecanoic (0.04%), linoleic (0.74%), linolenic (2.64%), myristic (0.18%), nonanoic (0.01%), octanoic (0.01%), palmitic (1.83%), pentanoic (0.03%), stearic (0.06%), phenylacetic (0.06%), and trace amount of viridiflorene.

8. Omer et al. (1997) from Lawrence (2003). Egypt; one sample from the cv. Balady.
9. Lota et al. (2001b). Corsica, France; one sample each hydrodistilled from the following cvs.: Avana Apireno, de Chios, Willow leaf, Commune, Tardivo di Caiculli; GC/FID and GC/MS on capillary columns coated with BP-20 and BP-1; LRI on BP-20 and BP-1 are reported; relative percentage of peak areas. Lota et al. also found α-bisabolene* (0–tr).
10. Gancel et al. (2003). Corsica, France; one sample from the cv. Willow leaf extracted with pentane/diethyl ether (1:1); GC/FID on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax and DB-1; LRI on DB-Wax and DB-1 are reported; the results of this paper are expressed as micrograms per gram of dry leaves, the percentage composition was calculated based on the original data; Gancel et al. also found hexanal (0.09%), (E)-2-hexenal (0.18%).
11. Fanciullino et al. (2005). Corsica, France; one sample from the cv. Willow leaf extracted with pentane/diethyl ether (1:1); experimental conditions and results expression are reported as in Gancel et al. (2003). Fanciullino et al. also found mentha-1,8-dien-4-ol* (0.14%), *trans*-limonene oxide (0.03%).
12. Tomi et al. (2008). Corsica, France; one sample hydrodistilled from the cv. Willow leaf; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Tomi et al. also found ethyl *N*-methyl anthranilate (0.2%).

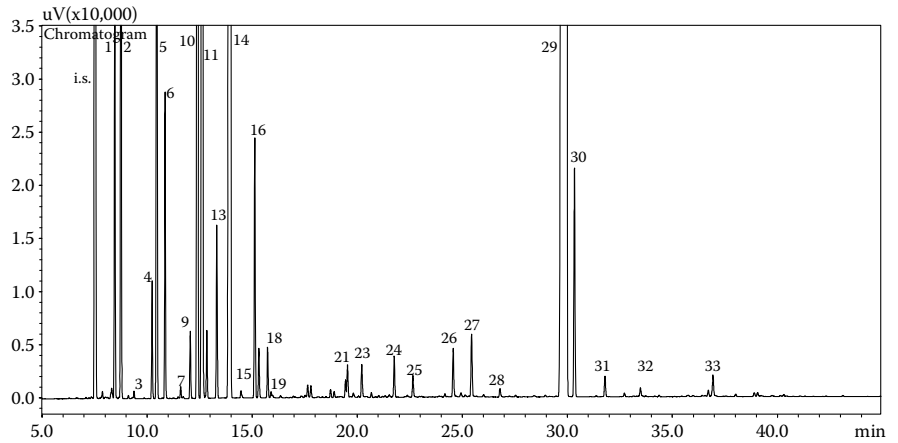

FIGURE 5.2 GC-FID chromatogram of a mandarin petitgrain oil from Egypt. Peak identification: **i.s.** internal standard; **1** α-thujene, **2** α-pinene; **3** camphene; **4** sabinene; **5** β-pinene; **6** myrcene; **7** α-phellandrene; **8** δ-3-carene; **9** *a*-terpinene; **10** *p*-cymene; **11** limonene; **12** (*Z*)-β-ocimene; **13** (*E*)-β-ocimene; **14** γ-terpinene; **15** *cis*-sabinene hydrate; **16** terpinolene; **17** *p*-cymenene; **18** linalol; **19** *trans*-sabinene hydrate; **20** nonanal; **21** terpinen-4-ol; **22** *p*-cymen-8-ol; **23** α-terpineol; **23** decanal; **24** thymol methyl ether; **25** linalyl acetate; **26** thymol; **27** 4-vinyl guaiacol; **28** methyl anthranilate; **29** methyl *N*-methyl anthranilate; **30** β-caryophyllene; **31** α-humulene; **32** α-selinene; **33** caryophyllene oxide*. (From Sciarrone, 2009. Personal communication.)

The composition of the Egyptian oil (Sciarrone, 2009, personal communication) is very close to that of Italian industrial oils (Dugo et al., 1996; Mondello et al., 1997c). The chromatogram of the Egyptian oil is reported in Figure 5.2.

5.4.2 Laboratory Oils

In Table 5.5, the information relative to the laboratory-extracted oils from mandarin foliage is also reported.

The composition of the oils analyzed by Karawya and Hifnawy (1979), by Germanà et al. (1990), and by Omer et al. (1997), although the amount of methyl *N*-methyl anthranilate is in accordance with that of genuine mandarin petitgrain oils, resulted anomalous: for the three oils, the presence of γ-terpinene, usually the most represented monoterpene hydrocarbon in such oil, was not reported. The oil analyzed by Karawya and Hifnawy (1979) presented 3% of methyl anthranilate, probably misidentified. The presence of this component at levels higher than 0.1% in mandarin petitgrain oils is considered a sign of adulteration. Probably the presence of α-phellandrene at 12%, reported by Karawya and Hifnawy (1979) and by Omer et al. (1997), is due to a misidentification. The oil analyzed by Germanà et al. (1990) presented high values of limonene and α-terpinene.

Fleisher and Fleisher (1990b, 1991) analyzed an oil with terpene compounds and methyl *N*-methyl anthranilate content compatible with a mandarin petitgrain. In this oil, as well as in tangerine and sweet orange petitgrain oils analyzed by the same authors, is also indicated the percentage of numerous ethyl esters of carboxylic acids, unusual in citrus peel and leaf oils. It is worth mentioning that Guenther (1948) indicated the presence of fatty acids (probably esters) in solvent extracts from the water obtained by the distillation of bitter orange leaf.

Other studies reported in Table 5.5 are carried out on plants grown in the fields of "Station de Researches Agronomiques" of INRA-CIRAD in San Ghjulian (Corsica, France), focused on the chemical characterization of the oils obtained by different mandarin species (Lota et al., 2001b) and of mandarin hybrids with different citrus species (Gancel et al., 2003; Fanciullino et al., 2005; Tomi et al., 2008). In addition to the data reported in Table 5.5, Lota et al. (2001b) also published

results on the composition of oils extracted from four cvs. of the species *C. deliciosa* as indicated by the authors (Peau rugueuse, Emperor, Empress, Late Emperor), which presented high content of sabinene (37.4%–48.3%) similar to sweet orange leaf oils.

More information on volatile components in mandarin leaf oil is reported by Adami et al. (2000) and by Alonzo et al. (2003). Adami et al. (2000) studied a sample of mandarin leaf oil extracted by supercritical CO_2 that showed a composition very similar to traditionally distilled oils but with very different olfactory notes. Alonzo et al. (2003) sampled and analyzed the head space of leaves of mandarin of the Ciaculli late variety using a polydimethylsiloxane (PDMS) SPME fiber. They observed high values of γ-terpinene (49.3%) and β-caryophyllene (14.2%), and a low value of methyl *N*-methyl anthranilate (7.1%), probably due to the low affinity of the fiber toward the polar components.

Manganaro and Mafrici (2000) carried out a study for the production of essential oil by distillation of microplants of *C. deliciosa* Ten. grown in a bioreactor. They observed that the content of methyl *N*-methyl anthranilate in the hexane extract of the microplant 30, 60, 90, and 135 days after their embryogenesis was respectively 0.003%, 0.047%, 0.076%, and 0.325%. The authors assumed that it was more convenient to stop the development of the plants at the stage when γ-terpinene and limonene did not accumulate but mainly produced methyl *N*-methyl anthranilate, in order to obtain an oil from which it was possible to isolate this component.

5.5 TANGERINE PETITGRAIN OIL (*CITRUS TANGERINE* HORT. EX TAN.)

This section will shortly describe some tangerine petitgrain oils laboratory extracted from foliage obtained from plants classifiable, in our opinion, as *C. tangerine* Hort. ex Tan. Although, in the original paper, these were indicated by a different botanical name and characterized by high content of linalol.

Attaway et al. (1966) identified in Murcott and Dancy tangerine leaf oils cultivated in Florida camphene^t, *p*-cymene, limonene, myrcene, β-ocimene*, α- and β-pinene, sabinene, α-terpinene, terpinolene, α-thujene^t, and linalool. In Murcott tangerine oil, β-caryophyllene and terpinen-4-ol were also identified, while in Dancy tangerine, *p*-cymenene, isoterpinolene^t, α-terpineol, thymol, and thymol methyl ether were also found.

The quantitative data on the composition of tangerine leaf oils of different geographic origins, reported in papers published after 1967, are summarized in Table 5.6.

The results of Fleisher and Fleisher (1990b, 1991) are not reported as variability ranges but singularly listed in Table 5.6 because the three samples studied presented important quantitative and qualitative difference in their composition. It must be highlighted, as done in mandarin petitgrain, that in all the oils analyzed by Fleisher and Fleisher, the presence of numerous ethyl esters of carboxylic acids is reported.

For the oils studied by Lota et al. (2001b) in Table 5.6, the ranges of three cvs. that presented similar composition are reported, while the cv. Dancy is reported separately due to quantitative differences such as the sensibly lower values of (*E*)-β-ocimene and γ-terpinene and the higher value of thymol methyl ether.

In addition to those reported in Table 5.6, Lota et al. (2001b), analyzed leaf oils extracted from cvs. Redskin and Swatow. The quantitative composition of these oils resulted significantly different from the others reported in Table 5.6, mainly for the high content of sabinene, with values of 43.1% and 37.8%, respectively, and the low amount of linalol with values of 22.1% and 25.6%, respectively. The level of sabinene is comparable with sweet orange leaf oils.

5.6 CLEMENTINE PETITGRAIN OILS (*CITRUS CLEMENTINA* HORT. EX TAN.)

The results relative to two articles on the composition of the leaf oil of clementine were shortly summarized by Lawrence (2002). In the literature, to our knowledge, only five articles are found

TABLE 5.6
Percentage Composition of Laboratory-Extracted Tangerine Petitgrain Oils

	1	2	3a	3b	3c	4	5	6a	6b
					Hydrocarbons				
Monoterpene									
Camphene	–	–	–	–	–	0.37	tr–0.02	–	–
p-Cymene	–	–	6.54	4.09	1.43	2.35	2.19–2.98	3.1	2.7–3.6
p-Cymenene	–	–	–	–	–	–	1.12–1.32	1.5	0.5–1.4
Limonene	0.8–1.9	–	1.00	0.39	0.23	8.32	2.34–2.64	4.5	2.6–4.3
Myrcene	0.3–0.6	–	0.34	0.11	0.25	0.05	0.71–0.78	0.4	0.7–0.8
β-Ocimene*	2.8–8.2	5.3	–	–	–	–	–	–	–
(*E*)-β-Ocimene	–	–	–	–	–	7.87	6.42–8.22	4.1	9.3–11.0
(*Z*)-β-Ocimene	–	–	–	0.10	2.31	–	0.28–0.40	0.3	0.5–0.6
α-Phellandrene	–	–	tr	–	–	–	0.04	–	–
β-Phellandrene	0.2–0.5	–	–	–	0.06	–	0–tr	0.1	tr–0.5
α-Pinene	1.1–2.7[a]	–	1.83	1.31	–	2.66	1.81–2.87	0.7	2.4–2.7
β-Pinene	1.4–2.6	–	1.51	1.18	0.11	0.86	2.29–2.71	1.1	2.7–2.9
Sabinene	–	4.1	–	0.41	1.41	2.81	0.27–0.29	0.3	0.2–0.3
α-Terpinene	0.1–0.3	–	0.11	0.21	0.07	1.95	0.22–0.25	0.1	0.3
γ-Terpinene	4.3–10.0	6.6	4.57	3.11	2.12	1.20	7.89–8.64	3.0	12.0–14.5
Terpinolene	0.7–1.3	–	0.34	–	–	1.49	0.59–1.27	0.7	1.1–1.2
α-Thujene	1.1–2.7[a]	–	0.78	0.65	–	1.26	0.82–1.31	0.3	1.1–1.3
Sesquiterpene									
α-Bergamotene	–	–	–	–	0.02[e]	0.11*	–	–	–
δ-Cadinene	–	–	–	–	–	0.08	0.06–0.19	–	–
β-Caryophyllene	–	–	5.44	0.24	2.00	0.24	0.07–0.22	–	0–0.4
β-Elemene	–	–	–	0.10	0.04	–	0.06–0.15	tr	–
δ-Elemene	–	–	–	–	–	0.22	0.07–0.18	–	–
(*E*,*E*)-α-Farnesene	–	–	0.71	0.50	1.00	–	0.04–0.07	–	–
Germacrene B	–	–	–	–	–	0.10	0.28–0.72	–	–
α-Humulene	–	–	0.51	0.25	0.11	0.08	0.06–0.11	–	0–tr
α-Muurolene	–	–	tr	tr	0.02	0.08	–	–	–
					Aldehydes				
Aliphatic									
Decanal	–	–	–	–	0.03	–	0–0.03	–	0–0.1
Monoterpene									
Citronellal	–	–	–	–	–	–	0.39–0.43	0.1	–
Neral	0.2–0.6[b]	–	–	–	–	6.05	–	–	–
Sesquiterpene									
α-Sinensal	–	0.9[d]	0.93	0.24	0.72	0.50	0.48–0.52	0.5	0.6–1.8
β-Sinensal	–	0.9[d]	–	0.41	2.98	–	–	–	

continued

TABLE 5.6 (continued)
Percentage Composition of Laboratory-Extracted Tangerine Petitgrain Oils

	1	2	3a	3b	3c	4	5	6a	6b
Alcohols									
Aliphatic									
(*Z*)-3-Hexenol	–	–	0.13	0.05	0.33	–	0–0.02	–	–
Monoterpene									
Citronellol	–	–	–	–	tr	–	0–0.10	–	0–tr
Geraniol	0.1–0.2	–	0.02	tr	0.03	0.16	–	tr	tr–0.2
Linalol	52–78	35.9	45.12	50.73	55.10	52.66	40.44–50.84	59.3	40.9–42.9
cis-Sabinene hydrate	–	–	–	–	–	0.08	0.02–0.04	–	–
trans-Sabinene hydrate	–	–	–	–	2.79	–	–	tr	tr
Terpinen-4-ol	–	–	–	–	–	–	0.11–0.14	0.1	0.1
α-Terpineol	0.2–0.6[b]	–	0.33	0.58	1.30	0.06	0.23–0.24	0.2	0.1–0.3
Thymol	2.6–6.9	4.9	14.43	11.70	8.21	–	6.67–7.48	8.4	12.2–15.3
Sesquiterpene									
Nerolidol	–	–	0.04*	tr*	0.04*	–	0.08–0.10[f]	–	–
Esters									
Monoterpene									
Citronellyl acetate	–	–	–	–	–	0.43	–	tr	–
Ethers and oxides									
cis-Linalol oxide	–	12.2[c]	0.06	0.44	–	–	0.02–0.06	–	–
trans-Linalol oxide	–	12.2[c]	–	0.21	–	–	–	–	–
Thymol methyl ether	1.1–16.0	–	–	4.63	–	–	7.43–9.10	8.8	0.4
Sesquiterpene									
Caryophyllene oxide*	–	–	0.31	0.33	–	–	0.05–0.19	–	–
Others									
Phytol	–	–	0.19	0.12	1.29	0.13	–	–	–

Notes: tr, traces; *, correct isomer not characterized; [a] α-pinene + α-thujene; [b] neral + α-terpineol; [c] *cis* + *trans*-linalol oxide; [d] α- + β-sinensal; [e] *trans*-α-bergamotene; [f] (*E*)-nerolidol.

Appendix to Table 5.6

1. Attaway et al. (1967). Florida, USA; Dancy tangerine; changes in composition from the first flush of growth (Marsh) and the time of fruit harvest (January); steam distillation; GC on packed column of Carbowax 20M; relative percentage of peak height. Attaway et al. also found *p*-iso propenyl toluene (0.6%–1.5%).
2. Lin and Hua (1992). China; Dahongpao tangerine; GC/FID and GC/MS on capillary column coated with SE-54; relative percentage of peak areas.
3. Fleisher and Fleisher (1990b, 1991). Israel; Yussuf Effendy (a), Dancy (b) and Maya (c) tangerine; water distillation; GC/FID and GC/MS on capillary column. Fleisher and Fleisher also found in Yussuf Effendy oil (a) γ-cadinene (0.06%), viridiflorene (0.31%), *p*-cymen-8-ol (0.05%), humulene oxide* (0.03%), ethyl esters of the following acids: decanoic

TABLE 5.6 (continued)
Percentage Composition of Laboratory-Extracted Tangerine Petitgrain Oils

(tr), heptadecanoic (0.09%), lauric (0.02%), linoleic (0.78%), linolenic (2.86%), myristic (0.11%), palmitic (2.31%), octanoic (tr), stearic (0.09%), and trace amounts of *cis*-carveol, myrtenol, terpinen-1-ol, phenylethanol; in Dancy oil (b) γ-cadinene (0.02%), viridiflorene (0.18%), *cis*-carveol (0.01%), anethole (0.02%), phenylethanol (0.02%), ethyl esters of the following acids: heptadecanoic (0.15%), lauric (tr), linoleic (0.39%), linolenic (0.68%), myristic (0.14%), palmitic (2.79%), stearic (0.25%), and trace amounts of (*E*)-β-farnesene, *p*-cymen-8-ol, myrtenol, terpinen-1-ol, sabinol, humulene oxide*; in Maya oil (c) *trans*-β-bergamotene (0.02%), bicyclogermacrene (0.06%), γ-cadinene (0.06%), α-copaene (0.02%), ethyl-2-butenoate* (0.04%), benzaldehyde (0.02%), ethyl esters of the following acids: heptadecanoic (0.07%), lauric (0.03%), linoleic (1.06%), linolenic (6.88%), myristic (0.05), palmitic (6.08%), octanoic (0.02%), stearic (0.87%), ethyl phenyl acetate (0.02%), anethole (0.01%), β-phenylethanol (0.03%), and trace amounts of (*E*)-β-farnesene, *p*-cymen-8-ol, myrtenol.

4. Blanco Tirado et al. (1995). Colombia; steam distillation; several samples extracted from leaves collected at different harvesting period of the fruits; GC/FID on capillary columns (60 m × 0.25 mm × 0.25 μm) coated with DB-1 and DB-Wax; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-1; LRI on DB-1 and DB-Wax are reported; relative percentage of peak areas. Blanco Tirado et al. also found δ-3-carene (0.04%), germacrene D (0.17%), longifolene (0.23%), β-selinene (1.01%), geranial (7.45%), nonanol (0.19%), 1,2-dihydrolinalol (0.03%), α-bisabolol (0.29%), δ-cadinol (0.06%), 1,4-cineole (0.27%), 1,8-cineole (0.70%), *cis*-limonene oxide (0.59%).
5. Huang et al. (2000). China; steam distillation; one sample each of the following cvs.: Chuanju, Dancy, Fuju; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas. Huang et al. also found γ-muurolene (0.06%–0.22%), valencene (0%–0.09%), (E)-2-hexenal (0%–0.06%), nonanal (0.03%–0.04%), carvacrol (0.04%–0.05%), elemol (0.04%–0.05%), β-eudesmol (0.04%–0.13%), spathulenol (0.04%–0.18%).
6. Lota et al. (2001b). Corsica, France; hydrodistillation; (a) one sample from the cv. Dancy; (b) one sample each of the following cvs.: Brickaville, Vohangisahy, Beauty of Glen Retreat; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; NIST and Wiley MS libraries; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found in (b) α-bisabolene* (0%–0.1%), bicyclogermacrene (0%–0.2%), *cis-p*-menth-2-en-1-ol (0%–0.2%), *trans-p*-menth-2-en-1-ol (0%–0.1%), nerol (0%–0.1%), methyl *N*-methyl anthranilate (0%–0.1%).

on this essential oil. Di Giacomo et al. (1982) analyzed three samples of Italian origin of not specified cv. characterized by the ratio of percentage of β-pinene/linalol approximately of 45/15. Ortiz Marcide et al. (1983) found that 10 samples of different cvs. of clementine leaf oil were characterized by high amount of linalol (16.7%–27.2%) associated to a high content of a not identified component (19.2%–48.8%). Lota et al. (2001b) made a reasonable hypothesis indicating that this compound could be sabinene. This is also confirmed by more recent papers were sabinene is reported at 33% to 50% in clementine leaf oil. Thus, the value reported by Di Giacomo et al. (1982) of β-pinene (45.05%) could be due to the coelution with sabinene caused by a not optimal chromatographic separation.

The more recent results on clementine leaf oil, relative to one sample of Chinese oil (Huang and Pu, 2000), to 16 oils extracted from the same number of different cvs. cultivated in Corsica (see Appendix to Table 5.7), and one oil of the same geographic origin (Tomi et al., 2008) are reported in Table 5.7. The results are in good agreement with each other and all the oils are characterized by high amounts of sabinene (33.1%–49.8%) and of linalol (19.4%–24.7%), while β-pinene is present at levels ranging from 1.7% to 2.3% confirming what was previously supposed for the results reported by Di Giacomo et al. (1982). These oils are also characterized by the presence of α-sinensal (0.58%–1.1%) and β-sinensal (0.8%–2.6%). In all the samples, terpinen-4-ol is also present at relatively high levels (2.95%–4.8%).

TABLE 5.7
Percentage Composition of Clementine Petitgrain Oils

	1	2	3
Hydrocarbons			
Monoterpene			
Camphene	0.05	tr–0.1	tr
δ-3-Carene	6.26	2.6–6.5	3.4
p-Cymene	0.28	tr–0.3	0.1
Limonene	3.63	2.5–6.9	2.9
Myrcene	2.50	2.8–3.3	3.0
(*E*)-β-Ocimene	3.46	2.5–5.6	5.2
(*Z*)-β-Ocimene	0.55	0.1–0.2	0.2
α-Phellandrene	0.14	0.3–0.7	0.4
β-Phellandrene	0.52	0.8–0.9	0.8
α-Pinene	1.53	0.7–1.6	1.5
β-Pinene	1.90	1.7–2.3	2.1
Sabinene	33.94	33.1–49.8	45.4
α-Terpinene	0.49	0.9–1.4	0.9
γ-Terpinene	1.64	1.5–2.1	1.4
Terpinolene	1.27	0.8–1.5	0.8
α-Thujene	0.54	0.3–0.4	0.4
Sesquiterpene			
Bicyclogermacrene	–	0–0.1	tr
α-Bisabolene	–	–	tr
δ-Cadinene	0.07	–	–
β-Caryophyllene	0.15	tr–0.2	0.1
(*E*,*E*)-α-Farnesene	0.03	–	–
(*E*)-β-Farnesene	–	0–0.1	–
(*Z*)-β-Farnesene	0.03	–	–
Germacrene B	0.04	–	–
α-Humulene	tr	–	tr
Aldehydes			
Aliphatic			
Decanal	0.06	0–0.1	tr
Nonanal	0.15	–	–
Monoterpene			
Citronellal	2.59	0.3–3.7	1.2
Geranial	0.42	0–0.8	–
Neral	0.30	tr–0.6	tr
Sesquiterpene			
α-Sinensal	0.58	0.3–1.1	0.9
β-Sinensal	1.49	0.8–2.6	1.8
Ketones			
Aliphatic			
6-Methyl-5-hepten-2-one	0.20	0–0.1	–

TABLE 5.7 (continued)
Percentage Composition of Clementine Petitgrain Oils

	1	2	3
Alcohols			
Monoterpene			
Carvacrol	0.02	–	–
Citronellol	1.85	0.1–0.7	0.5
Geraniol	0.68	tr–0.5	tr
Linalol	22.55	19.4–24.7	21.3
cis-p-Menth-2-en-1-ol	–	0.2–0.3	0.2
trans-p-Menth-2-en-1-ol	–	0.1–0.2	0.1
Nerol	0.60	tr–0.2	0.1
cis-Sabinene hydrate	1.02	–	–
trans-Sabinene hydrate	–	0.6–1.4	0.8
Terpinen-4-ol	2.95	3.3–4.8	3.0
α-Terpineol	0.92	0.7–1.4	0.3
Thymol	0.03	–	–
Sesquiterpene			
Elemol	0.06	–	–
β-Eudesmol	0.03	–	–
(*E*)-Nerolidol	0.14	tr–0.1	–
Esters			
Monoterpene			
Citronellyl acetate	0.05	–	–
Geranyl acetate	0.57	0.1–0.7	0.1
Methyl geranate	0.16	–	–
Neryl acetate	0.08	0–0.4	–
Ethers and oxides			
Monoterpene			
cis-Linalol oxide	0.21	–	–
Sesquiterpene			
Caryophyllene oxide*	0.04	–	–
Others			
Methyl *N*-methyl anthranilate	–	–	tr

Note: tr, traces.

Appendix to Table 5.7

1. Huang et al. (2000). China; one sample steam distilled; for analytical methods see point 13 of appendix to Table 5.2.
2. Lota et al. (2001b). Corsica, France; range of the composition of oils hydrodistilled from the cvs.: MA3, Nules, MA2, Hernandina, Tardia Villareal, Reina, Caffin, MacBean, Oroval, Monreal, Bruno, Tomatera, Commun, Marisol, Ragheb, Guillermina; for analytical methods see point 9 of appendix to Table 5.5.
3. Tomi et al. (2008). Corsica, France; one sample hydrodistilled; for analytical methods see points 12 of appendix to Table 5.5.

5.7 SWEET ORANGE PETITGRAIN OIL (*CITRUS SINENSIS* [L.] OSBECK)

5.7.1 Industrial Oils

Sweet orange petitgrain oil is of poor commercial value and therefore scantly produced. For this reason, not enough care is given to the selection of the foliage to be used for the distillation, and the cleanness of the stills, often used for the distillation of more valuable oils, is not controlled. The possible contamination by bitter orange, lemon, and mandarin leaves, are not, in fact, considered undesirable, and therefore it is quite difficult to find pure sweet orange petitgran oils (Mondello et al., 1997b).

The analyses on sweet orange petitgrain have been almost completely carried out on laboratory-extracted oils. The information on the composition of oils industrially produced are limited to the semiquantitative evaluation of major components reported by Peyron (1965) and to those relative to a more recent paper on Italian industrial sweet orange oils (Mondello et al., 1997b).

Peyron (1965) identified in sweet orange petitgrain the following components, listed in decreasing amount order: limonene, linalol, α-terpineol, β-pinene, and β-ocimene*.

The results obtained by Mondello et al. (1997b), using modern analytical techniques, GC/MS on SE-52 and Carbowax 20M columns, and HPLC-GC/MS on four samples industrially produced of Sicilian sweet orange petitgrain oils, are reported in Table 5.8.

As it can be seen from the values reported in Table 5.8, the variability ranges of some components are quite wide. The cause can be assigned to different factors, such as the distillation conditions used; the cultivar of *C. sinensis* from which originated the foliage. In Italy, numerous varieties of both blond and blood oranges are cultivated, and the poor selection of the foliage leads to the contamination by the presence of foliage of citrus species different from *C. sinensis*. The amount found by Mondello et al. (1997b) in industrial sweet orange leaf oils of methyl *N*-methyl anthranilate (1.26%–10.29%), characteristic of mandarin petitgrain oils, could be due to the poor selection of the foliage. This component was, in fact, determined only at trace levels in laboratory-extracted oils from carefully selected leaves of some sweet orange varieties grown in Italy. The composition of these oils was reported in the same paper (Mondello et al., 1997b) that generated the results for Sicilian industrial sweet orange petitgrain oils.

5.7.2 Laboratory Oils

Table 5.8 also summarizes most of the results reported in literature on the composition of sweet orange leaf oils extracted in laboratory. As reported in the table and in the appendix, they are very numerous and relative to different cultivars and geographic origins. Different oils, even if studied by the same authors, often present important qualitative and quantitative differences that render it impossible to summarize the data in variability ranges. It was therefore preferred to report the results for each single cultivars. Usually the major component is sabinene, ranging between ca. 16% and ca. 58%; only for two cvs.—Washington Navel and Portuguese (Baaliouamer et al., 1988)—sabinene was at lower values and the major components were linalol and limonene.

Additional information on sweet orange petitgrain oils can be found in literature. Moshonas and Shaw (1986) compared the oil extracted from Valencia foliage cultivated in Florida and obtained by plant treated with an abscission agent (5-Cl-3-methyl-4-nitro-1-H-pirazole) with that obtained from not treated plants. The author did not determine relevant qualitative and quantitative differences among the oils. The paper reported only qualitative information. The components identified were: δ-3-carene, *m*-cymene, *p*-cymene, limonene, myrcene, ocimene*, α-pinene, sabinene, γ-terpinene, terpinolene, α-thujene, β-caryophyllene, β-elemene, humulene*, citronellal, geranial, neral, α-sinensal, β-sinensal, 6-methyl-5-hepten-2-one, hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, citronellol, *p*-cymen-8-ol, geraniol, isopulegol, linalol, *cis*-*p*-menth-2-en-1-ol, nerol, terpinen-4-ol, α-terpineol, 4-vinyl guaiacol, citronellyl acetate, geranyl acetate, and neryl acetate.

TABLE 5.8
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

	Industrial	Laboratory									
	1a	1b	1c	1d	2	3a	3b	4	5a	5b	5c	5d
						Hydrocarbons						
Monoterpene												
Camphene	0.03–0.04	0.04	0.04	0.04		0.6	0.2	0.05–0.06	–	–	–	–
δ-3-Carene	3.46–5.91	4.45	10.28	5.51	2.0–8.0	3.2	5.2	4.66–5.23	0.76	1.66	6.47	0.63
o-Cymene	0.03–0.05	0.03	0.06	0.08	–	–	–	–	–	0.09	–	–
p-Cymene	0.59–2.89	0.76	0.59	1.68	–	0.1	0.9	0.20–0.23	3.39	3.17	1.18	2.28
p-Cymenene	–	–	–	–	–	tr	0.1	0–0.03	–	–	–	–
Limonene	5.12–8.37	2.90	3.67	4.04	2.3–6.4	4.9	2.6	2.53–5.57	14.39	9.76	5.00	16.63
Myrcene	2.66–3.85	3.49	4.40	3.72	3.9–6.1	4.9	3.3	3.76–4.57	0.36	0.40	4.18[c]	0.54
β-Ocimene*	–	–	–	–	6.2–9.3	–	–	4.73–8.88	–	–	–	–
(*E*)-β-Ocimene	4.53–9.21	6.14	9.73	4.99	–	4.3	3.2	–	–	–	5.39	–
(*Z*)-β-Ocimene	0.21–0.33	0.22	0.33	0.19	–	–	–	–	–	–	0.21	–
α-Phellandrene	0.23–0.50	0.37	0.69	0.22	–	–	–	–	–	–	4.18[c]	–
β-Phellandrene	0.78–1.06	0.65	0.70	0.74	1.1–1.8	0.4	1.3	0.71–0.91	0.01	0.01	0.01	0.06
α-Pinene	0.99–1.51	1.59	1.76	1.65	1.6–2.6[a]	2.7	3.1	2.19–2.79	0.69	0.95	2.19	0.81
β-Pinene	1.89–2.62	1.87	2.01	2.33	–	4.5	5.7	1.50–1.74	1.49	1.76	2.04	1.50
Sabinene	37.64–41.93	40.66	38.46	48.52	52.0–58.0	51.0	42.4	41.80–54.75	10.20	22.32	36.47	9.02
α-Terpinene	0.27–0.96	1.16	0.74	0.48	0.7–1.5	2.6	0.1	0.39–0.52	0.09[d]	0.08[e]	1.18[e]	0.06[e]
γ-Terpinene	2.19–2.98	2.43	1.41	1.46	1.1–2.4	3.0	2.8	0.84–1.04	0.12	0.11	2.15	0.04
Terpinolene	0.98–1.52	1.32	2.14	0.95	0.9–2.1	0.4	0.1	0.67–0.80	–	0.02	1.52	–
α-Thujene	0.21–0.39	0.34	0.34	0.33	1.6–2.6[a]	1.3	0.9	–	0.45	0.58	–	0.01
Sesquiterpene												
Bicyclogermacrene	0.01–0.24	0.05	0.09	–	–	–	–	–	–	–	–	–
β-Bisabolene	0–0.09	–	–	–	–	–	–	–	–	–	–	–

continued

TABLE 5.8 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

	Industrial	Laboratory									
	1a	1b	1c	1d	2	3a	3b	4	5a	5b	5c	5d
β-Caryophyllene	0.13–2.47	0.28	0.83	0.27	–	–	–	4.18–5.95	–	–	0.32	–
α-Copaene	tr–0.01	–	–	–	–	–	–	–	–	–	–	–
β-Cubebene	tr–0.10	–	–	–	–	–	–	–	–	–	–	–
β-Elemene	0.04–3.80	0.49	1.07	0.36	–	0.4	0.1	–	–	0.03	–	–
(*E*,*E*)-α-Farnesene	0.02–0.13	0.01	0.05	–	–	–	–	–	–	–	–	–
(*E*)-β-Farnesene	–	–	–	–	–	–	–	–	0.10	0.17	0.32[f]	0.06
(*Z*)-β-Farnesene	0.01–0.58	0.07	0.15	0.06	–	–	–	–	–	–	–	–
Germacrene B	–	–	–	–	–	–	–	–	–	–	–	–
α-Humulene	0.05–0.60	0.15	0.37	0.12	–	–	–	0.10–0.16	–	–	0.32[f]	0.06
β-Selinene	–	–	–	–	–	0.1	0.3	–	–	–	–	–
Aldehydes												
Aliphatic												
Decanal	0.02–0.07	0.02	0.01	0.02	–	tr	0.1	–	–	–	–	–
Dodecanal	–	–	–	–	–	tr	tr	–	–	–	–	–
Hexanal	tr	tr	tr	tr	–	–	–	–	–	–	–	–
(*E*)-2-Hexenal	–	–	–	–	–	tr	tr	–	–	–	–	–
Nonanal	tr–0.03	tr	0.01	0.05	–	tr	tr	–	–	–	–	–
Octanal	0.03–0.05	tr	tr	tr	–	–	–	–	–	–	–	–
Undecanal	–	–	–	–	–	–	–	–	–	–	–	–
Monoterpene												
Citronellal	0.43–4.44	0.47	0.51	0.48	0.2–3.6	1.1	2.9	1.60–2.13	1.82	1.52	0.77	1.58
Geranial	0.59–3.11	1.37	2.14	2.17	0.5–3.0	1.9	2.5	1.37–2.06	2.45	1.72	2.71	3.01
Neral	0.28–2.18	1.04	1.75	1.79	0.5–1.1[b]	1.3	2.7	0.08–0.17	1.56	1.01	1.62	1.70
Sesquiterpene												
α-Sinensal	0.04–0.32	0.27	0.46	0.08	–	–	–	–	–	–	0.67	–
β-Sinensal	0.23–1.29	1.38	1.27	1.44	–	–	–	–	–	–	1.30	0.46

Ketones												
Aliphatic												
6-Methyl-5-hepten-2-one	0.04–0.06	0.08	0.12	0.08	–	–	–	0.08–0.09	0.54	0.47	0.19	0.19
Alcohols												
Aliphatic												
(*Z*)-3-Hexenol	–	–	–	–	–	tr	tr	–	–	–	–	–
(*Z*)-2-Pentenol	–	–	–	–	–	tr	tr	–	–	–	–	–
Monoterpene												
Citronellol	0.10–0.22	0.13	0.26	0.23	–	0.2	0.4	0–0.24	0.08	1.30	0.80	1.23
p-Cymen-8-ol	tr-0.09	0.04	0.05	0.09	–	–	–	–	0.38	0.50	–	0.19
Geraniol	0.06–0.29	0.16	0.24	0.15	–	0.1	0.2	0.04–0.06	–	–	–	–
Isopulegol	–	–	–	–	–	tr	0.1	–	0.96	0.51	0.30	0.68
Linalol	4.34–10.71	15.12	6.29	6.52	1.5–16.0	8.7	14.5	3.90–8.55	11.26	12.09	3.79	15.78
cis-p-Menth-2-en-1-ol	0.04–0.11	0.25	0.16	0.19	–	–	–	–	–	–	–	–
trans-p-Menth-2-en-1-ol	0.03–0.07	0.22	0.14	0.18	–	–	–	–	–	–	–	–
Nerol	0.13–0.26	0.13	0.45	0.30	–	0.1	0.4	0.08–0.17	0.70	0.66	0.50	0.66
cis-Piperitol	0.01–0.07	0.06	0.05	0.06	–	–	–	–	0.23	–	0.44	0.48
trans-Piperitol	tr–0.05	0.16	0.07	0.09	–	–	–	–	–	–	–	–
cis-Sabinene hydrate	0.13–0.42	0.06	0.12	0.10	–	–	–	–	–	–	–	–
trans-Sabinene hydrate	–	–	–	–	–	–	–	–	–	–	–	–
Terpinen-4-ol	0.55–2.59	7.33	3.75	6.14	1.4–5.0	0.9	0.6	1.14–1.29	6.05	3.33	8.20	7.91
α-Terpineol	0.21–0.30	0.93	0.36	0.38	0.5–1.1[b]	0.2	0.6	0.17–0.30	1.98	1.09	0.80	1.35
Thymol	0.01–0.05	0.02	0.02	tr	–	–	–	–	–	–	–	–
Sesquiterpene												
Elemol	–	–	–	–	–	–	–	–	–	–	–	0.16
(*E*)-Nerolidol	0.01–0.05	0.04	0.01	0.02	–	–	–	–	–	–	–	–

continued

TABLE 5.8 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

	Industrial	Laboratory									
	1a	1b	1c	1d	2	3a	3b	4	5a	5b	5c	5d
Esters												
Monoterpene												
Citronellyl acetate	0.21–0.65	0.03	0.05	0.10	–	–	–	0.84–1.34	0.53	0.70	0.26	0.58
Geranyl acetate	0.15–0.28	0.04	0.16	0.15	–	0.1	0.1	0.23–0.53	1.52	0.32	0.52	0.70
Methyl geranate	0.05–0.07	0.04	0.04	0.10	–	–	–	–	0.08	0.20	0.10	0.35
Linalyl acetate	0.02–0.12	0.10	0.10	0.07	–	tr	0.2	0.11–0.16	0.78	0.30	0.37	0.76
Neryl acetate	0.29–0.38	0.04	0.11	0.15	–	–	–	–	0.27	0.08	0.14	0.09
α-Terpinyl acetate	–	–	–	–	–	–	–	–	–	0.12	–	–
Ethers and oxides												
Monoterpene												
1,8-Cineole	0–0.05	–	–	–	–	–	–	0.17–0.28	–	–	–	–
cis-Linalol oxide	tr–0.01	0.01	tr	tr	–	–	–	–	1.46	1.07	–	0.95
Thymol methyl ether	0–tr	–	–	–	–	–	–	0.18–0.47	–	–	–	–
Caryophyllene oxide*	0.02–0.24	0.04	0.07	0.10	–	–	–	–	0.49	0.10	–	0.70
Others												
Phytol	–	–	–	–	–	–	–	–	–	–	–	–

	Laboratory							
	6	**7a**	**7b**	**7c**	**8**	**9,10**	**11**	**12**
	Hydrocarbons							
Monoterpene								
Camphene	–	–	–	–	0.02	0.04–0.05	tr	–
δ-3-Carene	6.09–11.68	2.52	4.34	3.06	7.38	2.87–10.27	4.1	7.30
o-Cymene	–	–	–	–	–	–	tr	–
p-Cymene	0–12.31[g]	0.10	0.18	0.15	0.02	0.07–0.83	0.7	0.20
p-Cymenene	–	–	–	–	–	–	–	–
Limonene	3.71–7.38	1.70	1.25	2.96	5.95	2.62–9.48	4.6	12.13
Myrcene	1.37–3.84	2.79	5.63	3.64	3.73	3.59–4.23	5.3[r]	3.48
β-Ocimene*	–	–	–	–	–	–	–	–
(*E*)-β-Ocimene	0–6.36[h]	–	–	–	8.31	4.99–10.16	8.9	6.04
(*Z*)-β-Ocimene	–	0.18	0.06	0.50	–	0.19–0.34	tr	0.19
α-Phellandrene	0–0.84[i]	0.05	0.08	0.06	0.69	0.23–0.73	0.3	0.28
β-Phellandrene	0.41–0.85[j]	0.35	0.55	0.81	0.33	0.52–0.92	1.4	0.63
α-Pinene	1.48–3.09	1.75	0.88	1.02	0.55	1.35–1.92	1.1	1.11
β-Pinene	1.28–2.49	1.06	1.30	1.14	2.00	1.40–2.40	5.3[r]	1.07
Sabinene	16.03–29.48	18.66	32.58	15.81	47.68	30.19–52.11	40.6	23.29
α-Terpinene	–	–	–	–	0.12	0.13–0.66	1.2	–
γ-Terpinene	0–2.13[h]	7.52	7.41	10.50	0.65	0.34–1.19	2.9	0.06
Terpinolene	0–1.73[h]	1.31	0.75	1.51	1.57	0.68–1.78	0.4	0.76
α-Thujene	–	–	–	–	0.30	0.26–0.44	0.3	0.19
Sesquiterpene								
Bicyclogermacrene	–	0.06	0.08	0.06	–	–	–	0.35
β-Bisabolene	–	–	–	–	0.03	–	–	–
β-Caryophyllene	0–1.81[k]	7.87[q]	3.62[q]	6.95[q]	0.21	0.23–1.09	0.2	4.24

continued

TABLE 5.8 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

	Laboratory							
	6	**7a**	**7b**	**7c**	**8**	**9,10**	**11**	**12**
α-Copaene	–	–	–	–	–	–	–	0.07
β-Cubebene	–	–	–	–	–	–	–	0.09
β-Elemene	–	4.13	2.38	4.00	–	0.47–1.79	0.5	0.69
(*E*,*E*)-α-Farnesene	–	–	–	–	–	tr–0.08	–	–
(*E*)-β-Farnesene	–	–	–	–	–	–	–	1.46
(*Z*)-β-Farnesene	–	–	–	–	–	0.07–0.42	–	–
Germacrene B	–	–	–	–	0.12	0.04–0.09	–	–
α-Humulene	0–5.10[l]	0.11	0.98	1.19	0.06	0.14–0.41	tr	1.41
β-Selinene	–	0.02	tr	0.03	–	–	–	–
				Aldehydes				
Aliphatic								
Decanal	–	–	–	–	0.15	0.05–0.25	tr	0.06
Dodecanal	–	–	–	–	–	0.03–0.09	–	–
Hexanal	–	–	–	–	–	–	–	0.98
(*E*)-2-Hexenal	–	–	–	–	–	0–0.24	–	0.65
Nonanal	–	–	–	–	–	0.14–0.35	–	0.02
Octanal	–	–	–	–	0.04	–	–	0.02
Undecanal	–	–	–	–	–	tr–0.05	tr	–
Monoterpene								
Citronellal	–	1.82	1.32	1.65	2.83	1.34–3.81	2.7	4.76
Geranial	0.69–7.90	–	–	–	2.61	1.14–6.20	0.2	3.85
Neral	0–1.92[n]	–	–	–	1.87	0.88–4.82	0.2	2.72
Sesquiterpene								
α-Sinensal	–	0.03	0.02	0.07	0.13	0.13–0.39	0.1	0.57
β-Sinensal	–	1.22	0.43	2.35	–	0.95–1.75	1.0	3.11

Ketones								
Aliphatic								
6-Methyl-5-hepten-2-one	–	tr	tr	tr	–	0.17–0.52	–	–
Alcohols								
Aliphatic								
(*Z*)-3-Hexenol	–	1.25	0.41	1.93	–	0–0.03	–	0.09
(*Z*)-2-Pentenol	–	–	–	–	–	–	–	0.28
Monoterpene								
Citronellol	1.58–3.44	0.12	0.06	0.09	0.53	0.09–0.74	0.2	0.44
p-Cymen-8-ol	–	–	–	–	–	–	–	–
Geraniol	1.98–4.84	tr	tr	tr	0.21	0.09–0.95	tr	0.56
Linalol	9.67–17.59	8.21	5.13	20.92	4.38	5.03–12.27	15.2	6.32
Isopulegol	–	–	–	–	0.13	–	–	–
cis-*p*-Menth-2-en-1-ol	–	–	–	–	–	–	0.1	–
trans-*p*-Menth-2-en-1-ol	–	–	–	–	–	–	0.1	–
Nerol	1.79–4.92	–	–	–	–	0.08–1.26	0.1	0.28
cis-Piperitol	–	–	–	–	–	–	–	–
trans-Piperitol	–	–	–	–	–	–	–	–
cis-Sabinene hydrate	–	2.17	1.53	3.23	0.02	0.28–0.99	–	–
trans-Sabinene hydrate	0–0.63[o]	2.14	2.43	2.81	0.16	–	–	0.46
Terpinen-4-ol	0–11.43[p]	7.87[q]	3.62[q]	6.95[q]	–	0.82–2.17	5.6	–
α-Terpineol	0.62–2.24	0.13	0.09	0.18	0.10	0.10–0.65	0.1	0.30
Thymol	–	–	–	–	0.07	0.03–0.23	0.2	–
Sesquiterpene								
Elemol	–	–	–	–	–	0.03–0.05	–	–
(*E*)-Nerolidol	–	–	–	–	–	0.03–0.07	–	0.09
Esters								
Monoterpene								
Citronellyl acetate	–	–	–	–	0.06	0.19–1.07	0.2	0.61

continued

TABLE 5.8 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

	Laboratory							
	6	**7a**	**7b**	**7c**	**8**	**9,10**	**11**	**12**
Geranyl acetate	0–0.64[i]	–	–	–	0.16	0.30–2.06	tr	1.00
Methyl geranate	–	–	–	–	–	0.08–0.29	–	0.24
Linalyl acetate	0–0.26[m]	–	–	–	0.03	–	–	–
Neryl acetate	–	–	–	–	0.17	0.21–1.87	0.5	0.52
α-Terpinyl acetate	–	–	–	–	–	0–0.13	–	–
Ethers and oxides								
Monoterpene								
1,8-Cineole	–	–	–	–	0.16	–	–	0.50
cis-Linalol oxide	–	–	–	–	–	0.09–0.32	–	–
Thymol methyl ether	–	–	–	–	–	–	–	–
Caryophyllene oxide*	–	–	–	–	–	0.02–0.07	–	–
Others								
Phytol	2.04	2.04	0.91	1.07	0.22	–	–	–

Notes: tr, traces; *, correct isomer not characterized; [a] α-pinene + α thujene; [b] neral + α-terpineol; [c] myrcene + α-phellandrene; [d] α-terpinene + heptanal + 2-methylbut-2-enal; [e] α-terpinene + heptanal; [f] (*E*)-β-farnesene + α-humulene; [g] *p*-cymene is not present in cv. Hamlin, in other cvs. the minimum is 2.73%; [h] (*E*)-β-ocimene, γ-terpinene and terpinolene are not present in cvs.: Umudike and Hamlin, in other cvs. the minima of the three compounds are respectively 1.76%, 0.64%, 0.40%; [i] α-phellandrene and geranyl acetate are present only in cv. Etinam; [j] β-phellandrene is not present in cvs.: Umudike, Etinam and Hamlin, in other cvs. the minimum is 0.41%; [k] β-caryophyllene is not present in cv. Hamlin, in other cvs. the minimum is 0.92%; [l] α-humulene is not present in cvs.: Bendel and Meran, the value 5.10% was determined in cv. Agege, in other cvs. the range is 0.29%–0.71%; [m] linalyl acetate is present only in cv. Hamlin; [n] neral is not present in cvs.: Meran and Hamlin, in other cvs. the minimum is 0.38%; [o] *trans*-sabinene hydrate is present only in cv. Meran; [p] terpinen-4-ol is not present in cv. Umudike, in other cvs. the minimum is 7.20%; [q] β-caryophyllene + terpinen-4-ol; [r] myrcene + β-pinene.

Appendix to Table 5.8

1. Mondello et al. (1997b). Sicily, Italy; four samples industrially extracted (a), one sample each steam distilled from the cvs.: Valencia late (b), Biondo comune (c), Moro (d); GC/FID (quantitative analyses) on capillary column (30 m × 0.32 × 0.40–0.45 μm) coated with SE-52; GC/FID and GC/MS quadrupole on capillary columns (60 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52 and Carbowax 20M; coupled LC-GC/MS (ITD) on a LC column (10 cm × 2 mm) packed with

Spherisorb 5 μm silica and GC capillary columns as for GC/MS (quadrupole) analyses; relative percentage of peak areas. Mondello et al. also found α-fenchene (0.01%), isoterpinolene (0.09%–0.22%), methyl *N*-methyl anthranilate (1.26%–10.29%), and trace amounts of tricyclene, *cis*-α-bergamotene, α-selinene, valencene, hexanol, (*Z*)-3-hexenylbenzoate in samples (a); α-fenchene (0.01%), isoterpinolene (0.15%), hexanol (0.09%), and trace amounts of tricyclene, *cis*-α-bergamotene, α-selinene, valencene, (*Z*)-3-hexenylbenzoate, methyl *N*-methyl anthranilate in sample (b); α-fenchene (0.02%), isoterpinolene (0.36%), hexanol (0.09%), (*Z*)-3-hexenylbenzoate (0.01%), and trace amounts of tricyclene, α-selinene, valencene, methyl-*N*-methyl anthranilate in sample (c); α-fenchene (0.01%), isoterpinolene (0.14%), hexanol (0.11%), β-santalene (0.04%), and trace amounts of tricyclene, *cis*-α-bergamotene, α-selinene, valencene, (*Z*)-3-hexenylbenzoate, methyl-*N*-methyl anthranilate in sample (d).

2. Attaway et al. (1967). Florida, USA. Hamlin orange; change in composition from the first flush of growth (March) and time of fruit harvest (January); steam distillation; GC on packed column of Carbowax 20M; relative percentage of peak height.
3. Kamiyama and Amaha (1972). Japan; two samples extracted from the cvs.: Fukuhara orange (a), Washington Navel (b); solvent extraction with CH_2Cl_2, concentration of extract, steam distillation of residue and extraction with CH_2Cl_2; GC/FID on packed columns of PEG 20 M, Apiezon M, β-β′-oxydipropionitrile, UCON 50HB 280X, LAC-3R-728. Kamiyama and Amaha also found respectively in samples (a) and (b): caryophyllene* (0.4%–0.7%), β-farnesene* (0.2%–0.3%), humulene* (0.1%–tr), and trace amounts of (E)-2-hexenol.
4. Cheng and Lee (1981) (from Lawrence, 1993). Taiwan; three samples extracted from the cvs.: Snow orange and Pineapple and from an ordinary round orange; prefractionation techniques; GC; Cheng and Lee also found borneol (0.03%–0.06%).
5. Baaliouamer et al. (1988). Algeria; four samples extracted by hydrodistillation from the cvs.: Washington Navel (a), Valencia (b), Sanguine (c), Portuguese (d); GC/FID and GC/MS on capillary column (50 m × 0.2 mm) coated with FFAP; relative percentage of peak areas. Baaliouamer et al. also found in Washington Navel oil (a): *o*-mentha-1(7),5,8-triene (0.20%), cuminaldehyde (0.42%), dodecan-2-one (0.32%), tridecan-2-one (0.27%), piperitone (0.72%), octan-3-ol (1.96%), *cis*-carveol (0.32%), *trans*-carveol (0.27%), *p*-mentha-1,4-dien-7-ol (0.38%), myrtenol (0.15%), *trans*-pinocarveol (0.34%), sabinene hydrate* (0.50%), *trans*-linalol oxide (1.14%), methyl acetophenone (1.00%), *p*-isopropyl anisole (0.32%), toluene (0.11%), 2,6,6-trimethyl-2-vinyl-5-hydroxytetrahydropyran (0.65%); in Valencia oil (b): *o*-mentha-1(7),5,8-triene (0.12%), cuminaldehyde (0.60%), 2,6-dimethylhept-5-en-1-al (0.08%), myrtenal (0.50%), dodecan-2-one (0.31%), piperitone (1.04%), octan-3-ol (0.75%), *cis*-carveol (0.21%), *trans*-carveol (0.20%), *p*-mentha-1,4-dien-7-ol (0.29%), myrtenol (0.07%), sabinene hydrate* (0.61%), geranyl formate (0.31%), *trans*-linalol oxide (0.89%), perillene (0.16%), methyl acetophenone (0.09%), *p*-isopropyl anisole (0.46%), toluene (0.08%), 2,6,6-trimethyl-2-vinyl-5-hydroxytetrahydropyran (0.39%); in Sanguine oil (c): δ-4-carene (0.22%), octan-3-ol (0.17%); in Portuguese oil (d): piperitone (0.12%), octan-3-ol (2.83%), *trans*-carveol (0.02%), sabinene hydrate* (0.30%), nerolidol* (0.13%), *trans*-linalol oxide (0.62%), perillene (0.14%), *p*-isopropyl anisole (0.10%).
6. Ekundayo et al. (1990). Nigeria; six samples extracted by hydrodistillation from the cvs.: Agege I (a), Bendel (b), Meran (c), Umudike (d), Etinam (e), Hamlin (f); GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax. Ekundayo et al. also found in Agege I oil: camphor (1.44%), bornyl acetate (9.00%); in Meran and Umudike oils: octenyl acetate* respectively (0.57% and 0.56%).

continued

TABLE 5.8 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Sweet Orange Petitgrain Oils

7. Fleisher and Fleisher (1990a). Israel; three samples hydrodistilled from the cvs.: Valencia (a), Jaffa (b), Ruby (c); GC/FID and GC/MS on capillary column; Fleisher and Fleisher also found valencene (0.02%–0.04%), α-selinene (0.01%–0.02%), 3-methyl-2-butenol (0–0.02%), β-phenylethanol (tr–0.04%), anethole (0.05%–0.12%), ethyl esters of the following acids: benzoic (tr), (*E*)-2-butenoic (0.08%–0.11%), lauric (0.04%–0.05%), linoleic (0.47%–0.69%), linolenic (5.26%, 0.35%, 3.74% in samples (a), (b), (c) respectively), myristic (tr–0.05%), octanoic (tr–0.11%), palmitic (3.54%–6.77%), phenylacetic (0.08%–0.11%), stearic (0.07%–0.21%).
8. Blanco Tirado et al. (1995). Colombia; steam distillation; several samples extracted from the leaves collected at different harvesting period of the fruits; GC/FID on capillary columns (60 m × 0.25 mm × 0.25 μm) coated with DB-1 and DB-Wax; GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-1; LRI on DB-1 and DB-Wax are reported; relative percentage of peak areas. Blanco Tirado et al. also found δ-elemene (0.63%), longifolene (0.04%), nonanol (0.37%), octanol (0.27%), 1,2-dihydrolinalol (0.02%), isoborneol (0.20%), myrcenol (0.05%), methyl octanoate (0.12%), 1,4-cineole (0.48%), *cis*-limonene oxide (0.05%).

9,10. Huang and Chen (1998); Huang et al. (2000). China; range of 11 samples steam distilled from cvs.: Taoyecheng, Huangbaipitiancheng, Gailiangcheng, Jincheng, Hamlin, Washington, Ruby Blood, Valencia, Liucheng, Xuegan, Xinhuicheng; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas. These authors also found δ-cadinene (tr–0.05%), farnesal* (0.02%–0.12%), farnesol* (0.04%–0.07%), spathulenol (0%–0.10%).

11. Alonzo et al. (2000a). Italy; one sample; steam distillation; GC/FID and GC/MS on capillary column (30 m × 0.2 mm × 0.25 mm) coated with HP-5; MS libraries: NIST and Flavour and Fragrance Natural and Synthetic Compounds (FFNSC); LRI on HP-5 are reported; relative percentage of peak areas.
12. Gancel et al. (2005). Corsica, France; one sample solvent extracted with pentane/ether (1:1); GC/FID on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (30 m × 0.25mm × 0.25 μm) coated with DB-Wax and DB-1; Wiley 275.L MS library; LRI on DB-1 and DB-Wax are reported; the results of this paper are expressed as micrograms per gram of dry leaves, the percentage composition was calculated based on the original data. Gancel et al. also found germacrene A (5.80%), germacrene D (0.07%), *cis*-caryophyllene oxide (0.15%), *trans*-caryophyllene oxide (0.50%). The composition of this oil is also reported by Gancel et al. (2003).

Some papers reported an unusual composition for sweet orange leaf oil (Ogihara et al., 1990; Gurib-Fakim and Demarne, 1995; Haggag et al., 1999; Taufiq-Yap et al., 2001); none of these report the presence of sabinene. The first by Ogihara et al. (1990) was carried out on oils obtained from the foliage of diploid and tetraploid plants of three cultivars of sweet orange, Morita navel, Fukuhara, and Hamlin grown in Japan. These oils were obtained by extraction in pentane from the methanol extract of the foliage. The components identified in these oils were: camphene (19.4%–42.0%), *p*-cymene (0.3%–0.5%), limonene (3.6%–11.3%), α-phellandrene (5.5%–41.6%), γ-terpinene (0.1%–1.8%), β-bisabolene (0.6%–1.4%), caryophyllene* (1.3%–2.7%), α-elemene (0%–0.7%), β-elemene (0%–2.9%), and linalol (5.4%–8.6%). In these oils, the presence of phytol (10.8%–12.2%) and of β-amyrin (1.5%–9.9%) was also determined. On the basis of their results, these authors asserted that the absolute amount of lower terpenoids in leaves decreased with increasing ploid level and that only the amount of α-phellandrene in the monoterpene fraction increased, although those of other monoterpene content decreased. Gurib-Fakim and Demarne (1995) determined, for a sweet orange leaf oil of not specified cultivar from Mauritius, a composition extremely different from that commonly reported in other papers. In the oil, 53 components were identified, and only 2.81% of monoterpene hydrocarbons ([*E*]- and [*Z*]-β-ocimene, β-phellandrene, α- and β-pinene) were determined; a high amount of alcohols, among which the most abundant were *cis*-piperitol (26.42%) and 1-octen-3-ol (16.97%); and some esters, in particular cinnamyl formate (0.56%), citronellyl tiglate (0.37%), and geranyl tiglate (1.42%), unusual in citrus petitgrain oils. Haggag et al. (1999) also found, for a sweet orange leaf oil of not specified cultivar from Egypt, a not common qualitative and quantitative composition: δ-3-carene (2.39%), *m*-cymene (3.42%), *p*-cymene (1.45%), limonene (1.36%), myrcene (8.03%), α-pinene (1.71%), β-pinene (2.90%), γ-terpinene (4.61%), α-caryophyllene (3.93%), β-caryophyllene (4.61%), citronellal (3.42%), neral (5.47%), carvacrol (0.76%), citronellol (1.53%), eugenol (0.68%), geraniol (0.40%), linalol (21.37%), nerol (3.76%), α-terpineol (13.85%), thymol (0.17%), geranyl acetate (11.97%), neryl acetate (10.51%), and apiole (1.16%). The composition of a sweet orange leaf oil cultivated in Malaysia, analyzed by Taufiq-Yap et al. (2001), appears doubtful: *p*-mentha-1,5,8-triene (1.5%), (E)-β-ocimene (7.2%), α-terpinene (3.0%), bicyclogermacrene (2.0%), β-caryophyllene (3.0%), β-elemene (22.4%), germacrene A (3.8%), germacrene B (1.1%), α-humulene (2.8%), linalol (36.9%), 1,8-cineole (5.4%), and 4-vinyl-2-methoxyphenol (0.5%). The results relative to the composition of these oils are included only to provide complete information.

5.8 BERGAMOT PETITGRAIN OIL (*CITRUS BERGAMIA*)

Literature data on the industrial bergamot petitgrain oil composition are limited to semiquantitative results obtained by Peyron (1965) and to the results relative to oils produced in Calabria (Italy) reported by Calvarano (1968).

Peyron reported for petitgrain bergamot oil the presence of the following components, listed in decreasing amount order: linalyl acetate, linalol, limonene, α-terpineol, β-pinene, geranyl acetate with neryl acetate, and γ-terpinene.

The results of Calvarano are reported in Table 5.9. From the chromatogram reported in the paper, linalol and linalyl acetate are the major components of the oil.

In Table 5.9, information on the composition of bergamot leaf oil laboratory extracted is also reported. All these oils are characterized by high values of linalol and linalyl acetate. The only exception is given by an oil analyzed by Karawya et al. (1970), where linalol and linalyl acetate were only 1.33% and 0.83% respectively, while the major components were limonene, β-pinene, and nerol. The oil analyzed by Huang et al. (1986) is also characterized by high content of geraniol.

TABLE 5.9
Percentage Composition of Industrial and Laboratory-Extracted Bergamot Petitgrain Oils

	Industrial	Laboratory					
	1	2	3	4	5	6	7	8
Hydrocarbons								
Monoterpene								
Camphene	0.01	0.25	–	–	tr	–	tr	tr
δ-3-Carene	2.04[a]	–	–	–	0.05	–	tr	0.5
p-Cymene	1.02	–	0.31	–	tr	–	0.05	–
Limonene	2.80	10.91	0.63	1.21	1.16	1.0	1.79	1.8
Myrcene	2.04[a]	0.83	1.04	1.63	1.17	2.6	2.10	2.2
β-Ocimene*	0.18	3.25	1.33	1.54	0.32	–	–	–
(*E*)-β-Ocimene	–	–	–	–	–	2.4	0.92	1.4
(*Z*)-β-Ocimene	–	–	–	–	–	1.1	0.52	0.5
α-Phellandrene	0.04	–	1.99	–	tr	–	tr	0.3
β-Phellandrene	–	–	–	0.04	–	–	tr	0.1
α-Pinene	0.16	3.16	–	0.09	0.06	–	0.07	0.1
β-Pinene	1.11	8.24	0.37	1.00	0.81	0.9	0.54	0.5
Sabinene	0.12	–	0.05	0.15	0.17	–	0.17	0.3
α-Terpinene	0.09	–	tr	–	–	–	tr	0.1
γ-Terpinene	1.42	–	tr	–	0.07	–	tr	0.1
Terpinolene	0.42	–	0.21	0.31	0.06	0.6	0.17	0.2
α-Thujene	tr	–	–	–	tr	–	tr	tr
Sesquiterpene								
cis-α-Bergamotene	–	–	–	–	–	–	0.07	tr
β-Bisabolene	–	–	–	–	0.15	–	–	0.1
δ-Cadinene	–	–	–	–	–	–	0.12	tr
β-Caryophyllene	–	–	–	–	0.44		1.35	0.5
(E,E)-α-Farnesene	–	–	–	–	–	–	tr	tr
α-Humulene	–	–	–	0.90	0.06	–	0.17	0.2
Aldehydes								
Aliphatic								
Decanal	0.07	–	tr	–	0.01	–	tr	–
Nonanal	0.02	–	–	–	–		0.03	–
Monoterpene								
Citronellal	0.02	1.33	–	0.02	0.03	–	0.05	tr
Geranial	0.14	5.33	2.49[c]	3.15	1.24	–	0.44	2.6
Neral	0.03	1.50	tr	0.07	0.84	–	0.30	0.1
Ketones								
Monoterpene								
6-Methyl-5-hepten-2-one	–	–	–	0.03	0.06	–	tr	tr
Alcohols								
Aliphatic								
(*Z*)-3-Hexenol	–	–	1.02	–	tr	–	–	–

TABLE 5.9 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Bergamot Petitgrain Oils

	Industrial	Laboratory					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Monoterpene								
Citronellol	0.50	2.17	2.49[c]	3.27	–	–	0.07	–
Geraniol	0.09	0.92	1.76	2.77	22.51	6.8	5.58	2.1
Isopulegol	–	–	–	–	0.03	–	tr	–
Linalol	18.82	1.33	55.16	22.39	41.24	39.7	22.19	22.4
Nerol	1.74	10.16[b]	4.05	1.21	1.92	2.4	2.23	1.3
cis-Sabinene hydrate	–	–	–	–	–	–	0.02	tr
Terpinen-4-ol	0.21	–	1.60	2.53	0.11	–	0.13	1.8
α-Terpineol	5.69	0.67	5.17	4.21	7.89	12.1	9.80	6.2
Thymol	–	–	tr	–	–	–	tr	–
Sesquiterpene								
(*E*)-Nerolidol	–	–	–	–	0.20	–	0.21	0.1
Spathulenol	–	–	–	–	–	–	0.25	0.1
Esters								
Aliphatic								
Octyl acetate	+	–	–	–	0.02	–	–	–
Monoterpene								
Citronellyl acetate	+	–	–	0.42	0.14	–	0.08	0.1
Geranyl acetate	+	1.17	–	0.08	4.32	5.7	5.83	2.5
Linalyl acetate	+	0.83	22.30	51.64	11.31	19.9	28.21	49.6
Neryl acetate	+	–	–	0.27	3.00	3.3	6.87	1.3
Terpinyl acetate*	+	–	–	–	tr	–	–	–
α-Terpinyl acetate	–	–	–	–	–	–	0.14	0.1
Ethers and oxides								
Monoterpene								
1,8-Cineole	–	–	–	0.64	–	–	tr	tr
cis-Linalol oxide	–	–	–	–	tr	–	0.05	0.1
Sesquiterpene								
Caryophyllene oxide*	–	–	–	–	–	–	0.42	0.1

Notes: tr, traces; *, correct isomer not characterized; +, present, not quantified; [a] δ-3-carene + myrcene; [b] nerol + unknown; [c] geranial + citronellol.

Appendix to Table 5.9

1. Calvarano (1968). Calabria, Italy; six samples; steam distillation; chemical and TLC fractionation; GC/FID on stainlees steel capillary column (45 m × 0.5 mm) coated with UCON LB 550X; the original paper reports the composition of the fractions into which the oil (a mixture of the six samples) has been separated and the amount of each fraction; the relative percentage has been calculated on the basis of these values. Calvarano also found heptanal (0.01%), octanal (0.01%), methyl *N*-methyl anthranilate (6.80%–7.95%) and a total content of esters of 48.70%–55.40%. The last two results were obtained by conventional laboratory procedures.
2. Karawya et al. (1970). Giza, Egypt; one sample; steam distillation; column chromatography; TLC; GC on stainless steel capillary column (90 m × 0.25 mm) coated with Nujol; wt%.

continued

TABLE 5.9 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Bergamot Petitgrain Oils

3. Ortiz et al. (1978). USA; one sample; steam distillation; GC on packed column of LAC 446; relative percentage of peak areas.
4. Cheng and Lee (1981) (from Lawrence, 1993). Taiwan; prefractionation techniques; GC. Cheng and Lee also found borneol (0.02%).
5. Huang et al. (1986). China; one sample; steam distillation; GC/FID on capillary column (50 m × 0.25 mm) coated with OV-101; GC/MS on capillary column (50 m × 0.25 mm) coated with SE-54; LRI on OV-101 are reported; relative percentage of peak areas. Huang et al. also found pentadecane (0.06%), α-bergamotene* (0.06%), (*E*)-β-farnesene (0.04%), pulegone (0.06%), endo-fenchol (0.01%), sabinene hydrate* (0.07%), farnesol* (0.05%), (*Z*)-nerolidol (0.03%), decyl acetate (0.03%), and trace amounts of hexanol, *n*-butyl acetate, 1,4-cineole.
6. de Rocca Serra et al. (1998). Corsica, France; one sample hydrodistilled from the cv. Castagnaro; GC on polar and apolar capillary columns; ^{13}C-NMR; relative percentage of peak areas.
7. Huang et al. (2000). China; one sample; steam distillation; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; relative percentage of peak areas. Huang et al. also found germacrene B (0.18%), undecanal (0.02%), and trace amounts of β-elemene, octanol, methyl geranate.
8. Kirbaslar and Kirbaslar (2006). Antalya, Turkey; one sample; steam distillation; GC/FID and GC/MS on capillary columns (60 m × 0.25 mm × 0.25 μm) coated with DB-5 and Carbowax; Wiley and NIST MS libraries; relative percentage of peak areas. Kirbaslar et al. also found *trans*-α-bergamotene (0.1%), *cis*-*p*-menth-2-en-1-ol (0.1%), and trace amounts of isoterpinolene, *trans*-*p*-menth-2-en-1-ol, β-terpineol*, α-bisabolol, δ-cadinol, linalyl butyrate.

5.9 GRAPEFRUIT PETITGRAIN OIL (*CITRUS PARADISI* MACF.)

From the literature data at that time available, Peyron (1965) observed that grapefruit leaf oil contained from 65% to 73% of monoterpene hydrocarbons.

Attaway et al. (1966), during a qualitative study on different citrus leaf oils, found that in the laboratory-extracted oil obtained from Duncan and Marsh grapefruit foliage cultivated in Florida, the following components were present: camphene^{t}, δ-3-carene, *p*-cymene, limonene, myrcene, β-ocimene*, α- and β-pinene, sabinene, α-terpinene, β-caryophyllene, citronellal, geranial, neral, 6-methyl-5-hepten-2-one, linalol, and terpinen-4-ol. In the oil obtained from the Duncan variety were also present terpinolene, cadinene*^{t}, geraniol, and nerol, while in the Marsh variety were also present isoterpinolene^{t}, humulene*^{t}, α-terpineol, and neryl acetate.

The results found in literature, on quantitative analysis of grapefruit petitgrain all relative to laboratory-extracted oils, are not homogeneous enough, and therefore do not permit to define the typical composition of this oil. Some papers report sabinene as major component, present at levels up to 62% of the whole oil; in other papers, sabinene is present in small amounts or totally absent. In these cases, the main components are *p*-cymene and or citronellol, linalol, and terpinene-4-ol. The results relative to these papers are summarized in Table 5.10.

5.10 KEY LIME PETITGRAIN OIL (*CITRUS AURANTIFOLIA* [CHISTM.] SWING.)

Kumar and Banerjee (1957) determined, in a oil extracted by steam distillation from the leaves obtained from Indian trees, the presence of: limonene (20.5%), geranial + neral (36.0%), geraniol + linalol (13.2%), esters calculated as linalyl acetate (23.8%), acids calculated as acetic acid (2.0%), and citropten (2.0%). The analyses were carried out by common laboratory procedures on the fractions obtained by distillation of the oil.

From the literature available at that time, Peyron (1965) observed that Key lime petitgrain oil contained from 20% to 50% of monoterpene hydrocarbons, and from 30% to 60% of citral. Peyron (1965, 1966) also studied the composition of the petitgrain oil obtained from a citrus (*limonette*)

TABLE 5.10
Percentage Composition of Laboratory-Extracted Grapefruit Petitgrain Oils

	1	2	3	4	5	6	7a	7b	8
					Hydrocarbons				
Monoterpene									
Camphene	–	0.2	0.06	–	–	–	0.05	0.05	–
δ-3-Carene	0.1–0.2	6.1	0.12	–	–	–	tr	0.02	–
p-Cymene	–	1.4	0.05	–	21.32	–	0.21	0.09	–
Limonene	1.6–3.3	6.4	4.38	2.12	11.37	0.14	2.82	2.60	4.51
Myrcene	2.7–4.3	4.9	4.02	1.25	–	–	3.53	3.18	1.93
β-Ocimene*	8.1–13.0	–	7.18	–	–	–	–	–	–
(*E*)-β-Ocimene	–	4.6	–	–	–	–	9.23	10.47	3.93
(*Z*)-β-Ocimene	–	–	–	–	–	0.18	0.38	0.46	0.20
α-Phellandrene	–	–	–	–	–	tr	0.09	0.09	–
β-Phellandrene	0.7–2.0	2.3	0.88	–	–	0.21	0.75	0.65	0.27
α-Pinene	1.6–2.7	2.4	2.98	–	3.09	–	1.70	1.25	1.02
β-Pinene	–	3.1	4.50	–	3.17	–	3.58	3.30	1.25
Sabinene	42.0–59.0	18.5	61.91	10.22	2.49	–	50.57	50.38	22.35
α-Terpinene	0.7–1.8	10.7	0.55	0.49	–	tr	0.67	0.61	–
γ-Terpinene	1.2–3.8	12.0	0.82	–	–	–	1.68	1.20	–
Terpinolene	0.4–0.8	2.2	0.23	–	1.56	0.33	0.36	0.29	–
α-Thujene	–	4.0	–	–	–	–	0.40	0.41	0.14
Sesquiterpene									
Caryophyllene*	–	0.9	–	0.99	–	–	–	–	–
β-Caryophyllene	–	–	3.14	–	1.83	–	0.39	1.04	2.81
β-Elemene	–	tr	–	–	–	–	0.69	1.44	0.57
α-Humulene	–	–	0.28	–	1.60	–	0.22	0.53	0.76
					Aldehydes				
Aliphatic									
Decanal	–	0.1	–	–	–	–	0.20	0.23	0.02
Dodecanal	–	tr	–	–	–	–	0.05	0.07	–
(*E*)-2-Hexenal	–	0.3	–	–	–	–	–	–	0.43
Nonanal	–	tr	–	–	–	0.36	0.02	0.02	–
Monoterpene									
Citronellal	0.1–12.0	3.1	0.60	–	–	–	1.51	1.71	7.87
Geranial	0.1–3.3	0.6	0.20	5.33	1.70	0.10	0.24	0.46	0.43
Neral	0.4–1.2[a]	1.2	0.06	3.07	–	0.81	0.12	0.31	0.25
Sesquiterpene									
β-Sinensal	–	–	–	–	–	–	2.39	2.22	1.66
					Ketones				
Aliphatic									
6-Methyl-5-hepten-2-one	–	–	0.15	–	–	–	0.03	0.07	0.02

continued

TABLE 5.10 (continued)
Percentage Composition of Laboratory-Extracted Grapefruit Petitgrain Oils

	1	2	3	4	5	6	7a	7b	8
Alcohols									
Aliphatic									
(*Z*)-3-Hexenol	–	0.1	–	–	–	tr	–	–	0.04
Octanol	–	–	–	–	–	0.64	tr	tr	–
(*Z*)-2-Pentenol	–	tr	–	–	–	–	–	–	0.18
Monoterpene									
Carvacrol	–	–	–	–	–	0.22	0.06	0.06	–
Citronellol	–	1.4	–	–	3.83	8.60	0.22	0.26	0.88
Geraniol	–	0.3	0.04	3.74	1.69	2.73	0.09	0.09	0.35
Isopulegol	–	0.6	–	–	–	–	0.12	0.06	–
Linalol	5.9–24.0	4.4	3.33	26.66	12.78	22.93	8.18	8.51	3.32
Nerol	–	0.3	0.05	3.49	1.11	–	0.06	0.05	0.16
trans-Sabinene hydrate	–	–	–	–	0.85	–	–	–	0.27
Terpinen-4-ol	1.4–14.0	0.8	0.99	–	17.02	20.00	3.47	2.01	–
α-Terpineol	0.4–1.2[a]	0.3	0.12	13.56	1.61	0.30	0.24	0.20	0.10
Sesquiterpene									
Nerolidol	–	–	–	–	–	tr*	0.21[b]	0.24[b]	–
Esters									
Monoterpene									
Citronellyl acetate	–	–	0.12	–	–	4.84	0.43	0.18	1.66
Geranyl acetate	–	–	0.09	7.65	0.53	0.29	0.15	0.10	1.37
Linalyl acetate	–	–	0.08	–	0.57	–	0.09	0.09	–
Neryl acetate	–	–	–	3.92	–	–	0.28	0.11	0.61
Ethers and oxides									
Monoterpene									
cis-Linalol oxide	–	–	–	–	–	0.13	0.09	0.04	–
Sesquiterpene									
Caryophyllene oxide*	–	–	–	–	2.28	–	0.26	0.28	–

Notes: tr, traces; *, correct isomer not characterized; [a] neral + α-terpineol; [b] (*E*)-nerolidol.

Appendix to Table 5.10

1. Attaway et al. (1967). Florida, USA; Marsh grapefruit; change in composition from the first flush of growth (March) and time of fruit harvest (January); steam distillation; GC on packed column of Carbowax 20M; relative percentage of peak height.
2. Kamiyama and Amaha (1972). Japan; extraction with methylene chloride, concentration of extract, steam distillation of the residue and extraction with methylene chloride; GC on packed columns of PEG 20 M, Apiezon M, β, β'-oxydipropionitrile, UCON 50 HB, LAC-3R-728; relative percentage of peak areas. Kamiyama and Amaha also found β-farnesene* (0.7%), humulene* (0.5%), β-selinene (1.2%), and trace amounts of *p*-cymenene, hexanol, (*E*)-2-hexenol.
3. Cheng and Lee (1981) (from Lawrence, 1993). Taiwan; prefractionation techniques; GC. Cheng and Lee also found 1,8-cineole (0.36%), thymol methyl ether (0.16%).

TABLE 5.10 (continued)
Percentage Composition of Laboratory-Extracted Grapefruit Petitgrain Oils

4. Germanà et al. (1990). Sicily, Italy; Marsh grapefruit; one sample; steam distillation; GC on packed column of WEAS; relative percentage of peak areas. Germanà et al. also found farnesol* (5.63%), benzaldehyde (2.94%), methyl anthranilate (7.97%).
5. Ekundayo et al. (1991a). Ibadan, Nigeria; cv. Redblush; hydrodistillation; GC on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax; relative percentage of peak areas.
6. Gurib-Fakim and Demarne (1995). Mapou, Mauritius; steam distillation; GC/FID on capillary column (50 m × 0.2 mm × 0.32 μm) coated with HP-101; GC/MS on capillary column (50 m × 0.32 mm × 0.3 μm) coated with HP-101; LRI on HP-101 are reported; relative percentage of peak areas. Gurib-Fakim and Demarne also found β-copaene (0.62%), α-cubebene (0.30%), menthone (0.96%), verbenone (0.36%), *cis*-carveol (0.18%), isomenthol (0.10%), *trans*-piperitol (7.40%), *cis*-β-terpineol (6.98%), γ-terpineol (0.64%), bornyl acetate (1.17%), geranyl formate (0.23%), *trans*-rose oxide (0.82%), cinnamyl aldehyde (11.11%), cinnamyl formate (0.18%), citronellic acid (0.41%), and trace amounts of *trans*-β-bergamotene, citronellyl tiglate.
7. Huang et al. (2000). China; steam distillation; one sample each from the cvs.: (a) Marsh, (b) Duncan; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas. Huang et al. also found, in samples (a) and (b) respectively, *cis*-α-bergamotene (0%, 0.10%), δ-cadinene (0.04%, 0.09%), (*E*,*E*)-α-farnesene (0.09%, 0.07%), (*Z*)-β-farnesene (0.24%, 0.66%), germacrene B (0.13%, 0.33%), γ-muurolene (0%, 0.04%), *cis*-sabinene hydrate (0.45%, 0.36%), thymol (0.04%, 0.07%), elemol (0.13%, 0.15%), β-eudesmol (0%, 0.31%), spathulenol (0.25%, 0.24%).
8. Gancel et al. (2005). Corsica, France; one sample of Star Ruby grapefruit leaf oil extracted by a mixture of n-pentane and diethyl ether; GC/FID on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax and DB-1; LRI on DB-Wax and DB-1 are reported. The results of this paper are expressed as micrograms per gram of dry leaves, the percentage composition was calculated based on the original data. Gancel et al. also found bicyclogermacrene (0.45%), α-copaene (0.12%), β-cubebene (0.12%), (E)-β-farnesene (0.96%), germacrene A (2.42%), germacrene D (0.08%), hexanal (0.45%), 2-hexen-1-ol* (0.02%), *cis*-caryophyllene oxide (0.27%), *trans*-caryophyllene oxide (0.14%).

belonging to the group of lime (*C. aurantifolia*) largely diffused in Morocco. The author noticed that this oil resembled bitter orange petitgrain for its high level of linalyl acetate, the main component of the ester fraction (59% of the whole oil). In the oil, the following components were identified: camphene, cymene*, limonene, myrcene, β-ocimene*, α- and β-pinene, γ-terpinene, saturated linear aldehydes C_2, C_5, C_6, C_8–C_{12}, isopentenal, citronellal, geranial, neral, bezaldehyde, furfural, carvone, citronellol, geraniol, linalol, nerol, α-terpineol, farnesols*, nerolidol*, citronellyl acetate, citronellyl formate, geranyl acetate, geranyl formate, linalyl acetate, linalyl formate, methyl geranate, neryl acetate, neryl formate, α-terpinyl acetate, terpinyl formate, esters (not precisely indicated) of farnesol* and nerolidol*, 1,8-cineole, methyl anthranilate, methyl *N*-methyl anthranilate, the esters (not precisely indicated) of saturated linear chained carboxylic acids C_1–C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, and iso isomers C_5, C_6, C_8.

The quantitative results on Key lime petitgrain oil composition, reported in papers published from 1972 to now are summarized in Table 5.11. These are almost all relative to laboratory-extracted oils, with a wide variability range of composition. Only the oils analyzed by Dugo et al. (2010b) are industrial or semi-industrially obtained.

In most of the oils reported in Table 5.11, limonene, geranial, and neral were the major components. In the cv. Likeland oil, analyzed by Zollo Amvam et al. (1998), limonene (5.12%), geranial (0.36%), neral (0.12%) are at low values, while (*E*)-β-ocimene (8.42%) and γ-terpinene (3.24%) are at values higher than all oils. This oil is also characterized by a rich and complex sesquiterpene fraction (hydrocarbons and alcohols). Some oils, particularly those analyzed by Ekundayo et al. (1991b); Jantan et al. (1996); and Haggag et al. (1998), and that from the cv. Mexicaine analyzed by

TABLE 5.11
Percentage Composition of Industrial and Laboratory-Extracted Key Lime Petitgrain Oils

	Industrial		Laboratory											
	1a	1b	2	3	4	5	6	7	8	9a	9b	10	11	12	13
							Hydrocarbons								
Monoterpene															
Camphene	–	0.01	tr	–	tr	–	–	–	–	–	–	tr	–	–	–
δ-3-Carene	0.04	0.02	0.4	–	–	0.67	0.03	–	–	–	–	0.04	–	tr	0.03
p-Cymene	0.25	0.14	tr	–	0.68	–	–	0.28	–	0.10	1.04	2.94	–	0–tr	–
Limonene	45.22	45.29	24.7	21.6	28.93	33.76	16.41	32.07	34.1	43.09	5.12	33.41	26.0	22.1–34.1	27.01
Myrcene	0.78	0.45	1.0	0.6	0.99	0.56	0.79	–	1.0	1.31	0.46	0.85	1.0	0.6–1.0	0.93
Allo-ocimene	0.02	–	–	–	–	–	–	–	–	–	–	–	–	0–tr	–
β-Ocimene*	–	–	–	0.14	1.58	–	–	–	–	–	–	–	0.7	–	–
(*E*)-β-Ocimene	2.33	0.50	1.4	–	–	–	1.86	–	2.4	1.98	8.42	0.66	–	1.1–2.5	2.01
(*Z*)-β-Ocimene	0.45	0.23	–	–	–	–	0.41	–	0.7	–	1.54	0.17	–	0.4–0.8	0.40
α-Phellandrene	0.02	–	–	–	–	–	0.03	–	–	–	–	–	–	–	–
β-Phellandrene	–	–	0.1	–	–	1.81	–	–	–	–	–	0.20	–	0–0.3[b]	0.09
α-Pinene	0.30	0.11	0.3	–	0.20	0.61	0.04	4.11	–	0.25	0.51	0.55	0.1	0.1–1.1	0.15
β-Pinene	0.37	0.32	0.3	–	0.31	5.83	0.93	0.14	–	0.42	0.52	0.11	0.4	0.2–0.5	0.17
Sabinene	0.89	0.14	2.3	–	0.38	–	0.06	–	–	0.51	0.88	0.14	0.3	0.1–0.6	0.15
α-Terpinene	0.03	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01
γ-Terpinene	1.09	–	0.3	–	2.20	.–	–	–	–	0.08	3.24	0.02	–	tr–0.1	0.03
Terpinolene	0.10	–	0.1	–	0.05	–	0.04	–	–	0.06	0.21	tr	0.1	0–tr	0.04
α-Thujene	0.05	0.01	0.2	–	0.01	–	–	–	–	–	0.19	0.14	–	0–0.2	–
Sesquiterpene															
α-Bergamotene*	–	–	–	–	–	–	tr	–	–	–	–	–	0.1	–	–
cis-α-Bergamotene	0.02	0.04	–	–	–	–	–	–	–	–	–	0.12	–	–	–
trans-α-Bergamotene	0.28	0.25	–	–	–	–	–	–	–	0.08	0.62	–	–	0–0.1	1.06
Bicyclogermacrene	–	–	–	–	–	–	–	–	–	0.09	4.11	–	–	–	0.25
β-Bisabolene	0.58	0.48	–	–	–	–	0.14	–	–	0.18	1.90	0.16	–	–	–

β-Bournenene	0.01	0.09	–	–	–	–	–	–	–	–	–	–	–	–	0.14
β-Caryophyllene	2.72	2.63	0.7	–	–	–	5.68	–	–	–	3.40	1.09	0.4	0–0.4	6.97
δ-Cadinene	–	–	–	–	–	–	–	–	–	–	0.71	0.14	–	–	–
β-Elemene	0.81	0.67	–	–	–	–	1.59	–	–	0.25	1.77	0.23	–	0–0.2	–
δ-Elemene	0.57	0.58	–	–	–	–	0.80	–	–	0.23	1.52	–	–	–	0.03
(*E*,*E*)-α-Farnesene	0.99	0.48	–	–	–	–	–	–	–	0.23	–	0.06	–	–	2.52
(*E*)-β-Farnesene	–	–	–	–	–	–	1.82	–	–	–	0.36	–	–	–	0.22
(*Z*)-β-Farnesene	–	–	–	–	–	–	0.06	–	–	–	0.32	–	–	–	–
Germacrene B	1.33	0.31	–	–	–	–	–	–	–	0.46	5.22	0.08	–	–	3.13
Germacrene D	0.29[i]	–	–	–	–	–	–	–	–	0.16	9.65	–	–	0–0.3	1.28
α-Humulene	0.39[h]	0.02	–	–	–	–	0.77	–	–	0.14	1.23	0.20		0–01	0.76
Santalene	0.39[h]	–	–	–	–	–	–	–	–	–	0.11*	–	–	–	–
α-Selinene	0.06	0.16	–	–	–	–	–	–	–	0.05	1.22	–	–	–	0.32
β-Selinene	–	0.15	0.1	–	–	–	–	–	–	–	–	–	–	–	0.30
Aldehydes															
Aliphatic															
Decanal	0.28	0.18	0.2	–	0.81	–	0.61	–	–	–	–	0.06	0.8	0.2–0.6	0.41
Dodecanal	0.11	0.15	–	–	–	–	0.47	–	–	–		0.06	–	–	–
(*E*)-2-Hexenal	–	–	tr	–	–	–	–	–	–	–	–	0.02	–	–	0.12
Nonanal	0.04	–	tr	–	tr	–	–	–	–	–	–	0.08	–	0–0.1	0.05
Octanal	0.07	0.01	–	–	0.10	–	–	–	–	–	–	–	–	0–0.1	0.13
Undecanal	0.05	0.27	–	–	–	–	–	–	–	–	–	0.06	–	0–0.1	–
Monoterpene															
Citronellal	0.74	1.64	0.5	0.03	2.50	–	0.97	2.64	1.8	–	–	1.55	1.1	1.2–3.8	0.46
Geranial	13.96	10.75	40.0	17.4[j]	25.34	21.54	19.42	+	23.1	14.49	0.36	22.95	33.6	23.1–26.9	25.81
Neral	10.38	7.45	23.8	12.4	19.72	–	11.43	+	16.1	3.84	0.12	15.86	24.0	16.0–20.5	15.64
Sesquiterpene															
β-Sinensal	–	–	–	–	–	–	–	–	–	0.05	0.40	–	–	0–tr	–

continued

TABLE 5.11 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Key Lime Petitgrain Oils

	Industrial		Laboratory												
	1a	1b	2	3	4	5	6	7	8	9a	9b	10	11	12	13
Ketones															
Aliphatic															
6-Methyl-5-hepten-2-one	0.87	0.34	–	0.02	–	–	–	–	1.7	4.39	0.04	0.63	–	1.1–2.5	0.02
Alcohols															
Aliphatic															
Hexanol	0.04	–	tr	–	–	–	–	–	–	–	–	–	–	–	–
(*Z*)-3-Hexenol	0.06	–	tr	–	–	–	–	–	–	0.09	–	–	–	–	0.04
2-Hexenol	0.02[g]	–	tr[g]	–	–	–	–	–	–	–	–	–	–	–	0.03*
Octanol	0.03	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–
Monoterpene															
Citronellol	3.27[e]	0.59	–	0.04[a]	–	0.42	–	21.39	0.8	–	–	0.39	–	0.4–1.1	–
Geraniol	3.91[f]	1.82	0.6	–	0.25	3.95	7.53	10.38	2.4	4.76	0.19	3.45	–	1.6–3.8	0.90
Isopulegol	–	–	0.1	0.09	–	–	–	–	–	0.78	–	0.33	–	–	–
Linalol	1.20	0.61	0.8	0.4	1.24	1.93	1.07	3.97	1.2	1.34	1.43	1.26	1.0	1.1–1.5	0.46
Nerol	3.27[e]	1.12	0.2	–	1.80[t]	6.15	9.54	1.48	2.2	11.75	0.29	1.73	–	1.4–3.1	0.38
Terpinen-4-ol	0.47[d]	0.01	0.1	–	0.15	0.81	0.82	–	–	0.14	0.22	0.31	–	0–0.1	–
α-Terpineol	0.16	0.18	0.2	–	0.34	1.25	0.19	–	–	0.32	0.65	0.14	0.3	0.1–0.6	0.10
Thymol	–	–	–	–	–	–	–	0.50	–	–	–	0.09	–	–	–
Sesquiterpene															
Elemol	–	–	–	–	–	–	0.54	–	–	–	8.29	–	–	–	–
β-Eudesmol	–	–	–	–	–	–	0.34	–	–	0.11	5.16	–	–	–	–
Nerolidol*	–	–	–	–	–	–	–	–	–	–	0.72	–	0.4	–	–

(*E*)-Nerolidol	–	–	–	–	–	–	–	–	–	–	–	0.15	–	0–0.3	–
(*Z*)-Nerolidol	–	0.29	–	–	–	–	2.05	–	–	–	–	–	–	–	–
Spathulenol	–	0.58	–	–	–	–	–	–	–	0.16	0.49	0.20	–	0–tr	–
Esters															
Monoterpene															
Citronellyl acetate	0.06	0.53	–	–	–	–	–	–	–	0.07	–	0.16	–	0–0.3	0.02
Geranyl acetate	1.31	6.22	0.5	17.4[j]	5.49	0.98	6.61	+	4.3	2.12	0.27	4.75	2.1	2.2–8.3	1.33
Linalyl acetate	f	0.12	tr	–	0.55	–	–	+	–	–	–	–	–	–	–
Methyl geranate	0.01	0.17	–	–	–	–	–	–	–	–	–	0.13	–	–	–
Neryl acetate	0.45	2.11	–	6.9	0.62	–	0.72	–	1.6	–	0.04	2.14	1.1	0.6–3.1	0.28
Terpinyl acetate	–	–	–	–	0.74*	–	–	–	–	0.60*	0.22*	–	–	0–0.1[c]	–
Ethers and oxides															
Monoterpene															
1,8-Cineole	0.14	–	–	–	–	–	–	–	–	–	–	0.46	–	0.1–1.0	0.11
cis-Limonene oxide	–	0.37	–	–	–	–	–	–	–	0.11	–	–	–	0–0.1	0.02
trans-Limonene oxide	0.03	0.25	–	–	–	–	–	–	–	–	–	–	–	–	0.02
cis-Linalol oxide	–	0.01	–	–	–	–	–	–	–	–	–	10.06	–	–	–
Sesquiterpene															
Caryophyllene oxide*	0.15	2.09	–	–	–	1.03	–	–	–	–	–	0.14	–	0.1–0.4	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, present, not quantified; [a] citronellol + unknown; [b] β-phellandrene + 1,8-cineole (cvs.: Antelaise, Mexicaine, Kirk, Nouvelle Calédonie), in the cv. Ambilobe β-phellandrene is absent and 1,8-cineole is 1.0%; [c] α-terpinyl acetate; [d] (*E*)-isocitral + terpinen-4-ol; [e] citronellol + nerol; [f] geraniol + linalyl acetate; [g] (*Z*)-2-hexenol; [h] α-humulene + β-santalene; [i] germacrene D + γ-muurolene; [j] geranial + geranyl acetate.

continued

TABLE 5.11 (continued)
Percentage Composition of Industrial and Laboratory-Extracted Key Lime Petitgrain Oils

Appendix to Table 5.11

1. Dugo et al. (2010b). (a) One sample from Mexico steam distilled in a pilot plant; (b) one industrial sample from Egypt; for analythical methods see point 11 of appendix to Table 5.1. Dugo et al. also found aromadendrene (0.01%), γ-elemene (0.20%), γ-muurolene (0.29%), (*Z*)-isocitral (0.24%), *trans*-*p*-mentha-2,8-dien-1-ol (0.01%), geranyl formate (0.05%), neryl formate (0.03%), and trace amounts of γ-cadinene in sample (a), tetradecane (0.08%), 4,8-dimethyl-1,3(*E*),7-nonatriene (0.02%), aromadendrene (0.05%), γ-muurolene (0.11%), 7-epi-α-selinene (0.03%), (*E*)-isocitral (0.16%), (Z)-isocitral (0.04%), 6-methyl-3,5-heptadien-2-one (0.04%), nonanol (0.01%), *trans*-isocarveol (0.04%), isopulegol (0.04%), *cis*-*p*-mentha-1(7),8-dien-2-ol (0.06%), *trans*-*p*-mentha-2,8-dien-1-ol (0.05%), γ-terpineol (0.08%), α-bisabolol (0.09%), α-cadinol (0.09%), selin-11-en-4 α-ol (0.17%), *cis*-sesquisabinene hydrate (0.03%), octyl acetate (0.02%), geranyl formate (0.11%), neryl formate (0.05%), neryl isobutyrate (0.09%), α-pinene oxide (0.04%), rosefuran oxide (0.10%), humulene epoxide II (0.28%), bergamal (0.03%), geranyl acetone (0.34%), and trace amounts of γ-cadinene.
2. Kamiyama and Amaha (1972). Japan; one sample; extraction with methylene chloride, concentration of extract, steam distillation of the residue and extraction with methylene chloride; GC on packed column of PEG 20M, Apiezon M, β,β'-oxydipropionitrile, UCON 50HB 280X, LAC-3R-728; relative percentage of peak areas. Kamiyama and Amaha also found trace amount of *p*-cymenene.
3. Lund et al. (1982). Florida, USA; one sample; steam distillation; prefractionation by preparative GC on packed column of DEGS; GC on packed column of Carbowax; IR; GC/MS; relative percentage of peak areas. Lund et al. also found iso isopulegol (0.01%).
4. Calvarano et al. (1982). Brazil; one sample of Galego lime leaf oil; steam distillation; GC on stainless steel capillary column (45 m × 0.5 mm) of UCON LB 550X; relative percentage of peak areas.
5. Ekundayo et al. (1991b). Nigeria; one sample; water distillation; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax; relative percentage of peak areas.
6. Jantan et al. (1996). Malaysia; one sample; water distillation; GC/FID and GC/MS on capillary column (25 m × 0.2 mm) coated with SE-30; GC/FID analysis was also performed on capillary column (25 m × 0.2 mm) coated with PEG 20M; LRI on SE-30 are reported; relative percentage of peak areas. Jantan et al. also found α-guaiene (0.93%), α-eudesmol (0.28%), phytol (1.03%).
7. Haggag et al. (1998). Egypt; one sample; steam distillation; TLC; GC; relative percentage of peak areas. Haggag et al. also found terpineol* (12.63%), carvacrol (0.38%), eugenol (0.70%).
8. de Rocca Serra et al. (1998). Corsica, France; one sample hydrodistilled; GC on polar and apolar capillary columns; ^{13}C-NMR; relative percentage of peak areas.
9. Zollo Amvam et al. (1998). Yaoundè, Cameroon; one sample each hydrodistilled from the cvs.: (a) Mexicaine, (b) Likeland; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 and Carbowax 20M; GC/MS on capillary column (25 m × 0.22 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found (*Z*,*E*)-α-farnesene (0.05%), menthone (0.37%), borneol (0.56%), terpinen-1-ol (0.12%), γ-terpineol (0.33%), sabinene hydrate* (0.10%), bornyl acetate (0.05%), cubenol (0.05%) in sample (a) and bicycloelemene (0.06%), cadina-1,4-diene (0.44%), α-cadinene (1.31%), γ-cadinene (0.71%), α-copaene (0.05%), α-cubebene (0.08%), (*Z*,*E*)-α-farnesene (0.24%), β-gurjunene (0.33%), viridiflorene (1.56%), α-sinensal (0.06%), γ-terpineol (0.05%), bergamotol* (0.16%), T-cadinol (1.33%), cubenol (1.76%), γ-eudesmol (3.48%), globulol (0.89%), (*E*,*E*)-farnesol (0.03%), (*Z*,*E*)-farnesol (0.96%), (*Z*,*Z*)-farnesol (0.06%), α-humulene oxide (0.88%) in sample (b).
10. Huang et al. (2000). China; one sample steam distilled from the cv. Mexico; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17; Adams MS library; relative percentage of peak areas. Huang et al. also found *cis*-sabinene hydrate (0.02%).

11. Selvaraj et al. (2002). India; one sample hydrodistilled of Kagzi lime leaf oil; GC/FID on capillary column coated with Carbowax 20M (25 m × 0.2 mm × 0.2 μm) and HP-101 (50 m × 0.32 mm). LRI on HP-101 are reported. Selvaraj et al. also found bisabolene* (0.2%).
12. Lota et al. (2002). Corsica, France; hydrodistillation; one sample each from the cvs.: Nouvelle Calédonie, Antillaise, Ambilobe, Kirk, Mexicaine; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 × 0.25 μm) coated with BP-20; LRI on BP-1 and BP-20 are reported; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found santal-10-en-2-ol (0.1% in the cv. Ambilobe, 8.5% in the cv. Nouvelle Calédonie), manool oxide 0.7% in the cv. Mexicaine.
13. Gancel et al. (2005). Corsica, France; one sample of Mexican Key lime leaf oil extracted by a mixture of n-pentane and diethyl ether; GC/FID on capillary columns coated with DB-Wax (60 m × 0.32 mm × 0.25 μm) and DB-1 (30 m × 0.32 mm × 0.25 μm); GC/MS on capillary columns (30 m × 0.25 mm × 0.25 μm) coated with DB-Wax and DB-1; LRI on DB-Wax and DB-1 are reported; the results of this paper are expressed as micrograms per gram of dry leaves, the percentage composition was calculated based on the original data. Gancel et al. also found α-bisabolene* (0.41%), germacrene A (2.36%), germacrene C (0.96%), γ-selinene (0.08%), hexanal (0.05%), (*Z*)-2-pentenol (0.02%), *trans*-sabinene hydrate (0.02%), *cis*-caryophyllene oxide (0.08%), acetic acid (0.04%).

Zollo Amvam et al. (1998), are characterized by high values of monoterpene alcohols. In this last oil, a high value of 6-methyl-5-hepten-2-one (4.39%) is also reported.

The Egyptian industrial oil, compared to the one extracted by a pilot plant in Mexico, is mainly characterized by lower content of (*E*)-β-ocimene, by the absence of γ-terpinene, by lower amount of alcohols, and a higher content of esters (Dugo et al., 2010b).

5.11 PERSIAN LIME PETITGRAIN OIL (*CITRUS LATIFOLIA* TANAKA)

All the data found in literature are related to laboratory-extracted oils; most of these data are reported in Table 5.12. The oils reported in Table 5.12 show a quite homogeneous composition. The major components are limonene, geranial, and neral. These oils are also rich in monoterpene alcohols (mainly nerol) and esters, with the exception of that analyzed by Selvaray et al. (2002), where nerol is not present.

Overall, the characterization of Key and Persian lime petitgrain oils should require further investigation, particularly to differentiate the two oils.

TABLE 5.12
Percentage Composition of Laboratory-Extracted Persian Lime Petitgrain Oils

	1	2	3	4	5	6a	6b
				Hydrocarbons			
Monoterpene							
Camphene	tr	–	tr	–	–	–	–
δ-3-Carene	–	–	0.03	–	tr	–	0.01
p-Cymene	0.24	0.23	0.06	–	tr	0.08	0.08
Limonene	34.47	49.72	35.35	35.6	37.3–53.4	30.56	25.18
Myrcene	0.93	1.26	0.88	1.2	0.9–1.5	0.52	1.26
β-Ocimene*	0.80	–	–	1.3	–	–	–
(*Z*)-β-Ocimene	–	–	–	–	0.2–0.3	0.23	0.20
(*E*)-β-Ocimene	–	1.35	0.71	–	1.3–2.2	1.14	1.69
β-Phellandrene	–	–	0.08	–	0–1.2[a]	+	+
α-Pinene	0.23	0.29	0.19	0.2	0.2–0.3	0.14	0.33
β-Pinene	0.22	0.34	0.13	0.1	0.2–0.3	0.51	0.25
Sabinene	0.83	0.91	0.64	0.8	0.7–1.1	1.17	2.01
α-Terpinene	–	–	tr	–	tr	–	–
γ-Terpinene	1.39	0.21	0.03	0.1	0.1–0.3	0.23	0.07
Terpinolene	0.04	0.06	0.02	–	0–0.1	0.05	0.03
α-Thujene	tr	–	tr	–	tr	0.04	tr
Sesquiterpene							
trans-α-Bergamotene	–	0.06	–	–	0–tr	–	–
β-Bisabolene	–	0.12	0.57	–	–	–	0.06
β-Caryophyllene	–	–	1.34	1.0	0.1–0.3	0.04	0.12
β-Elemene	–	0.13	0.17	–	0–0.2	–	–
(*E*,*E*)-α-Farnesene	–	0.08	0.31	–	0–tr	–	–
Germacrene B	–	0.17	0.06	–	–	–	–
Germacrene D	–	0.13	–	–	0–tr	–	–
α–Humulene	–	0.07	0.16	0.1	tr	–	–

TABLE 5.12 (continued)
Percentage Composition of Laboratory-Extracted Persian Lime Petitgrain Oils

	1	2	3	4	5	6a	6b
Aldehydes							
Aliphatic							
Decanal	0.55	–	0.25	0.2	0.1–0.4	0.18	0.19
Nonanal	0.12	–	0.09	–	0–0.2	0.43	0.36
Octanal	0.08	–	–	–	tr–0.1	0.10	0.20
Undecanal	–	–	0.07	–	0–0.1	0.06	–
Monoterpene							
Citronellal	2.06	–	1.72	0.1	0.9–2.7	3.51	3.32
Geranial	19.54	13.71	18.07	24.3	11.8–22.5	18.21	17.73
Neral	14.95	2.60	13.77	21.3	8.3–16.1	15.43	16.23
Ketones							
Aliphatic							
6-Methyl-5-hepten-2-one	–	2.25	0.60	–	1.1–2.2	2.05	3.97
Alcohols							
Aliphatic							
Octanol	–	–	tr	–	–	0.06	0.12
Monoterpene							
Citronellol	–	–	0.47	–	0.3–0.7	0.69	0.78
Geraniol	0.39	2.16	0.84	–	1.3–2.5	2.16	2.20
Isopulegol	–	1.42	0.30	–	–	–	–
Linalol	1.07	1.39	1.02	1.0	0.9–1.5	2.41	3.27
Nerol	6.48[t]	11.24	1.89	–	1.1–2.4	2.76	3.57
Terpinen-4-ol	0.06	0.30	0.07	–	0.1–0.2	0.21	0.32
α-Terpineol	0.47	0.67	0.46	0.7	0.6–0.9	1.04	1.40
Sesquiterpene							
(*E*)-Nerolidol	–	–	0.07	–	tr–0.1	–	–
Spathulenol	–	0.10	0.08	–	–	–	–
Esters							
Monoterpene							
Citronellyl acetate	–	0.19	0.24	–	0.1–0.2	0.06	0.44
Geranyl acetate	3.64	3.45	4.31	2.5	2.7–4.5	1.51	2.23
Neryl acetate	0.32	–	7.70	2.6	3.5–6.1	8.34	3.85
Terpinyl acetate*	0.40	2.80	–	–	–	–	–
α-Terpinyl acetate	–	–	0.10	–	0–tr	tr	0.15
Ethers and oxides							
Monoterpene							
1,8-Cineole	–	–	0.99	–	0–2.5[b]	2.69	5.70
cis-Limonene oxide	–	0.17	–	–	0–0.1	0.07	0.27

continued

TABLE 5.12 (continued)
Percentage Composition of Laboratory-Extracted Persian Lime Petitgrain Oils

	1	2	3	4	5	6a	6b
Sesquiterpene							
Caryophyllene oxide*	–	–	0.23	–	tr–0.1	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, present, not quantified; [a] β-phellandrene is present only in the cv. Tahiti; [b] 1,8-cineole is absent only in the cv. Tahiti, in the other cultivars ranges from 1.3% to 2.5%.

Appendix to Table 5.12

1. Calvarano et al. (1982). Brazil; one sample steam distilled from the cv. Tahiti; GC/FID on capillary column (45 m × 0.5 mm) coated with UCON LB 550X; relative percentage of peak areas. Calvarano M. et al. also found linalyl acetate (0.21%).
2. Zollo Amvam et al. (1998). Yaoundè, Cameroon; one sample hydrodistilled from the cv. Tahiti; GC/FID on capillary columns (25 m × 0.25 mm) coated with OV-101 and Carbowax 20M; GC/MS on capillary column (25 m × 0.22 mm) coated with DB-1; relative percentage of peak areas. Zollo Amvam et al. also found δ-elemene (0.23%), menthone (0.37%), borneol (0.51%), terpinen-1-ol (0.16%), γ-terpineol (0.15%), bornyl acetate (0.08%), thymol (0.06%).
3. Huang et al. (2000). China; one sample hydrodistilled from the cv. Tahiti; GC/FID and GC/MS on capillary columns (50 m × 0.25 mm) coated with OV-101 and OV-17, Adams MS library; relative percentage of peak areas. Huang et al. also found *cis*-α-bergamotene (0.42%), δ-cadinene (0.06%), (Z)-β-farnesene (0.06%), γ-muurolene (0.11%), dodecanal (0.05%), methyl geranate (0.07%), and trace amounts of α-phellandrene, *cis*-sabinene hydrate, *cis*-linalol oxide.
4. Selvaraj et al. (2002). India; one sample hydrodistilled of Persian seedless lime; GC/FID on capillary columns coated with Carbowax 20M (25 m × 0.2 mm × 0.2 μm) and HP-101 (50 m × 0.32 mm); relative percentage of peak areas. Selvaraj et al. also found α-bergamotene* (0.4%), bisabolene* (0.3%), nerolidol* (0.1%).
5. Lota et al. (2002). Corsica, France; one sample each hydrodistilled from the cvs.: Bearss, De Perse, IAC, El Kseur, Tahiti; GC/FID and GC/MS on capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-1; GC/FID analysis was also performed with capillary column (50 m × 0.22 mm × 0.25 μm) coated with BP-20; LRI on BP-1 and BP-20 are report; ^{13}C-NMR; relative percentage of peak areas. Lota et al. also found in one or more of analyzed samples trace amounts of β-sinensal, *cis-p*-menth-2-en-1-ol, *trans*-sabinene hydrate, santal-10-en-2-ol, octyl acetate.
6. Smadja et al. (2005). Reunion Island, France; one sample each hydrodistilled from the cvs.: (a) De Perse, (b) Tahiti; GC/FID and GC/MS on capillary columns coated with Supelcowax (60 m × 0.20 mm × 0.20 μm) and HP-5 (30 m × 0.20 mm × 0.25 μm); LRI on Supelcowax and HP-5 are reported; relative percentage of peak areas; Smadja et al. also found in sample (a) piperitone (0.25%), *trans*-limonene oxide (0.04%), and trace amounts of 2,6-dimethylhept-5-en-1-al, α-bisabolol; in sample (b) nonanol (0.11%), *trans*-limonene oxide (0.20%), and trace amounts of 2,6-dimethylhept-5-en-1-al.

5.12 FINAL REMARKS

Papers published on industrially produced petitgrain oils, as it can be noticed from the data summarized in Tables 5.1 through 5.12, are scant. Some are obsolete (published in the 60s and the 70s) and obtained by unreliable chromatographic separation. Most of the studies on leaf oils were carried out on laboratory-extracted samples from numerous cultivars and different geographic origins; it is easy to imagine that these oils were obtained in different period of the year and from plants of different age. In many papers, the identification of the components, mainly the numerous minor ones, is obtained by the use of commercial MS libraries, leading to doubtful results and requiring further confirmation. The more or less drastic experimental conditions of the length of distillation can lead to more or less evident modification of the composition of the essential oil. Thus, it is difficult to directly compare the available data, since doubts arise if differences among them are due to the different vegetable matrices or to the extraction process. It must also be underlined that often the incompetent or careless gatherer can cause contamination in industrial oils.

TABLE 5.13
Main Quali-Quantitative Differences among Different Citrus Petitgrain Oils

	Bitter Orange[1] (Industrial)	Lemon[2] (Industrial)	Mandarin[3] (Industrial)	Dancy Tangerine[4] (Laboratory)	Clementine[5] (Laboratory)	Sweet Orange[6] (Industrial + Laboratory)	Bergamot[7] (Laboratory)	Grapefruit[8] (Laboratory)	Key Lime[9] (Industrial)	Persian Lime[10] (Laboratory)
δ-3-Carene	tr–1.15	0.11–1.08	tr–0.10	–	2.6–6.5	3.46–10.28	tr–0.05	tr–0.12	0.02–0.04	tr–0.03
p-Cymene	tr–0.12	0.02–0.91	2.96–5.19	2.35–4.09	tr–0.3	0.59–2.89	tr–0.31	0.05–21.32	0.14–0.25	tr–0.24
Limonene	0.3–5.43	28.41–37.20	7.18–12.59	0.39–4.5	2.5–6.9	2.90–8.37	0.63–1.80	1.6–4.51[k]	42.79–45.22	25.18–53.4
(*E*)-β-Ocimene	0–3.64	1.25–2.43	0.35–0.92	4.1–6.42	2.5–3.3	4.53–9.73	0.92–2.4	3.93–10.47	0.50–2.33	0.71–2.2
β-Phellandrene	tr–0.10	2.22–2.60[a]	0.03–0.05	0.1	0.52–0.9	0.65–1.06	tr–0.1	0.21–2.3	–	0.08[g]
Sabinene	0–0.45	1.05–4.22	0.22–0.90	0.28–0.41	33.1–49.8	37.64–48.52	0.15–0.3	2.49–61.91[l]	0.14–0.89	0.64–2.01
γ-Terpinene	0.01–0.18	0.01–3.19[b]	23.94–28.48	3.0–7.89	1.5–2.1	1.41–2.98	tr–0.1	0.82–1.68	0–1.09	0.03–0.3[h]
Valencene	–	–	–	–	–	tr	–	–	–	–
Geranial	tr–0.67	9.87–18.41	tr–0.10	–	0–0.8	0.59–3.11	0.44–3.15	–	10.75–13.96	11.8–22.5
Neral	tr–0.43	6.48–12.63	tr–0.06	m	tr–0.6	0.28–2.18	tr–0.84	–	7.45–10.38	2.60–16.23[i]
α-Sinensal	–	–	–	0.24–0.52	0.3–1.1	0.04–0.67	–	–	–	–
β-Sinensal	–	–	–	0–0.41	0.8–2.6	0.23–1.44	–	–	–	tr
Linalol	12.30–23.70	0.87–3.87	0.10–1.10	40.44–59.3[d]	19.4–24.7	4.34–15.12	22.19–55.16	3.32–26.66	0.61–1.20	0.9–3.27
Geranyl acetate	1.9–4.5	0.19–2.92	tr–0.02	–	0.1–0.7	0.04–0.28	0.08–5.83[e]	0.09–7.65	1.31–6.22	1.51–3.64
Linalyl acetate	39.75–71.0	0.02–0.42[c]	0.02–0.10	–	–	0.02–0.12	19.9–51.64	0.08–0.57	tr–0.12	0.21
Neryl acetate	1.04–3.0	3.75–7.44	tr–0.05	–	0–0.4	0.04–0.38	0.27–3.00[f]	0.11–3.92	0.45–2.11	0.32–7.70[j]
1,8-Cineole	0.02–0.06	1.12–2.68	0.01–0.02	–	–	0–0.05	tr–0.64	–	0–0.14	0–5.70
Phenyl ethyl alcohol	0.20	–	–	tr	–	–	–	–	–	–
Thymol	–	–	0.11–0.17	7.10–11.70	0–0.03	tr–0.05	–	0.04–0.07	–	0.06
Thymol methyl ether	–	–	–	4.63–8.8	–	0–tr	–	0.16	–	–
Methyl anthranilate	0.10	–	tr–0.03	–	–	–	–	n	–	–

continued

TABLE 5.13 (continued)
Main Quali-Quantitative Differences among Different Citrus Petitgrain Oils

	Bitter Orange[1] (Industrial)	Lemon[2] (Industrial)	Mandarin[3] (Industrial)	Dancy Tangerine[4] (Laboratory)	Clementine[5] (Laboratory)	Sweet Orange[6] (Industrial + Laboratory)	Bergamot[7] (Laboratory)	Grapefruit[8] (Laboratory)	Key Lime[9] (Industrial)	Persian Lime[10] (Laboratory)
Methyl *N*-methyl anthranilate	tr–0.31	tr–0.78	41.61–51.93	–	–	tr°	–		–	–
Methyl *N*-dimethyl anthranilate	–	–	tr–0.04	–	–	–	–	–	–	–

Notes:

1. Prager and Miskiewicz (1981); Formàček and Kubeczka (1982); Boelens and Sindreu (1988); Haubruge et al. (1989); Boelens and Oporto (1991); Mondello et al. (1996a,b); Dugo et al. (1996, 2010a).
2. Di Giacomo et al. (1985); Goretti et al. (1986); Verdi et al. (1993); Mondello et al. (1996a, 1997c); Dugo et al. (1996).
3. Dugo et al. (1996); Mondello et al. (1997a); Sciarrone (2009, personal communication).
4. Fleisher and Fleisher (1990b, 1991); Huang et al. (2000); Lota et al. (2001b).
5. Huang et al. (2000); Lota et al. (2001b); Tomi et al. (2008).
6. Mondello et al. (1997b).
7. Ortiz et al. (1978); Cheng and Lee (1981); Huang et al. (1986, 2000); de Rocca Serra et al. (1998); Kirbaslar and Kirbaslar (2006).
8. Cheng and Lee (1981); Germanà et al. (1990); Ekundayo et al. (1991a); Gurib-Fakim et al. (1995); Huang et al. (2000); Gancel et al. (2005).
9. Dugo et al. (2010b).
10. Calvarano et al. (1982); Zollo Amvam et al. (1998); Huang et al. (2000); Selvaraj et al. (2002); Lota et al. (2002); Smadja et al. (2005).

[a] The presence of β-phellandrene in industrial lemon petitgrain oils is reported only by Dugo et al. (1996) and by Mondello et al. (1997c); [b] Very low values of γ-terpinene (0.01%–0.17%) were determined in Turkish oils (Verdi et al., 1993), in all the others the minimum is 0.34%; [c] Not considered were the high values of linalyl acetate determined by Goretti et al. (1986) and by Mondello et al. (1996b) probably due to coelutions with other components; [d] Attaway et al. (1967) reported values up to 78%; [e] The very low value of geranyl acetate (0.08%) is reported by Cheng and Lee (1981), for the other oils the minimum is 2.5%; [f] the very low value of neryl acetate (0.27%) is reported by Cheng and Lee (1981), in the other papers the minimum reported is 1.3%; [g] Lota et al. (2002) reported the presence of β-phellandrene (1.2%) only in one of the numerous cvs. analyzed; [h] Calvarano (1982) reported for γ-terpinene the value of 1.39%; [i] The value of 2.60% for neral is reported by Smadja et al. (2005), in all the other samples the minimum is 8.3%; [j] The minimum value 0.32% is reported by Calvarano (1982), the lowest value for the other oils was 2.6%; [k] Not included are the low value reported by Gurib-Fakim and Demarne (1995) (0.14%) and the high ones 6.4% and 11.37% reported respectively by Kamiyama and Amaha (1972) and by Ekundayo et al. (1991) (1995); [l] The minimum (2.49%) is reported by Ekundayo et al. (1991), for the other oils it was 10.22%; [m] Blanco Tirado et al. (1995) reported the presence of neral (6.06%), only Attaway previously reported the presence of this component (0.2%–0.6%) coeluted with α-terpineol; [n] Not included is the value of 7.97% reported by Germanà et al. (1990) probably due to a wrong identification; [o] Not included are the values determined for methyl *N*-methyl anthranilate (1.26%–1.29%) found by Mondello et al. (1997b) and considered by the authors significant of contamination during gathering.

The great variability of the composition of the oils of the same species is therefore inevitable, making it difficult to determine reasonable quantitative variability ranges and to find selective markers for the different citrus oils.

Although the limitations reported above, if only some of the papers reported in Tables 5.1 through 5.12 are taken in consideration, and only selected components are examined, it will be possible to draw some conclusions. In Table 5.13, the percentages of selected components are reported for the different citrus species. When possible, oils of industrial production were considered. Otherwise, if data were unavailable, those obtained in laboratory were considered.

The petitgrain oils of lemon, Key lime, Persian lime all are characterized by high values of limonene, geranial, and neral, and relatively abundant geranyl and neryl acetates. In lemon, and in some of the Persian lime petitgrain oils, high levels of 1,8-cineole are also determined. In industrial lemon petitgrain oils, Dugo et al. (1996) and Mondello et al. (1996a, 1997) reported appreciable amounts of β-phellandrene.

Bitter orange and bergamot petitgrain oils are mainly characterized by their high content of linalol and linalyl acetate. The Spanish bitter orange leaf oils also contain a small amount of methyl *N*-methyl anthranilate and of phenyl ethyl alcohol. In mandarin petitgrain, the main components are methyl *N*-methyl anthranilate and γ-terpinene, and also present are large amounts of limonene and *p*-cymene and small amounts of thymol, methyl anthranilate, and methyl *N*-dimethyl anthranilate.

Tangerine petitgrain oils contain high amount of linalol and relatively high values of (*E*)-β-ocimene and of *p*-cymene. These oils are also characterized by the presence of a considerable amount of thymol and thymol methyl ether. In addition to mandarin and tangerine, thymol is also present in a very low amount in clementine, sweet orange, grapefruit, and Persian lime petitgrain oils, while thymol methyl ether is also detected in sweet orange and grapefruit petitgrain oils. Sweet orange, clementine, and grapefruit petitgrain oils contain high amount of sabinene, linalol, and (*E*)-β-ocimene. Sweet orange petitgrain oil is also characterized by high values of δ-3-carene.

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6 Extracts From the Bitter Orange Flowers (*Citrus aurantium* L.): Composition and Adulteration

Giovanni Dugo, Louis Peyron, Ivana Bonaccorsi

CONTENTS

6.1 INTRODUCTION

Peyron (2002) gives a detailed description of the systems used for the production of neroli oil, bitter orange flower water absolute, and bitter orange flower absolute. This chapter describes the composition of the following products obtained from bitter orange flowers and their possible adulterations:

- Neroli oil obtained by steam distillation or by hydrodistillation from the flowers
- Bitter orange flower water absolute obtained from bitter orange flower by solvent extraction of the water phase produced by distillation
- Bitter orange flower absolute obtained by extraction in alcohol from the solvent extract of the flowers

The text and tables will use the same symbols and criteria applied in Chapter 1.

6.2 COMPOSITION OF NEROLI OIL

Guenther (1949) reported the physicochemical indices of neroli oils of different origins, and, for some of these, the esters content (expressed as linalyl acetate), the free alcohols content (expressed as linalol), and the content of methyl anthranilate as well. These values were as follows:

	Esters	Free Alcohols	Methyl Anthranilate
France	6.7%–21.0%	–	0.4%–1.2%
Italy	7.3%–14.7%	49.9%–67.5%	0.6%–1.4%
Spain	12.3%–32.3%[a]	–	–
Haiti	8.7%–18.7%	–	–

[a] The maximum value was considered by Guenther as too high, probably due to the presence of leaves in the raw material used for distillation.

For the neroli oil produced in France, Guenther (1949) also reported the following approximated composition:

Hydrocarbons (α-pinene, camphene, limonene, heptacosane)	35%
(–)-Linalol	30%
Geraniol + nerol	4%
(+)-α-Terpineol	2%
Linalyl acetate	7%
Geranyl acetate + neryl acetate	4%
(+)-Nerolidol	6%
Indole	<0.1%
Methyl anthranilate	0.6%
Acetic acid + palmitic acid	0.1%
Others (decanal[t], esters of phenyl acetic acid and of benzoic acid, jasmone*, farnesol*)	11.2%

Examining the literature available at that time, Guenther also reported in neroli oil β-ocimene*, phenylethyl alcohol, and trace amounts of phenols.

The same quantitative data reported by Guenther were later included by Gildemeister and Hoffmann (1959) and by Bigi (1962) in their respective reviews.

Calvarano (1963) determined by classical methods the following composition, relative to six samples of Italian neroli oils:

Esters (as linalyl acetate)	11.98%–25.99%
Free alcohols (as linalol)	49.14%–58.75%
Aldehydes (as decanal)	0.62%–1.06%
Methyl anthranilate	0%–0.72%
Indole	0.08%–0.10%

The gas chromatographic analysis on a neroli sample obtained by mixing some of the six samples, carried out on a stainless steel capillary column coated with UCON LB-550X, revealed the presence of *p*-cymene (2.53%), limonene (5.25%), myrcene (0.8%), α-pinene (0.15%), β-pinene (6.09%), γ-terpinene (0.15%), and also, not quantified, decanal, geraniol, linalol, nerol, α-terpineol, farnesol*, nerolidol*, phenylethyl alcohol, geranyl acetate, linalyl acetate, neryl acetate, and α-terpinyl acetate.

Using gas chromatography on packed columns, Peyron (1965) determined in neroli oils the presence of the following components, listed in decreasing amount order: linalol, β-pinene, linalyl acetate, limonene, β-ocimene*, nerolidol*, α-terpineol, neryl acetate, geranyl acetate, and α-pinene.

Few years later, McHale (1971) determined that the β-ocimene, identified in neroli oil, was the *trans* isomer, and described the alkaline isomerization to the conjugated triene of this component. Corbier and Teisseire (1974) isolated and identified in neroli oil *cis*-heptadec-8-ene and 2,5-dimethyl-2-vinyl-hex-4-enal. As reported by Lawrence (1997), Balboa et al. (1972) tentatively identified in an Egyptian oil limonene, geranial, neral, linalol, nerol, α-terpineol, linalyl acetate, and methyl anthranilate. However, it should be noted that Anonis (1985), for the oil analyzed by Balboa et al. (1972), reports a quantitative composition characterized by unusually high percentages of camphene (5.50%) and δ-3-carene (2.46%), with very low amounts of linalol (2.52%) and linalyl acetate (0.87%).

Toyoda et al. (1993) identified in neroli oil *p*-cymene, limonene, myrcene, β-ocimenes*, β-phellandrene, β-caryophyllene, benzyl aldehyde, geraniol, linalol, nerol, α-terpineol, nerolidol*, 2-phenylethyl alcohol, linalyl acetate, linalol oxides*, indole, methyl anthranilate, and phenylacetonitrile.

Since the beginning of the 1970s, many papers have been published on the quantitative composition of neroli oil. The numerous results are reported in Table 6.1.

The composition of the oils reported in Table 6.1 varies within very wide ranges, and some of these should not be considered genuine if related to the regulatory limits (Association Francaise de Normalisation [AFNOR], International Organization for Standardization [ISO], and the European Pharmacopoeia [EP]) listed later in this chapter.

TABLE 6.1
Percentage Composition of Neroli Oil

	1	2a	2b	3	4	5	6	7	8	9	10,11	12a	12b	13a	13b	14
								Hydrocarbons								
Monoterpene																
Camphene	–	–	–	–	0.02	0.05	–	–	0.05	–	0.04	0.04	0.02	0.05– 0.08	0.07	0.01
δ-3-Carene	–	–	–	–	–	0.1	–	–	0.05	–	0.52	–	–	–	–	0.03–0.09
p-Cymene	–	–	–	0–0.56	–	tr	–	–	0.22	0.14	1.04	–	–	0.01–0.05	0.12	0.06–0.13
Limonene	16.1[a]	9.2–16.6	1.1–12.2	12.58–18.12	9.13	16.6	1.06	7.15	12.88	9.34	24.57	8.02	10.87	12.88–17.89	12.20	7.87–11.89
Myrcene	1.6	0.3–2.2	0–0.5	1.97–9.34[f]	6.26	1.7	0.07	1.34	2.49	0.34	2.33	1.75	1.77	1.40–3.09	2.08	1.33–1.74
(*E*)-β-Ocimene	6.0	0.9–8.2	0–1.9	–	–	6.0	0.40	–	5.60	–	3.60	6.39	3.72	5.60–7.00	6.24	3.31–5.11
(*Z*)-β-Ocimene	16.1[a]	0–1.0	–	0.66–1.02[c]	–	0.5	–	–	0.82	–	0.34	0.68	0.55	0.10–0.43	0.73	0.47–0.61
α-Phellandrene	–	–	–	–	–	0.05	–	–	0.04	–	0.09	–	–	–	–	0.01–0.02
β-Phellandrene	–	–	–	0.66–1.02[c]	–	0.4	–	–	–	–	–	0.20	0.17	–	–	–
α-Pinene	0.8	0.8–6.7[o]	0–1.2	0.86–1.35	0.28	1.1	–	0.25	0.75	0.24	1.31	0.60	0.01	0.75–1.13	0.99	0.15–0.26
β-Pinene	15.0	11.2–23.4	1.7–8.3	7.42–14.08[g]	0.53	11.8	0.13	4.85	0.52	3.10	20.22[e]	9.80	5.15	10.52–13.00	14.56	1.89–3.70
Sabinene	–	1.0–3.3	0–0.8	5.27–9.53	–	2.8	–	0.66	2.26	0.33	20.22[e]	1.07	4.10	1.40–2.80	1.17	0.85–1.56
α-Terpinene	–	–	–	0–0.74[h]	–	0.5	–	5.64	0.18	–	0.51	0.08	0.07	0.18–0.48	0.10	0.04–0.13
γ-Terpinene	–	–	–	2.75–8.34[i]	–	1.0	–	–	0.33	–	3.71	0.11	0.17	0.01–0.51	0.18	0.11–0.28
Terpinolene	–	–	–	0.17–0.71	–	0.4	–	–	0.42	–	0.53	0.35	0.25	0.42–0.60	0.42	0.26–0.43
α-Thujene	–	–	–	–	tr	0.1	–	–	0.05	–	0.25	–	–	0.01–0.05	+	0.01–0.02
Tricyclene	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	0–tr
Sesquiterpene																
Aromadendrene	–	–	–	–	–	–	0.30	–	–	–	–	–	–	–	–	tr–0.01
δ-Cadinene	–	–	–	–	–	tr	–	–	0.03	–	0.03	–	–	0.01–0.03	0.03	0.02–0.04
Caryophyllene*	–	–	–	–	–	0.5	–	1.23	–	–	–	–	–	–	–	–
β-Caryophyllene	–	0.3–1.6[b]	0–0.3	–	0.13	–	–	–	0.54	–	0.72	0.35	0.23	0.54–0.60	0.73	0.56–0.94
β-Elemene	–	–	–	–	–	0.1	–	–	0.05	–	0.31	–	–	–	–	0.09–0.18
δ-Elemene	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	0.03–0.06

continued

TABLE 6.1 (continued)
Percentage Composition of Neroli Oil

	1	2a	2b	3	4	5	6	7	8	9	10,11	12a	12b	13a	13b	14
(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	–	–	–	0.07	–	–	0.05–0.10	0.11	0.01–0.05
(*E*)-β-Farnesene	–	–	–	–	–	–	–	–	0.13	–	–	–	–	0.05–0.10	0.17	0.09–0.22
(*Z*)-β-Farnesene	–	–	–	–	–	–	–	–	0.08	–	0.14	–	–	–	–	–
Germacrene D	–	–	–	–	–	0.1	–	–	0.05	–	0.05	–	–	–	–	0.03–0.09
α-Humulene	–	–	–	–	tr	0.1	0.06	–	0.18	–	0.10	–	–	–	–	0.06–0.11
Valencene	–	–	–	–	–	tr	–	–	0.05	–	–	–	–	–	–	–
Aldehydes																
Aliphatic																
Nonanal	–	–	–	–	tr	–	–	–	0.01	–	–	–	–	–	–	–
Monoterpene																
Citronellal	–	–	–	–	–	–	–	–	0.01	–	0.06	–	–	–	–	–
Geranial	–	–	–	–	0.04	tr	–	1.05	0.10	0.48	0.65	–	–	0.05–0.10	0.06	0.05–0.07
Neral	–	–	–	–	0.58	tr	–	3.7	0.03	0.23	0.41	–	–	0.01–0.03	0.03	0–0.03
Ketones																
Aliphatic																
6-Methyl-5-hepten-2-one	–	–	–	–	–	–	–	–	–	–	0.11	–	–	–	–	0.02
Monoterpene																
Carvone	–	–	–	–	–	–	–	–	–	0.14	–	–	–	–	–	0.01–0.03
Alcohols																
Monoterpene																
Citronellol	0.2	–	–	2.21–4.08	–	–	–	–	–	–	–	–	–	–	–	–
Geraniol	2.0	1.2–2.9	0.8–3.1	1.50–4.09	tr	4.25	–	0.45	2.18	–	–	3.15	4.20	0.80–2.28	2.00	2.94–3.83
Linalol	30.6	30.7–40.5	27.9–43.4	25.48–38.81	50.46	37.5	73.72	53.19	8.93	44.29	15.59	32.18	34.41	31.37–47.05	37.86	43.69–53.33

cis-p-Mentha-2-en-1-ol	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	tr–0.03
Nerol	0.5	0.5–1.2	0.4–1.3	0.48–0.81	tr	0.5	0.19	0.98	0.82	0.95	0.69	1.18	3.43	0.32–0.82	0.88	0.95–1.28
trans-Piperitol	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	0.01–0.02
Terpinen-4-ol	–	0.3–1.6[b]	1.3–2.4	0.17–1.90[j]	0.11	0.75	–	–	0.42	0.22	1.20	0.20	0.61	0.31–1.32	0.39	0.44–0.79
α-Terpineol	3.0	2.8–4.9	3.6–4.7	2.26–3.35	4.75	2.0	–	9.76	3.30	6.50	1.79	4.80	6.58	1.07–4.5	4.11	4.89–6.17
Sesquiterpene																
Farnesol*	4.0	0.9–2.8	1.4–8.3	0–2.17[k]	tr	1.0[d]	2.46	7.55	–	–	–	5.40	3.88	–	–	–
(*E,E*)-farnesol	–	–	–	–	–	–	–	–	1.48	–	0.01	–	–	–	–	–
(*Z,E*)-Farnesol	–	–	–	–	–	–	–	–	0.04	0.36	0.98	–	–	–	–	–
Globulol	–	–	–	–	–	–	–	–	–	–	0.01	–	–	–	–	0.01–0.02
Nerolidol*	4.0	1.4–5.6	2.9–6.9	2.76–6.88	–	2.6	–	–	2.58	0.60	–	1.46	0.40	–	–	–
(*E*)-Nerolidol	–	–	–	–	0.64	–	–	–	–	–	1.76	–	–	2.15–3.37	3.36	1.15–3.21
(*Z*)-Nerolidol	–	–	–	–	1.09	–	1.18	–	–	–	tr	–	–	–	–	–
Spathulenol	–	–	–	–	–	–	–	–	–	–	0.02	–	–	–	–	0.03–0.05
Esters																
Monoterpene																
Citronellyl acetate	–	–	–	–	–	–	–	–	0.09	–	0.03	–	–	–	–	0–0.02
Geranyl acetate	2.9	2.5–4.1	1.8–4.2	–	2.15	1.7	0.21	1.08	2.65	2.73	1.63	4.01	3.42	–	–	3.01–3.08
Linalyl acetate	9.1	3.4–7.6	1.3–10.3	2.48–9.45[l]	18.39	2.8	16.53	–	6.37	–	9.76	12.05	11.33	0.58–10.00	3.30	2.19–14.57
Neryl acetate	1.7	1.3–2.0	2.1–3.7	–	1.09	0.9	0.45	0.48	1.36	–	0.92	1.50	1.76	0.29–1.55	1.53	1.43–1.45
α-Terpinyl acetate	–	–	–	–	tr*	0.2	–	–	0.06	–	0.05	–	–	0.01–0.30	0.14	0.05–0.07
Sesquiterpene																
Farnesyl acetate	–	–	–	0–0.03*	–	–	–	–	–	–	–	–	–	0.01–0.05[q]	0.09[q]	0.02–0.04
Ethers and oxides																
Monoterpene																
cis-Linalol oxide	–	–	–	0.05–0.11	–	0.1[n]	–	–	0.02	1.47	0.02	0.07	0.05	0.01–0.07	0.16	0.13–0.18

continued

TABLE 6.1 (continued)
Percentage Composition of Neroli Oil

	1	2a	2b	3	4	5	6	7	8	9	10,11	12a	12b	13a	13b	14
trans-Linalol oxide	–	–	–	0.02–0.24	0.15	0.1[n]	–	–	0.07	1.27	0.01	0.15	0.18	0.10–0.20	0.06	–
trans-Linalol oxide (pyranoid)	–	–	–	–	0.09	–	–	–	–	0.21	–	–	–	–	–	–
Perillene	–	–	–	–	–	tr	–	–	0.02	–	–	–	–	–	–	–
Sesquiterpene																
Caryophyllene oxide*	–	–	–	–	–	–	–	–	0.01	–	0.04	–	–	–	–	0.02–0.04
Others																
Geranyl acetone	–	–	–	–	–	0.05	–	–	0.05	–	–	–	–	–	–	0.02–0.04
Indole	0.1	–	–	–	–	0.1	0.83	–	0.16	–	0.06	0.70	0.30	0.10–0.16	0.16	–
cis-Jasmone	–	–	–	–	–	0.05	–	–	0.05	–	–	–	–	0.01–0.05	0.02	–
Methyl anthranilate	0.3	0–0.5	–	0.13–0.85[m]	tr	0.1	0.25	–	0.10	–	0.11	–	–	0.10–0.22	0.09	0.04–0.12
Methyl *N*-methyl anthranilate	–	–	–	–	–	tr	–	–	0.10	–	3.18	–	–	–	–	0.01–0.04
Methyl jasmonate	–	–	–	–	–	tr*	–	–	0.01*	–	–	–	–	0.01[p]	0.01[p]	–
Phenylethyl alcohol	–	–	–	–	–	0.2	–	–	0.20	–	0.01	–	–	0.06–0.25	–	–

Notes: tr, traces; *, correct isomer not characterized; t, tentative identification; +, present, not quantified; [a] limonene + (*Z*)-β-ocimene; [b] β-caryophyllene + terpinen-4-ol; [c] (*Z*)-β-ocimene + β-phellandrene; [d] farnesol isomers sum; [e] β-pinene + sabinene; [f] myrcene was 7.87% and 9.34% in Italian and Spanish oils, respectively, in other oils the maximum was 3.76%; [g] β-pinene was 14.08% in Tunisian oil, in the other oils the maximum was 9.97%; [h] α-terpinene was absent in Algerian, Spanish, and Tunisian oils, in Egyptian and Italian oils was 0.59% and 0.74% respectively; [i] γ-terpinene was 2.75% in Spanish oils, in the other oils the minimum was 6.98%; [j] terpinen-4-ol was 1.59% and 1.90% in Egyptian and Italian oils respectively, in the other oils the maximum was 0.53%; [k] farnesol* was absent in Algerian and Italian oils, in the other oils the minimum was 0.72%; [l] linalyl acetate was 9.45% in Egyptian oil, in the other oils the maximum was 5.39%; [m] methyl anthranilate was 0.85% in Egyptian oil, in the other oils the maximum was 0.30%; [n] *cis*- + *trans*-linalol oxide; [o] the very high value of α-pinene (6.7%) was observed only in one of the samples considered genuine, for the other oils the maximum was 1.9%; [p] (*Z*)-methyl jasmonate; [q] (*E*,*Z*)-farnesyl acetate.

Appendix to Table 6.1

1. Buccellato (1981). One sample.
2. Prager and Miskiewicz (1981). (a) Range of the composition of 16 commercial Mediterranean samples, considered genuine by the authors; (b) range of the composition of 3 commercial samples, considered adulterated by the authors due to one or more of the following anomalies: low content of monoterpene hydrocarbons, high content of farnesol*, nerolidol*, linalyl acetate, presence of 2.5%–3% of both *cis*- and *trans*-linalol oxide. GC/FID and GC/MS on capillary column (33 m × 0.5 mm) coated with Carbowax 20M; GC/MS; relative percentage of peak areas.
3. Srinivas (1986). Oils produced in Algeria, Egypt, Italy, Spain, and Tunisia; GC/MS. Srinivas also found β-terpineol* (0.32%–0.85%).
4. Lin et al. (1986). China; one sample; column chromatography on silica gel; IR; GC/FID on capillary column (36 m × 0.32 mm) coated with SP-2305 or SE-30; GC/MS on capillary column (30 m × 0.32 mm) coated with SE-30; LRI on SE-30 are reported; relative percentage of peak areas. Lin et al. also found β-ocimene* (3.91%), *cis*-linalol oxide, pyranoid form (0.09%), and trace amount of γ-muurolene.
5. Boelens and Sindreu (1988). Spain; GC/FID and GC/MS on capillary columns coated with Carbowax 20M, UCON or SE-54; GC/MS; relative percentage of peak areas. Boelens and Sindreu also found bisabolenes* (0.1%), farnesenes* (0.1%), *cis*- + *trans*-anhydrolinalol oxide (0.05%), and trace amounts of *p*-cymenene, selinenes*, perillene.
6. Ma et al. (1988). China; one sample; GC/MS and retention indices on two columns of differing polarity; relative percentage of peak areas. Ma et al. also found isocaryophyllene (0.09%), benzyl alcohol (0.15%), 3-cycloexenyl carbinol[t]* (0.43%), (*E*)-2-hexenol (0.09%), hexadecanoic acid (0.36%), phthalic acid[t] (0.09%).
7. Germanà et al. (1990b). Sicily, Italy; one sample; GC on packed column of WEAS; relative percentage of peak areas.
8. Boelens and Oporto (1991). Spain. Boelens and Oporto also found decanal (0.03%), octanal (0.02%), δ-cadinol (0.02%), 1,8(9)-menthadienyl acetate (0.02%), *cis*-limonene oxide (0.03%), *trans*-limonene oxide (0.03%), 2,2,6-trimethyl-6-vinyl tetrahydropyran (0.04%).
9. Zhu et al. (1993). China; GC/MS. Zhu et al. also found cuminaldehyde (0.10%), (*Z*,*E*)-farnesal (0.10%), *trans*-carveol (0.16%), linalyl anthranilate (16.38%).
10,11. Mondello et al. (1994, 1996). Sicily, Italy; one commercial sample; GC/FID (quantitative analyses) on capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; GC/FID and GC/MS quadrupole on capillary columns (60 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52 or Carbowax 20M; coupled LC-GC/MS (ITD) on a LC column (10 m × 2 mm) packed with Sferisorb 5 μm silica and GC capillary columns as for GC/MS (quadrupole) analyses; relative percentage of peak areas. Mondello et al. also found *trans*-α-bergamotene (0.02%), benzaldehyde (0.01%), *trans*-*p*-menth-2-en-1-ol (0.19%), *cis*-sabinene hydrate (0.03%), *trans*-sabinene hydrate (0.09%); α-cadinol (0.02%), 1,8-cineole (0.18%).
12. Boussaada (1995). (a) One sample from Chaina, Creta, Greece, (b) one sample from Tunisia; GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP-5; GC/FID analysis was also performed on capillary column (30 m × 0.25 mm × 0.25 μm) coated with HP Innowax; Wiley 275 MS library; relative percentage of peak areas. Boussaada also found farnesene* 0.04% and 0.06% in sample (a) and (b) respectively. The results relative to the Tunisian oil were reported also in a different paper (Boussaada and Chemli, 2006).
13. Boelens (1997). Hydrodistillation; (a) several samples from Spain, (b) one sample from Tunisia; GC/FID and GC/MS on capillary column (50 m × 0.32 mm) coated with SE-54; relative percentage of peak areas. Boelens also found γ-elemene (0.01%–0.02%), (*E*,*Z*)-farnesol (0.72%–1.59%), hexyl acetate (0.01%–0.05%), (*E*,*Z*)-farnesyl acetate (0.01%–0.05%), in samples (a); γ-elemene (0.12%), (*E*,*Z*)-farnesol (1.57%), hexyl acetate (0.05%), (*E*,*Z*)-farnesyl acetate (0.09%), 2-phenyl ethyl acetate (0.03%) in sample (b).
14. Dugo et al. (2010); range of 5 samples from Egypt: one produced in 2008 and 4 produced in 2009. GC/FID and GC/MS on capillary column (30 m × 0.25 mm × 0.25 μm) coated with SLB-5MS; MS libraries: Adams, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) home-made; LRI on SLB-5MS are reported; relative percentage of peak areas. Dugo et al. also found 4,8-dimethyl-1,3(*Z*),7-nonatriene + phenyl ethyl alcohol (0.03%), *cis*-α-bergamotene (0.01%), bicyclogermacrene (0.10%), 9-epi-β-caryophyllene (0.01%), α-muurolene (0.01%), β-sesquiphellandrene (0.01%), (*E*,*E*)-2,6-farnesal (0.03%), (*E*,*Z*)-2,6-farnesal (0.02%), α-sinensal (0.02%), β-sinensal (0.09%), tetradecanol (0.02%), *p*-cymen-8-ol (0.02%), trans-*p*-menth-2-en-1-ol (0.02%), *trans*-myrtanol (0.01%), cadin-4-en-10-ol (0.02%), 2,3-dihydrofarnesol (0.02%), (*Z*,*Z*)-2,6-farnesol (2.03%), (*Z*)-3-hexyl butanoate (0.01%), geranyl formate (0.03%), methyl geranate (0.01%), *cis*-dehydrolinalol oxide (0.02%), *trans*-limonene oxide (0.01%), geranyl acetone (0.04%), (*E*,*E*)-geranyl linalol (0.02%), (*E*)-jasmone (0.02%), and trace amounts of tetradecane, *o*-cymene, bicycloelemene, γ-cadinene, *cis*-carveol, fenchol*, *trans*-*p*-mentha-2,6-dien-1-ol, (*E*)-2-hexenyl acetate, bornyl acetate, geranyl propanate, linalyl propanate, perillyl acetate (average values).

To be noticed are the very low values of limonene (1.06%) and β-pinene (0.13%) and very high value of linalol (73.72%) in the Chinese oil analyzed by Ma et al. (1988); the very low value of β-pinene (ca. 0.5%) reported by Lin et al. (1986) and by Boelens and Oporto (1991); and the very high values of α-terpinene (5.64%) and farnesol* (7.55%) and the absence of linalyl acetate found by Germanà et al. (1990b). It appears strange that the total of the components identified and quantitatively determined by Boelens and Oporto (1991) is less than 60%. In the same paper, Boelens and Oporto, based on literature information and their researches, asserted that the composition in class of components of neroli oil is as follows: monoterpene hydrocarbons (40%), oxygenated monoterpenes (50%), sesquiterpene hydrocarbons (1%), oxygenated sesquiterpenes (7%), and others (2%). In the paper by Boelens (1997), in addition to the composition of Spanish and Tunisian oil obtained by hydrodistillation (see Table 6.1), the composition of an oil from Morocco extracted by supercritical CO_2 is also reported. The main differences between the two types of oils are reported below:

	Hydrodistillation (%)	**Supercritical CO_2 (%)**
Monoterpene hydrocarbons	38	28
Linalyl acetate	3–5	24
Linalol	38	35
Nitrogen derivatives	<0.5	2
Sesquiterpene alcohols	4	<2

Boelens (1997) explained the different contents in monoterpene hydrocarbons and in linalyl acetate between the two oils to some chemical change of linalyl acetate during the distillation process, which lead to the formation of monoterpene hydrocarbons and other monoterpenoids. The higher content of nitrogen-containing components in the oil extracted by supercritical CO_2 could be due to the water solubility of these components and to their consequent loss during the hydrodistillation process. Boelens also asserted that a group of perfumers preferred the oils extracted by supercritical CO_2, for their odor character and the color intensity (double if compared with the hydrodistilled ones).

The Egyptian oils analyzed by Dugo et al. (2010) were industrially produced by the same industry; one in 2008 and four between March 23 and April 12, 2010. Figure 6.1 reports the chromatogram of one of these samples. In the sample produced in 2008, the highest content of linalol (53.35%) and the lowest content of linalyl acetate (2.19%) were determined, with a ratio of these components equal to 24.35. In the samples produced in 2009, the amount of linalol decreases from 45.58% in the sample produced on March 23 to 43.69% in that produced on April 11 and 12th, while linalyl acetate presents an opposite trend, increasing in the same period from 8.77% to 14.57%. The ratio of linalol and linalyl acetate decreases from 5.20% to 3.00%. From the results described earlier, it seams that the extraction technology of this industrial plant has been improved in 2009 with respect to the previous year, and that significant variation of the composition of the oil is dependent on the harvest period of the raw material used for the extraction.

Other papers on the composition of neroli oil not included in Table 6.1 can be found in literature. Many report peculiar composition more or less evident. Kekelidze et al. (1977) analyzed a neroli oil characterized by low content of limonene (0.3%), high amounts of nerolidol* (13.0%), and methyl anthranilate (5.0%), and the total absence of linalyl acetate. Hethelyi et al. (1988) analyzed some commercial samples characterized by high amounts of δ-3-carene (2.6%–6.9%); in some of these, linalyl acetate was not present. Some commercial oils were analyzed also by Braun and Franz (2001); many of these, as reported by the same authors, were slightly or heavily adulterated. Alissandrakis et al. (2003), in an article on the characterization of the floral origin of honey, reported the main components of a neroli oil where limonene was only 1.6%, linalol 80.6%, linalyl acetate 10.6%, and indole 2.7%.

The results reported by Germanà et al. (1990a) are of interest. This article reports the results obtained by separate analyses of petals, stamens, and pistils of bitter orange flowers. The oil extracted by the stamens present the highest amount of oxygenated compounds compared to those

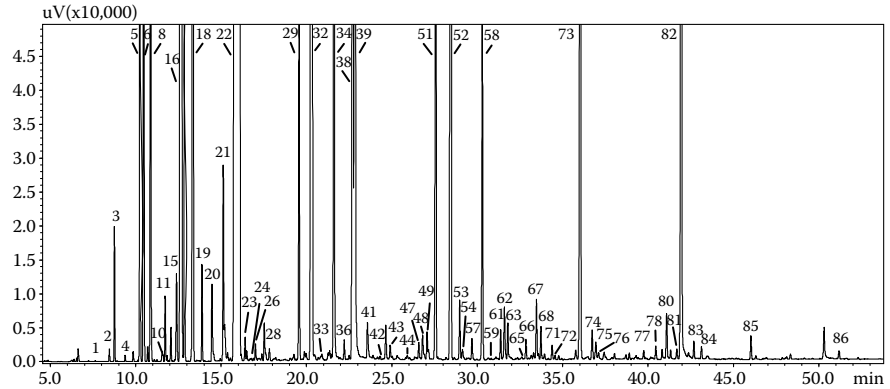

FIGURE 6.1 GC/FID chromatogram of a neroli oil from Egypt (Dugo et al., 2010). Peak identification: **1** tricyclene; **2** α-thujene, **3** α-pinene; **4** camphene; **5** sabinene; **6** β-pinene; **7** 6-methyl-5-hepten-2-one; **8** myrcene; **9** *cis*-dehydrolinalol oxide; **10** α-phellandrene; **11** δ-3-carene; **12** (*E*)-2-hexenyl acetate; **13** α-terpinene; **14** *o*-cymene; **15** *p*-cymene; **16** limonene; **17** (*Z*)-β-ocimene; **18** (*E*)-β-ocimene; **19** γ-terpinene; **20** *cis*-linalol oxide; **21** terpinolene; **22** linalol; **23** 4,8-dimethyl-1,3(*E*),7-nonatriene + phenylethyl alcohol; **24** fenchol*; **25** *trans*-*p*-mentha-2,8-dien-1-ol; **26** *cis*-*p*-menth-2-en-1-ol; **27** *trans*-limonene oxide; **28** *trans*-*p*-menth-2-en-1-ol; **29** terpinen-4-ol; **30** *p*-cymen-8-ol; **31** (*Z*)-3-hexenyl butanoate; **32** α-terpineol; **33** *trans*-piperitol; **34** nerol; **35** *cis*-carveol; **36** neral; **37** carvone; **38** linalyl acetate; **39** geraniol; **40** trans-myrtanol; **41** geranial; **42** bornyl acetate; **43** geranyl formate; **44** methyl geranate; **45** linalyl propanate; **46** bicycloelemene; **47** δ-elemene; **48** methyl anthranilate; **49** α-terpinyl acetate; **50** citronellyl acetate; **51** neryl acetate; **52** geranyl acetate; **53** *cis*-β-elemene; **54** (*E*)-jasmone; **55** tetradecane; **56** *cis*-α-bergamotene; **57** methyl *N*-methyl anthranilate; **58** β-caryophyllene; **59** perillyl acetate; **60** aromadendrene; **61** geranyl acetone; **62** (*E*)-β-farnesene; **63** α-humulene; **64** 9-epi-β-caryophyllene; **65** geranyl propanate; **66** germacrene D; **67** bicyclogermacrene; **68** α-muurolene; **69** (*E*,*E*)-α-farnesene; **70** γ-cadinene; **71** δ-cadinene; **72** β-sesquiphellandrene; **73** (*E*)-nerolidol; **74** spathulenol; **75** caryophyllene oxide*; **76** globulol; **77** cadin-4-en-10-ol; **78** tetradecanol; **79** 2,3-dihydrofarnesol; **80** β-sinensal; **81** (*E*,*Z*)-2,6-farnesal; **82** (*Z*,*Z*)-2,6-farnesol; **83** (*E*,*E*)-2,6-farnesal; **84** α-sinensal; **85** farnesyl acetate*; **86** (*E*,*E*)-geranyl linalol. (From Dugo, G., 2009. Personal communication.)

obtained by the other parts of the flower. Stamens count ca. 22% of the weight of the whole flower, and their yield in oil is lower (0.38%) than of pistils (1.39%) and of petals (2.22%).

6.3 COMPOSITION OF THE BITTER ORANGE FLOWER WATER ABSOLUTE

Guenther (1949) reported the results obtained by Naves in 1934 on two types of orange flower water oil; one representative of 500 liters of distillation water per 1000 kg of flowers (2 kg), and the other representative of 1000 liters of water for 1000 kg of flowers (kg/kg). The results are summarized below:

	2 kg	**kg/kg**
Hydrocarbons (camphene, limonene, pinene*, *n*-C_{27})	tr	tr
Geraniol + nerol	8%	6%
Linalol	32%	54%
α-Terpineol	13%	18%
Nerolidol*	1%	1%
Methyl anthranilate	22%	8%
Phenylethyl alcohol esterified with phenyl acetic acid	8%–10%	1%–2%
Benzyl alcohol	2%	tr
Linalyl acetate, geranyl acetate, neryl acetate, indole	tr	tr
Free acids and phenols	1%	tr

The composition of the bitter orange flower water absolute obtained by gas chromatographic techniques is reported in Table 6.2.

Remy et al. (1993) monitored the chemical changes of the bitter orange water absolute in function of the time and the conditions used for the storage of the distillation water obtained after the separation of the neroli oil. They determined, as shown in Table 6.2, a decrease of the free monoterpene alcohols content and of those esterified (geraniol, linalol, nerol), and of the sesquiterpene alcohols (farnesol* and nerolidol*), with an increase of α-terpineol and 6-methyl-5-hepten-2-one, as well as of another compound with a structure similar to 6-methyl-5-hepten-2-one, later identified by Jeannot et al. (2005) as 6-methyl-5-hepten-2-ol. These phenomena were more evident after long periods of storage if the water was kept in contact with air. In these conditions, the racemization of linalol and of α-terpineol would also occur. Moreover, the odor note would turn green. Such behavior would therefore suggest that, in order to obtain a good quality flower water absolute, it would be preferable to perform the extraction in short time (maximum 10 days) after the distillation.

Boelens and Oporto (1991) also reported the following composition in the class of components determined in bitter orange flower water absolute: monoterpene hydrocarbons (2%), oxygenated monoterpenes (80%), oxygenated sesquiterpenes (3%), and others (15%).

6.4 COMPOSITION OF BITTER ORANGE FLOWER ABSOLUTE

The quantitative composition of the bitter orange flower absolute is reported in Table 6.3.

In addition to the data reported in Table 6.3, more information can be found in literature on the composition of bitter orange flower absolute.

Kaiser and Lamparsky (1982) studied the nitrogen-containing components present in bitter orange flower absolute and in its headspace. The components determined were methyl anthranilate (6.5%), indole (5.0%), phenylacetaldoxime (2.0%), phenylacetonitrile (1.5%), 1-nitro-2-phenylethane (0.4%), methyl *N*-acetyl anthranilate (0.03%), ethyl anthranilate (tr), and methyl *N*-formyl anthranilate (tr). The following components were also identified: methyl *N*-methyl anthranilate, decanalaldoxime, nonanalaldoxime, undecanalaldoxime, geranialaldoxime, neralaldoxime, farnesaloxime*, methylheptenone oxime, geranylacetone oxime, methylheptenone isooxazoline, decylnitrile, nonylnitrile, undecylnitrile, 1-methyl-2-ethylpyridine, 2-acetylpyridine, quinoline, 2-methylquinoline, 6-methylquinoline, and 2-methylbutynylnitrile (the latter only in headspace). A few years later, one of these authors (Kaiser, 1986) identified in bitter orange flower absolute traces of 2-isobutyl-4-methylpyridine and di 2-isobutenyl-4-methylpyridine.

Yang and Lee (1988), in three samples from Egypt, France, and Morocco, found, respectively, 2.650, 545, and 680 ppm of bergapten and a undetermined amount of epoxibergamotin. The authors attributed to bergapten the phototoxic activity of bitter orange flower water absolute.

Toyoda et al. (1993) identified in bitter orange flower water absolute the same components identified in neroli oil previously reported in this chapter.

During the same year, Surburg et al. (1993) identified the heterocyclic nitrogen-containing components following listed 0.01% of 3-phenylquinoline and 2-phenyl-2-propylpyridine; 0.001% of 4-methyl-3-phenylpyridine, 2-methyl-5-phenylpyridine, and 2-ethyl-5-phenylpyridine; 0.0005% of 3-phenyl-4-propylpyridine; and 0.0002% of 4-ethyl-3-phenylpyridine.

Boelens and Oporto (1991) also reported the following composition in the classes of components for bitter orange flower absolute: monoterpene hydrocarbons (7%), oxygenated monoterpenes (60%), sesquiterpene hydrocarbons (1%), oxygenated sesquiterpenes (15%), and others (18%).

6.5 ADULTERATION

The bitter orange flower oil (or neroli) is essential for perfumery. For a long time, its production has been limited and its cost on the market has been considerably high. The annual production of neroli in Tunisia and Morocco is ca. 1500 kg, representing more than 90% of the worldwide production. A

TABLE 6.2
Percentage Composition of Bitter Orange Flower Water Absolute

	1	2a	2b	3a	3b	4	5
δ-3-Carene	–	–	–	–	–	–	–
Limonene	0.5[a]	0–2.2	0–2.0	–	–	–	–
(*E*)-β-Ocimene	0.2	–	–	–	–	–	–
(Z)-β-Ocimene	0.5[a]	–	–	–	–	–	–
α-Pinene	–	–	–	–	–	0.2	–
β-Pinene	1.1	0–1.1	–	–	–	0.3	–
β-Caryophyllene	–	0.6–2.0[b]	1.2–3.2[b]	–	–	–	–
Geranial	–	–	–	–	–	0.4	–
6-Methyl-5-hepten-2-one	–	–	–	0.32	21.0	0.4	0.5–6
6-Methyl-5-hepten-2-ol	–	–	–	–	3.60	–	0–10
Citronellol	0.2	–	–	–	–	2.5	–
Eugenol	0.5	–	–	–	–	–	–
Geraniol	6.4	3.8–7.6	4.8–11.3	8.25	0.11	5.0	0.5–7
Linalol	44.2	39.7–53.1	40.8–44.3	47.80	27.0	70.1	40–60
Nerol	2.6	1.7–2.7	1.8–3.7	2.86	0.97	0.4	1.2–3.5
Terpinen-4-ol	–	0.6–2.0[b]	1.2–3.2[b]	–	–	1.4	0.5–1.5
α-Terpineol	18.5	13.3–22.1	10.7–22.6	19.80	25.0	10.1	15–25
Farnesol	0.5*	0–1.9*	0.2–4.6*	1.0*	0.06*	–	0–0.5[d]
Nerolidol	1.7*	0–1.5*	0.2–3.5*	0.70*	0.23*	–	0–1[e]
Phenylethyl alcohol	1.9	0.9–3.9	0.6–1.2	2.54	0.57	–	0.5–5
2,6-Dimethyl-7-octen-2,6-diol	–	–	–	–	–	–	0–1
p-Mentha-1,8-diol	–	–	–	–	–	–	0–0.5
Geranyl acetate	0.5	0–1.1	0.7–1.3	0.70	0.13	–	0–0.2
Geranyl formate	–	–	–	–	–	0.6	–
Linalyl acetate	–	0–2.4	0–9.4	0.18	0.05	3.0	–
Neryl acetate	0.5	0–0.6	–	0.33	0.07	–	1–5
cis-Linalol oxide	–	0.6–2.3	0.8–1.7	5.10[c]	6.0[c]	1.3	–
trans-Linalol oxide	–	1.0–3.7	1.4–2.7	5.10[c]	6.0[c]	0.7	0.5–3
Indole	0.1	0–3.2	0.6–5.4	1.50	1.40	0.3	0–2
Methyl anthranilate	4.1	2.0–11.4	2.9–4.5	2.97	4.30	1.9	1–6
Phenylacetonitrile	–	0.8–5.0	1.5–3.2	–	–	–	1–5

Notes: tr, traces; *, correct isomer not characterized; [a] limonene + (*Z*)-β-ocimene; [b] β-caryophyllene + terpinen-4-ol; [c] *cis*- + *trans*-linalol oxide, [d] (*E*,*E*)-farnesol; [e] (*E*)-nerolidol.

Appendix to Table 6.2

1. Buccellato (1981). One sample.
2. Prager and Miskiewicz (1981). (a) Range of the composition of 11 commercial Mediterranean samples, considered genuine by the authors; (b) range of the composition of 5 commercial samples, considered adulterated by the authors due to the high content of one or more of the following components: geraniol, terpinen-4-ol, farnesol*, nerolidol*, linalyl acetate, indole; GC/FID and GC/MS on capillary column (33 m × 0.5 mm) coated with Carbowax 20M; relative percentage of peak areas.
3. Remy et al. (1993). Tunisian oils from the productive seasons 1990–1992; (a) oils extracted after a storage period shorter than 10 days, (b) oils extracted after one month of storage.
4. Ayadi et al. (2004). Tunisia; one laboratory sample; GC/FID and GC/MS on capillary column coated with DB-5; Adams 2001, NIST 98* and Wiley 6* MS libraries; relative percentage of peak areas.
5. Jeannot et al. (2005). Morocco; 10 samples from the productive seasons 2001–2004; GC/FID and GC/MS on capillary columns coated with apolar and polar stationary phases; relative percentage of peak areas.

TABLE 6.3
Percentage Composition of Bitter Orange Flower Absolute

	1	2a	2b	3
Limonene	5.1[a]	0–3.6	1.1–9.6	2.8
Myrcene	0.1	–	–	–
(*E*)-β-Ocimene	0.6	0–2.2	0.2–1.3	–
(*Z*)-β-Ocimene	5.1[a]	–	–	–
α-Pinene	tr	–	–	–
β-Pinene	0.4	0–2.7	0.2–1.3	2.4
β-Caryophyllene	–	0–0.8[b]	–	–
Citronellol	0.5	–	–	–
Eugenol	0.3	–	–	–
Geraniol	1.6	0–2.0	0.2–4.0	–
Linalol	32.0	34.7–47.5	26.9–37.0	55.2
Nerol	0.9	–	–	3.7
Terpinen-4-ol	–	0–0.8[b]	–	–
α-Terpineol	2.4	1.5–3.7	0.3–4.0	–
Farnesol	7.7*	3.6–15.4*	0–8.5*	2.1[c]
Nerolidol	7.6*	4.8–8.9*	0–9.8*	3.7[d]
Phenylethyl alcohol	4.5	0–2.1	1.0–12.2	tr
Citronellyl acetate	0.1	–	–	–
Geranyl acetate	0.6	0–1.3	0.3–2.5	3.7
Linalyl acetate	16.8	14.2–20.9	12.2–25.8	–
Neryl acetate	0.8	–	–	1.9
Indole	1.0	2.6–9.9	1.4–4.4	–
Methyl anthranilate	3.0	1.0–4.3	1.8–5.0	–
Phenylacetonitrile	–	0–1.1	0–1.2	–

Notes: *, correct isomer not characterized; [a] limonene + (*Z*)-β-ocimene; [b] β-caryophyllene + terpinen-4-ol; [c] (*E*,*E*)-farnesol; [d] (*E*)-nerolidol.

Appendix to Table 6.3

1. Buccellato (1981). One sample.
2. Prager and Miskiewicz (1981). (a) Range of the composition of 11 commercial Mediterranean samples, considered genuine by the authors; (b) range of the composition of 7 commercial samples, considered adulterated by the authors due to the high content of: limonene and/or phenylethyl alcohol and/or a fatty alcohol (C_{20} or C_{22}) or due to the absence of nerolidol* and farnesol*; the high content of indole (9.9%) was not considered indicative of adulteration but caused by the production process (flowers packed too tightly or processed too slowly during solvent extraction); in the other oils considered genuine the maximum content of indole was 6.1%; GC/FID and GC/MS on capillary column (33 m × 0.5 mm) coated with Carbowax 20M; relative percentage of peak areas.
3. Hethelyi et al. (1998). One sample. These authors also analyzed two samples of concrete with very similar composition. Major differences were due to the presence of linalyl acetate (5%) in both samples and of phenylethyl alcohol (1.7%) in only one sample.

small amount of neroli is also produced in Egypt, Spain and Comorros (not exceeding 150 kg totally). The annual production of neroli depends on the domain of the market of bitter orange flowers concrete, which subtracts the raw material for the production of neroli. The production of concrete is ca. 800 kg from the same geographic origins. The price of neroli actually ranges from 3,400 USD/kg to 3,700 USD/kg and, depending on the quality, can increase up to 4,700 USD/kg for organic neroli.

Due to this high cost, over the years the producers and the users of natural flowers have tried to obtain less expensive products with odor characters similar to neroli oil to use as substitutes and sometimes as adulterants.

In the last decade, the adulteration has become more subtle, along with the development of the analytical techniques and the increased availability of the raw material. These are mainly of the following types:

- The first, more or less rough, consists of the adjustment of the composition of main components and of the correction of the physicochemical indices, to imitate the genuine oils.
- The second, more refined, also consists of the reproduction of the odor character of the natural oil.

The products commonly used as adulterants are numerous:

- Citrus essential oils, different from neroli, that are present on the market at a lower price
- Single components natural or synthetic
- Reconstituted essential oils that present chemical composition and sensorial properties close to those of genuine neroli oils

A detailed list of the most common ingredients used to prepare a synthetic neroli oil has been reported by Anonis (1985). The adulteration can also be performed by distilling, along with bitter orange flowers, sweet orange flowers, bitter orange foliage, and any other vegetable material containing components of the same nature as those normally present in neroli oil. Obviously, the presence of vegetable material different from the bitter orange flowers can occur by accident or be simply due to the poor selection of the raw material. Some anomalies of the composition and the odor character can be also due to the technology and the inadequate condition used, the unclearness of the stills and containers, and the modification of the natural composition during the storage of the oil.

The techniques and the analytical methods used for the detection of adulterations in neroli oil, also common for other essential oils, consist of the determination of classical indices mentioned by the International Regulations and by the Pharmacopoeias (refractive index, optical rotation, density, solubility, acidity number, esters number, etc.), indicating for each oil the minimum and maximum values or the ranges' variability. Modern instrumental methods, in particular the chromatographic and spectroscopic ones, are also applied in order to determine the content of each component and specific characters of some of these, as the enantiomeric distribution and the isotopic ratios.

Guenther (1949) asserted that the adulterations most commonly used for neroli oils are the additions of bitter orange petitgrain, either as a whole or deterpenated. These additions could be detected by measuring the total amount of esters. In fact, this adulterant is characterized by amounts of linalyl acetate much higher than those in neroli oil. Guenther suggested, however, to proceed by an accurate sensorial test, since the chemical analyses at that time could be inadequate for revealing the adulteration. Guenther also reported comments published by Naves (1948), who asserted that the enantiomeric distribution of linalol and the amount and composition of the free alcohols' fraction are influenced by the duration of the distillation. Moreover, analyzing the esterified alcoholic fraction after saponification, the addition of synthetic linalyl acetate (presence of partially racemic linalol) and of terpinyl acetate could be revealed.

The EP (2004), AFNOR (1995), and ISO (2002) regulations provide the following limits for neroli oil:

	EP 2006	**AFNOR 1995**	**ISO 3517:2002**
Relative density	0.863–0.880	0.864–0.876	0.863–0.876
Refractive index	1.464–1.474	1.468–1.474	1.464–1.474
Optical rotation	(+)1.5°–(+)11.5°	(+)2°–(+)11°	(+)2°–(+)11°
Acid value	max. 2.0	max. 2.0	max. 2.0
Ester value	–	26–60	26–60

continued

Chromatographic profile			
Limonene	9.0%–18.0%	9%–18%	9%–18%
Myrcene	–	1%–4%	1%–4%
(*E*)-β-Ocimene	–	3%–8%	3%–8%
α-Pinene	–	max. 2%	tr–2%
β-Pinene	7.0%–17.0%	7%–17%	7%–17%
Sabinene	–	–	tr–3%
Linalol	28.0%–44.0%	28%–44%	28%–44%
α-Terpineol	2.0%–5.5%	2%–5.5%	2%–5.5%
(*E*,*E*)-Farnesol	0.8%–4.0%	1%–4%	1%–4%
(*E*)-Nerolidol	1.0%–5.0%	1%–5%	1%–5%
Linalyl acetate	2.0%–15.0%	3%–15%	3%–15%
Geranyl acetate	1.0%–5.0%	1%–5%	1%–5%
Neryl acetate	max. 2.5%	max. 2.5%	tr–2.5%
Methyl anthranilate	0.1%–1.0%	–	–
Chiral purity			
S (+)-Linalol	max. 30%	–	–
S (+)-Linalyl acetate	max. 5%	–	–

max.: maximum.

Prager and Mishiewicz (1981) esteemed some samples of neroli oil to be adulterated due to their low content of monoterpene hydrocarbons (2.8%, 14.8%, and 22.1%, respectively) when compared with the average content of more than 39% determined by these authors in genuine oils. One or more of the oils considered adulterated presented high content of farnesol*, nerolidol*, and linalyl acetate. In one of these, ca. 2.5% and 3% of *cis*- and *trans*-linalol oxide were also present. The authors considered adulterated some samples of bitter orange flower water absolute for the high content of linalyl acetate (5.9%–9.4% vs. the average value of 1% determined in genuine samples); and/or of terpinen-4-ol (2.6%, 3.2%); and of nerolidol* (2.9%, 3.5%), farnesol* (2.8%, 4.6%), indole (5.4%), and geraniol (11.3%) that in genuine sample reached the maximum of 2%, 1.5%, 1.9%, 3.2%, and 7.6% respectively. In the same paper, those samples of bitter orange flower absolute that presented a high level of phenylethyl alcohol (4.7%–12.2% vs. the range of variation of 0.3%–2.1% determined in genuine samples) were also considered adulterated. Two of the adulterated oils also presented high values of limonene (6.2% and 9.6% vs. the range of 0%–3.6% determined in genuine oils), and one of these oils contained high amount (13.5%) of one linear chained alcohol of 20 or 22 carbon atoms. One of the oils considered adulterated also did not contain nerolidol*, which in genuine samples was present with a range of 4.9%–8.9%. The high amount of indole (9.9%) in one sample was not considered sign of adulteration, but a consequence of the processing conditions. In fact, it was Guenther's opinion (1949) that if the flowers were packed too tightly or processed too slowly an increase of the indole content would result.

Frey (1988) suggested using the determination of dihydrolinalol to reveal the addition to neroli oil of synthetic linalol, most of which contain dihydrolinalol in amounts ranging from 0.5% to 2%. Frey asserted that by applying the selective ion monitoring in GC/MS it is possible to determine 50 ppm of dihydrolinalol in neroli oil, corresponding to the addition of 0.5% to 1% of synthetic linalol.

Juchelka et al. (1996) and Mosandl and Juchelka (1997), from the results obtained analyzing numerous samples of neroli oil extracted in laboratory by distillation and solvent extraction, samples of industrially processed oils of ensured origin, and samples of commercial oils, come to the conclusion that the presence of the *R*-(–)-linalyl acetate (>95%), of *S*-(–)-β-pinene (>98%), and *S*-(+)-*E*-nerolidol (>98%) can be considered tools of extreme importance for the quality control of neroli oils. Even if the enantiomeric distribution of limonene ranged widely, it could be used as a characteristic marker of genuineness. For this compound was indicated a value of the enantiomer *R*-(+) >

93% for a genuine neroli oil. The enantiomeric distributions of linalol, α-terpineol, terpinen-4-ol, and α-pinene were not considered by these authors as reliable for the neroli oil characterization. In fact, the enantiomeric distribution of linalol presented a ratio of the *R*-(–) and *S*-(+) isomers of about 90:10 in the solvent cold-extracted flowers, but this ratio shifted toward racemization up to 70:30 in the oils obtained by distillation, depending on the length of the process. The α-terpineol present in neroli oil ranged between 2% and 7%, representing an artifact generated during the distillation process. This is, in fact, almost absent in the extracts obtained by solvent cold extraction. The enantiomeric distribution of the S-(–) and R-(+) isomers was about 70:30. Terpinen-4-ol is usually present at trace levels as racemic mixture. Lastly, the enantiomeric distribution of α-pinene varied between wide ranges. Dugo et al. (2010) determined values of the enantiomeric distribution of β-pinene and of linalyl acetate in agreement with those determined by Juchelka et al. (1996) and by Mosandl and Juchelka (1997). The same authors found that the enantiomeric distribution of limonene in Egyptian oils varied within narrow ranges. More information is reported in Chapter 7.

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7 The Chiral Compound of Citrus Oils

Luigi Mondello, Rosaria Costa, Danilo Sciarrone, Giovanni Dugo

CONTENTS

7.1 INTRODUCTION

Chirality is a chemical concept that finds wide application in nature. A great part of plants present a highly typical distribution of enantiomers, therefore resulting as a very useful tool for identification, characterization, and detection of human interference. The enantiomeric distribution depends upon partially investigated biochemical pathways, which lead to the formation of chiral

molecules that are synthesized by plants not for a chance function, but quite often for a specific one. Enantiomers can work as growth regulators, support plant reproduction, as well as defend the plants from external attacks. Within a vegetable individual, enantiomers show not only quantitative differences—although sometimes they can be present as racemic mixtures—but they also exert a different biological activity on animal organisms based on their stereochemical configuration. The highly specific interaction between the tridimensional structure of chiral molecules with the biological receptors located at the specialized districts of the human body is demonstrated by the fact that, for a couple of enantiomers, it is possible to observe different activity or sometimes none at all for one of them. This observation is in some cases very easily seen, especially when the interaction between enantiomer and receptor occurs through the olfaction. Enantiomers can differ in odor (Theimer et al., 1977), such as (+)-(2R,4S)-carveol, which smells musty, and (–)-(2S,4R)-carveol, which smells minty (Friedman and Miller, 1971).

Today, an enormous amount of information is available on this topic, boosted by the recent (in 2004) Nobel Prize in Physiology or Medicine awarded to Buck and Axel for their discovery of odorant receptors and the organization of the olfactory system. If the specificity of interaction at olfactive level has been intuitive enough, the same has not been so under a pharmacological point of view: the eclatant case of thalidomide, with its (R)-enantiomer having sedative action and its (S)-enantiomer teratogenic, has shocked the scientific community and brought attention to the use of chiral mixes in therapy or in food.

Therefore, research in stereochemistry has suddenly received an impressive stimulus, crossing the edges of technological hindrance and exploiting all the resources available for the separation and purification of optically active compounds. In chromatography, a big step forward has been made by the introduction of new stationary phases, the most popular being α-, β-, and γ-cyclodextrin derivatives. Pioneer works in the field were carried out by Armstrong et al. (1990); Li et al. (1990); and Schurig and Novotny (1988).

In this chapter, data present in literature on the enantiomeric distribution of some components of the volatile fraction of citrus oils are reported. Many papers are related to more than one oil, or sometimes the same authors published a series of papers where the same (or a similar) analytical method was used to determine the enantiomeric distribution of some components in the different oils. Since in this chapter, for an easier and clearer approach, the single citrus oils will be treated separately, a brief summary of the most used analytical techniques available in literature will be given. In this way, the section dedicated to the single oils will focus mainly on the results reported in literature and their discussion.

Because the enantiomers present in essential oils are embedded in complex matrices, and quite often in low amount, it is sometimes necessary to carry out a preseparation step before injecting samples in a GC system. To this end, traditional techniques such as high-performance thin layer chromatography (HPTLC) or high performance liquid chromatography (HPLC), off-line or online coupled to GC, are still successfully employed. But other, more advanced techniques have been introduced in the past years that allow for the isolation and separation of enantiomers through the application of multidimensional gas chromatography (MDGC).

In this field, Mosandl's research group produced a series of papers (Mosandl et al., 1990; Hener et al., 1990a,b; Kreis et al., 1991; Mosandl, 1995) based on the use of a fully automated MDGC system (Siemens Sichromat 2) with a "live switching" coupling piece ("live-T-piece") that was applied to determine some of the chiral components (α-pinene, β-pinene, and limonene) in citrus oils. The MDGC system used in this case consisted of a double oven, having a Carbowax 20M as precolumn and a heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin as main column. Portions of eluate were transferred from the first dimension to the second one by exploiting the heart-cutting method. Figure 7.1 shows the chiral separation of α-pinene, β-pinene, and limonene in a standard mixture, as well as in real samples of citrus oils using the heart-cutting MDGC system. Two articles published in 1997 by the same research group (Mosandl and Juchelka, 1997a,b), where they applied the same analytical technique and eventually a preseparation by HPTLC for trace level compounds, report interesting

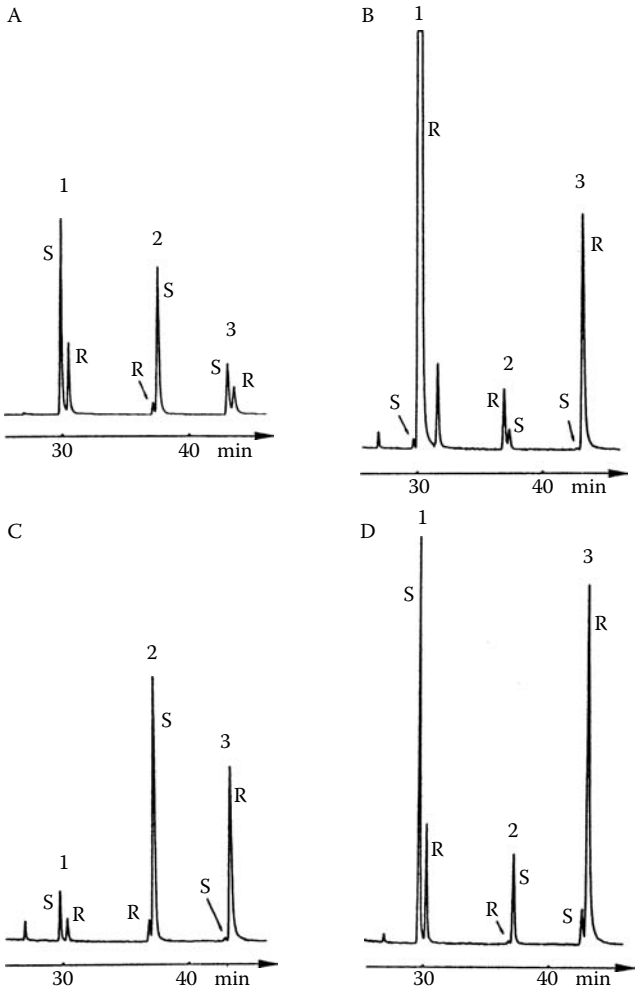

FIGURE 7.1 (A) MDGC analysis of α-pinene (1), β-pinene (2), limonene (3), using heptakakis (2,3-tri-*O*-β-cyclodextrin as chiral stationary phase. (B) MDGC analysis of self-prepared orange oil. (C) MDGC analysis of self-prepared lemon oil. (D) MDGC analysis of lime oil. (This image originally appeared in Mosandl, A., and V. Schubert, *J. Essent. Oil Res.* 2, 121–132, 1990. Printed with permission of Allured Business Media.)

results relative to characteristic enantiomeric purities of selected compounds in citrus essential oils. Mosandl and Juchelka reported the following results:

	Bitter Orange Oil	**Bitter Orange Petitgrain Oil**	**Neroli Oil**	**Sweet Orange Oil**	**Lemon Oil**	**Bergamot Oil**
Linalyl acetate	R-(–)>96	R-(–)>97	R-(–)>95	–	–	R-(–)>99
Linalol	R-(–)>70	R-(–)>70	R-(–)>70	S-(+)>90	R-(–)50–80	R-(–)>99
Limonane	R-(+)>99	–	R-(+)>93	R-(+)>99	R-(+)>97	R-(+)>97
α-Pinene	R-(+)>85	S-(–)>80	S-(–)>85	R-(+)>99	S-(–)67–80	S-(–)66–74
β-Pinene	S-(–)>96	S-(–)>98	S-(–)>96	–	S-(–)>93–	S-(–)>91
Sabinene	R(+)>93	–	–	R-(+)>99	–	–
(*E*)-Nerolidol	S-(+)>80	S-(+)>70	S-(+)>98	–	–	S(+)>80

Around the same time, Casabianca and his co-workers (Casabianca et al. 1995; Casabianca and Graff, 1994, 1996) drew on the same apparatus for the investigation of chiral components present in citrus oils.

Much work was carried out in the following years by Dugo's research group, although some preliminary papers were already presented earlier (Dugo et al., 1992a,b, 1993; Cotroneo et al., 1992). The group developed an LC-GC method based on the use of a Dualchrom 3000 Series (Fisons), the core consisting of an on-column type interface and partially concurrent eluent evaporation. This led to the separation and quantification of chiral alcohols present in various citrus oils, such as linalol (Dugo et al., 1994a,b; Mondello et al., 1996). Fractions of analytes with the same polarity were consecutively and selectively transferred to the GC, where they underwent separation.

Around the end of 1990s, the same research group published several papers on the enantio-GC analysis of citrus oils, performed by means of a newly designed MDGC system (Mondello et al., 1997, 1998a,b,c,d, 1999; Dugo et al., 2000). Completely developed in laboratory, the innovative feature of this system was the transfer device that used mechanical valves instead of flow-switching devices. The new MDGC system made possible the separation and characterization of sabinene, β-pinene, limonene, linalol, linalyl acetate, terpinen-4-ol, and α-terpineol on chiral stationary phases, mainly by applying the heart-cutting technique shown in Figure 7.2 for the chiral separation of some monoterpene hydrocarbons and oxygenated monoterpenes in cold-pressed lemon oil.

It is also worth remembering that during this time, Coleman et al. (1998) proposed to use solid-phase microextraction (SPME) to overcome the use of solvent necessary for sample dilution prior to GC analysis. The paper demonstrated that fiber exposure into the headspace of the essential oil produces results quantitatively comparable to those of conventional injection without compromising the enantiomeric ratios. More recently, Sciarrone et al. (2010) evaluated a newly developed high-performance MDGC system employed in the analysis of a series of chiral compounds present in mandarin essential oils. The system, previously used for the investigation of noncitrus oils, consisted of a simple transfer device for the rapid and sequential re-injection of analytes from the first to the second (chiral) column. The novelty of such a system, when compared to the valve-based ones, was the absence of restrictions due to the temperature of the transfer device, very low dead volumes, and the possibility of multiple heart cuts through a pressure balance mechanism.

Within this chapter, each citrus oil will be singularly treated. For each oil, data published approximately between 1990 and 2001, and already revised by Mondello et al. (2002), will be summarized in a table and in a schematic appendix containing useful information and comments. Then, the most recent results on the enantiomeric distribution of the components of citrus oils will be reported in detail in the tables and discussed. The data in the tables, drawn from the original paper, will represent the composition of a single sample, mean values, or, when possible, variability ranges. These are expressed by two decimal figures, or by one or no decimal if this is the approximation in the original paper. When available, the appendix to the tables will provide information regarding geographical and botanical origin, the production technology, the number of samples relative to the given data, and the analytical technique.

In the appendix, if the column dimensions are reported in parentheses, the first number indicates the length (meters), the second indicates the internal diameter (millimeters), and the third indicates the film thickness of the stationary phase (micrometers). Unless differently specified, all the columns were of fused silica. For the chiral columns, if reported in the original paper, the stationary phase used to dilute the chiral stationary phase has also been indicated.

7.2 LEMON (*CITRUS LIMON* [L.] BURM) OIL

7.2.1 1990–2001

Table 7.1 reports a summary of the results published after 1990 on the enantiomeric distribution of the components of lemon oil, already revised by Mondello et al. (2002). The components are listed separately according to industrial and laboratory-extracted oils.

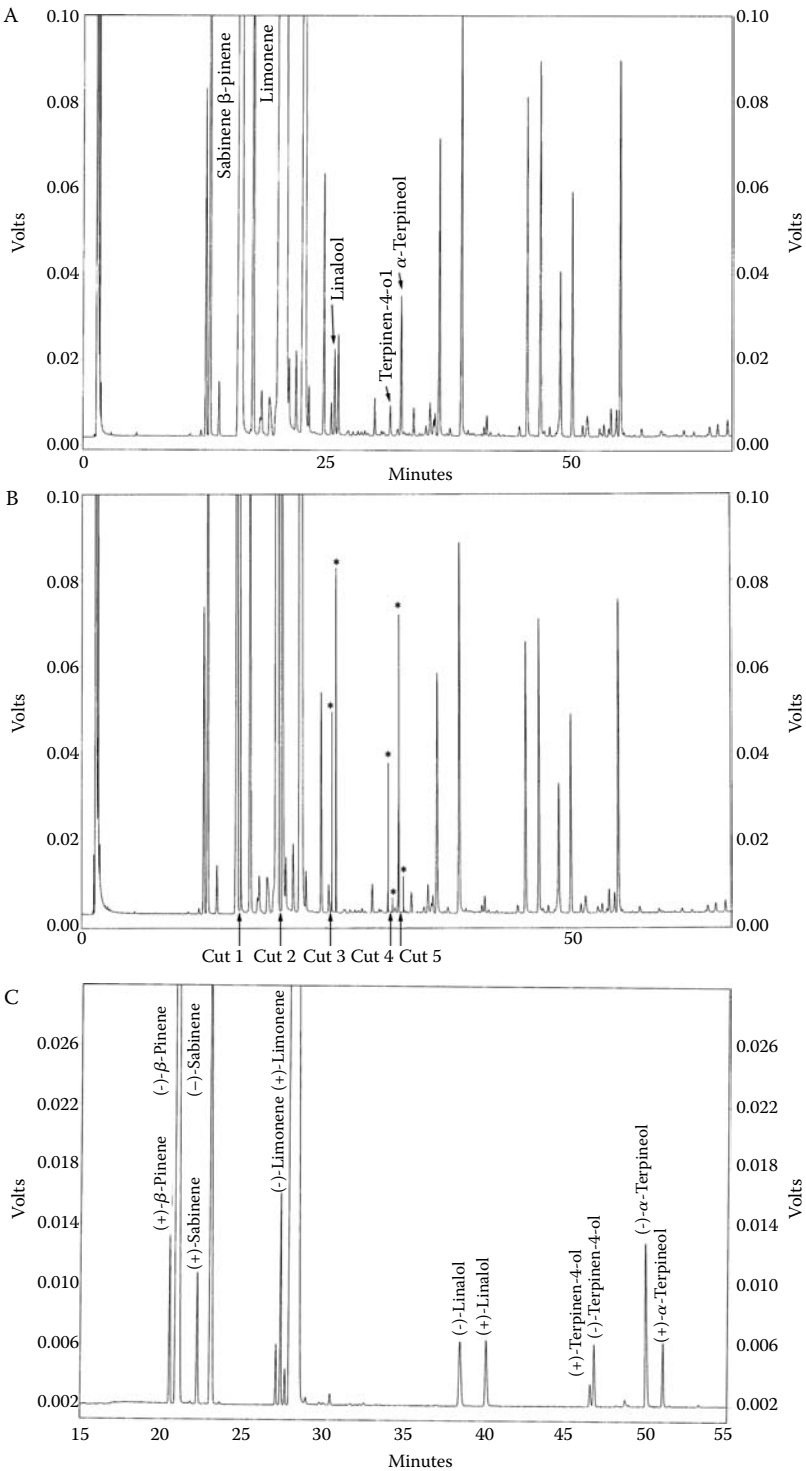

FIGURE 7.2 (A) GC chromatogram of a cold-pressed lemon oil obtained with a SE-52 column. (B) GC chromatogram of a cold-pressed lemon oil obtained with the SE-52 column, with the five heart cuts. (C) GC-GC chiral chromatogram of the transferred components. (From Mondello, L., et al., *J. High Resolut. Chromatogr.* 22, 350–356, 1999. Reproduced with permission of Wiley-VCH Verlag GmbH.)

TABLE 7.1
Enantiomeric Distribution of Some Volatile Components of Lemon Oils (1990–2001)

		Industrial Oils			Laboratory Oils		
		Cold-Pressed	**Distilled**	**Commercial**	**Solvent**	**Distilled**	**SFE**
α-Pinene	R-(+)	30–38			23–33	28–34	
	S-(–)	70–62			77–67	72–66	
β-Pinene	R-(+)	4.2–7.0	6.4–6.6	3.5–7.8	4–6	5–6	
	S-(–)	95.8–93.0	93.6–93.4	96.5–92.2	96–94	95–94	
Sabinene	R-(+)	12.5–15.5	12.7–14.6	16.9–94.0			
	S-(–)	87.5–84.5	87.3–85.4	83.1–6.0			
Limonene	S-(–)	1–2.6	1.7	5.0–14.5	1–2	1–2	
	R-(+)	99–97.4	98.3	95.5–85.5	99–98	99–98	
Linalol	R-(–)	49.5–71.5	51–60.0	5.7–23.3			32–46
	S-(+)	50.5–28.5	49–40.0	94.3–76.7			68–54
Citronellal	S-(–)			72			
	R-(+)			28			
Terpinen-4-ol	S-(+)	13.7–32.5	28–28.5	28.9–75.4			
	R-(–)	86.3–67.5	72–71.5	71.1–24.6			
α-Terpineol	S-(–)	64.2–82.0	76.4–77.0	8.8–67.1			
	R-(+)	35.8–18.0	23.6–23.0	91.2–32.9			

Appendix to Table 7.1

- The results reported in Table 7.1 and in this appendix, for the different categories of lemon oils, are taken from the following original papers:
  - Cold-pressed industrial oils: Mosandl et al. (1990); Hener et al. (1990a); Mosandl (1995); Rocca et al. (1992); Dugo et al. (1992a, 1994a, 2001); Bicchi et al. (1994); Mondello et al. (1996, 1997, 1998a, 1999); Dellacassa et al. (1997a).
  - Distilled industrial oils: Dugo et al. (1994a, 2001).
  - Commercial oils: Werkhoff et al. (1993); Mondello et al. (1999); Dugo et al. (2001).
  - Laboratory solvent-extracted oils: Mosandl et al. (1990); Hener et al. (1990a); Kreis et al. (1991); Mosandl (1995).
  - Distilled laboratory oils: Kreis et al. (1991).
  - SFE-extracted oils: Casabianca and Graff (1996).
- Most of the results summarized in Table 7.1 were obtained by multidimensional gas chromatography (MDGC), with the exception of the results reported by Bicchi et al. (1994) obtained by direct analysis on chiral column, those by Dugo et al. (1992a, 1993) by coupling of conventional and chiral columns and those by Mondello et al. (1994a, 1996) obtained by online coupling HPLC-HRGC.
- Not included in the table are the results relative to the enantiomeric distribution of lemon concentrates obtained by Ravid et al. (1995) for α-terpineol (4S(–)/4R(+): 67/33) and Werkhoff et al. (1993) for methyl jasmonate ((–)-(1R,2R)/(+)-(1S,2S): 99/1), for methyl epijasmonate ((+)-(1R,2S)/(–)-(1S,2R): 99/1) and for citronellal (S(–)/R(+): 89/11). Also not considered in the table is the enantiomeric distribution of α-terpineol (S(+)/R(–): 38.4/61.6), obtained by monodimensional GC by Bicchi et al. (1994) probably influenced by coelutions.
- The data reported in the table relative to industrial oils and to laboratory-extracted oils, both by distillation and by solvent extraction are in good agreement with each other. It should be pointed out that Mondello et al. (1996) observed a slight tendency to racemization of terpinen-4-ol for all the Italian industrial oils obtained by distillation from the residues of the cold extraction, and that the oils from Uruguay (Dellacassa et al. 1997a) showed enantiomeric distributions similar to the distilled ones, probably due to some drawback of the extraction technology (e.g., heating of the centrifuge during the oil separation). Mondello et al. (1999) also observed that in Italian industrial oils the enantiomeric distribution of limonene, sabinene and β-pinene showed constant values during the entire productive season; the enantiomeric distribution of α-terpineol and of terpinen-4-ol varied with a similar behavior within quite wide ranges; the enantiomeric distribution of (–)-linalol ranged widely: at the beginning of the season (October) the enantiomeric excess of (–)-linalol was ca. 33, decreasing from October to May, and at the end of the season reached the initial values.
- Commercial oils reported in the table are clearly adulterated as revealed by one or more of the enantiomeric ratios.

7.2.2 1999–2009

Table 7.2 reports the results of most of the research published after the review by Mondello et al. (2002) on the enantiomeric distribution of the components of lemon oil. Papers relative to columns 1 and 2, although published before 2001, are here inserted because they were not revised in the previous edition of this book. As can be seen, the papers published on this topic during these years are very few (only five). The first paper (Hara et al., 1999), is relative to a single sample and some selected chiral components have been determined, such as α-pinene, β-pinene, limonene, linalol, α-terpineol, and citronellal. The results for all the couples of enantiomers are in good agreement with those reported for cold-pressed oils or extracted laboratory solvent presented in Table 7.1. In particular, the enantiomeric ratio of linalol shows that the sample has similar values to those of the SFE-extracted oil. The paper by Mitiku et al. (2001) is relative to different cultivars (Eureka, Monachello, and Femminello) grown in Japan and reports the chiral ratio of limonene, the major monoterpene present in this oil, in agreement with data previously reviewed by Mondello et al. (2002) (see Table 7.1) for cold-pressed lemon oils, while the enantiomeric ratio of α-pinene, β-pinene, and sabinene presents a wider range when compared to the cold-pressed genuine oil reported in the same table. One year later, Lorenzo et al. (2002) presented some data obtained with a MDGC system based on valves. The results were comparable to those previously obtained by the same group (Dellacassa et al., 1997a) on Uruguayan lemon oils. However, in this last paper, the enantiomeric ratio of α-pinene was also reported, and was very similar to that of Italian genuine cold-pressed oils reported in the past (see Table 7.1). Gionfriddo et al. (2004) studied the enantiomeric distribution of different cultivars of Sicilian lemon oils extracted in laboratory. In particular, 10 samples of Femminello Comune and 9 samples of Monachello (fruits collected every 15 days from October 2002 until January 2003) were investigated. The results are grouped together in column 4a of Table 7.2, from where it can be concluded that the values reported (with the exception of limonene for verdelli oil) fall in a range wider when compared to that of industrial lemon oils (see Table 7.1). The Femminello fruits were also sampled after the summer (September 2003) when they are usually green (Verdelli). The results obtained are reported in column 4b of Table 7.2. They appear quite similar to those of Femminello sampled during fall/winter, with the exception of linalol and α-terpineol, which resulted in a higher average value for the green fruits.

It seems also noteworthy that the paper by Nhu-Trang et al. (2006) reports the enantiomeric ratio for citronellal. Even if the paper is limited to the determination of only this compound, it deserves mention because literature on chiral components in lemon oil generally lacks this information. To our knowledge, only one paper (Werkhoff et al., 1993) reports the enantiomeric ratio of citronellal. The results obtained by Nhu-Trang et al. (2006) demonstrated that S-(–)-citronellal predominated in lemon essential oils and lower amounts reveal adulteration with citronellal coming from other natural sources (e.g., citronella, lemongrass and sweet orange oils and *Litsea cubeba*).

Finally, Dugo P. (2009, Personal communication) have investigated 87 industrial samples of cold-pressed lemon oil. In this study, the chiral ratio of α-thujene, camphene, and α- and β-phellandrene have been reported for the first time. Chiral ratios determined for the other components are similar to those previously reported in literature, and the enantiomer excess trends throughout the production season are equal to the values determined by Mondello et al. (1999) for the same periods in two productive seasons (see Figure 7.3). For the components not subjected to study in this previous research, no variations were noted in the enantiomer excess of α-thujene, which remained constant, while the values for (–)-citronellal were found to be moderately higher in the oils extracted from February to May, with respect to the oils produced in the previous months. With regard to (–)-camphene, its enantiomer excess values resulted moderately higher in the samples from January to March, while lower values were found for (+)-β-pinene during the same period. The enantiomer excess values for (+)-β-phellandrene increased considerably during the production season with an opposite trend compared to linalol.

TABLE 7.2
Enantiomeric Distribution of Some Volatile Components of Lemon Oils (1999–2009)

		1	2	3	4a	4b	5	6
α-Thujene	S-(+)*							0.7–1.2
	R-(–)*							99.3–98.8
α-Pinene	R-(+)	35.3	24.39–48.34	30.2				25.5–31.5
	S-(–)	64.7	75.61–51.66	69.8				74.5–68.5
Camphene	1S,4R-(–)							86.2–92.4
	1R,4S-(+)							13.8–7.6
β-Pinene	R-(+)	7.7	3.42–10.39	5.5	4.8–10.9	4.4–5.8		4.3–6.8
	S-(–)	92.3	95.58–89.61	94.5	95.2–89.1	95.6–94.2		95.7–93.2
Sabinene	R-(+)		13.56–23.33	14.2	14.1–16.2	13.6–14.7		12.4–15.0
	S-(–)		84.44–76.77	85.8	86.9–83.8	86.4–85.3		87.6–85.0
α-Phellandrene	R-(–)							46.9–52.6
	S-(+)							53.1–47.4
β-Phellandrene	R-(–)							31.1–53.9
	S-(+)							68.9–46.1
Limonene	S-(–)	1.7	1.12–1.54	1.6	1.8–2.4	2.4–2.7		1.4–1.6
	R-(+)	98.3	98.88–98.46	98.4	98.2–97.6	97.6–97.3		98.6–98.4
Linalol	R-(–)	53.5		58.2	50.2–57.2	56.2–62.9		52.0–74.5
	S-(+)	46.5		41.8	49.8–42.8	43.8–37.1		48.0–25.5
Citronellal	S-(–)	88.1					82.2–92.1	89.5–94.8
	R-(+)	11.9					17.8–7.9	10.5–5.2
Terpinen-4-ol	S-(+)			21.9	11.4–23.8	10.2–24.9		12.0–26.2
	R-(–)			78.1	88.6–76.2	89.8–75.1		88.0–73.8
α-Terpineol	S-(–)	74.7		79.9	70.2–76.1	75.7–77.8		66.4–82.0
	R-(+)	25.3		20.1	29.8–23.9	24.3–22.2		33.6–18.0

Note: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil.

Appendix to Table 7.2

1. Hara et al. (1999). Japan; one laboratory sample; double oven MDGC.
2. Mitiku et al. (2001). One sample laboratory cold-pressed from each of the cvs. Femminello and Monachello (Italy), Eureka (Kenya). The enantiomeric ratios of α-pinene, β-pinene, and limonene were determined on capillary column (60 m × 0.25 mm × 0.25 μm) coated with Thermon 660T coupled to a main column (30 m × 0.25 mm × 0.25 μm) coated with 2,3,6-tri-*O*-methyl-β-CD; the enantiomeric ratio of sabinene was determined on the same chiral column without precolumn.
3. Lorenzo et al. (2002). Uruguay; one laboratory sample cold extracted; double oven MDGC; precolumn: capillary column (30 m × 0.32 mm × 0.40–0.45 μm) coated with SE-52; main column: capillary column coated with 2,3-di-*O*-ethyl-6-*O*-*ter*-butyldimethylsilyl-β-CD or 2,3-di-*O*-*tert*-butyldimethylsilyl-β-CD or 2,3-di-*O*-methyl-6-*O*-pentyl-β-CD in PS-086 or in OV-1701.
4. Gionfriddo et al. (2004). Sicily, Italy; laboratory samples cold-pressed; (a) range of the values of 10 samples from fruits of the cv. Femminello comune and 9 samples from fruits of the cv. Monachello collected from October to January 2003; (b) range of the values of 5 samples from fruits (verdelli) of the cv. Femminello comune collected in September 2003; double oven MDGC; precolumn: capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5; main column: 2,3-di-*O*-ethyl-6-*O*-tert-butyldimethylsilyl-β-CD.
5. Nhu-Trang et al. (2006). Two laboratory samples from Spain and 15 commercial samples from Spain, Italy and unknown origin.
6. Dugo P. (2009, Personal communication). Eighty-seven industrial cold-pressed oils from Italy with different extraction technologies; direct enantio-GC on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.

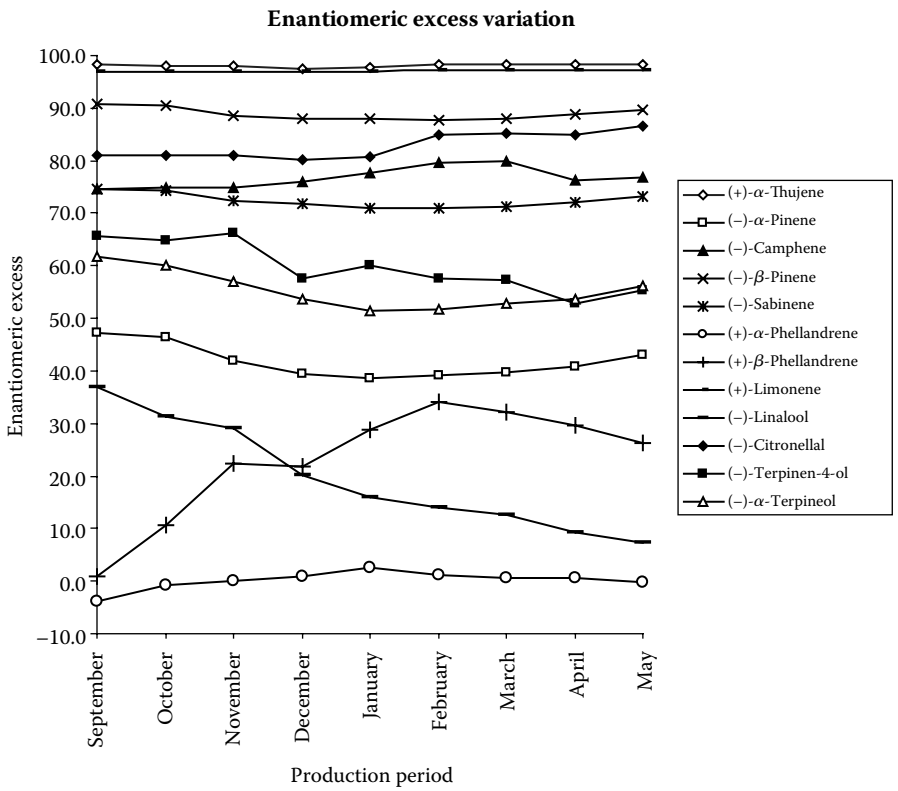

FIGURE 7.3 Enantiomeric excess variations during the production period in lemon essential oil for (+)-α-thujene; (–)-α-pinene; (–)-camphene; (–)-β-pinene; (–)-sabinene; (+)-α-phellandrene; (+)-β-phellandrene; (+)-limonene; (–)-linalol; (–)-citronellal; (–)-terpinen-4-ol; and (–)-α-terpineol. (From Dugo, G., 2009. Personal communication.)

7.3 MANDARIN (*CITRUS DELICIOSA* TEN.) OIL

7.3.1 1990–2001

In Table 7.3, the results on the enantiomeric distribution of the components of mandarin oil published after 1990, already revised by Mondello et al. (2002), separately for industrial and laboratory-extracted oils, are summarized.

7.3.2 1997–2009

Table 7.4 reports the results relative to the papers published after the review by Mondello et al. (2002) on the enantiomeric distribution of the components of mandarin. The paper by Faulhaber et al. (1997) has been listed here because it was not previously revised by Mondello et al. (2002). It reports on different samples of mandarin oils. The first group was represented by 10 Italian genuine oils, while the second group was represented by 10 commercial oils of unknown origin from Italy, Greece, Brazil, and Argentina. The first group showed enantiomer ratio of the components analyzed to be very similar to the oils reported in Table 7.3 for genuine samples, while in the second group, three oils appear to be adulterated: the first sample is from the values of the α-pinene (R-(+)/S-(–) = 36.6/63.4) and of β-pinene (R-(+)/S-(–) = 57.1/42.9), the second sample is from the value of α-pinene

TABLE 7.3
Enantiomeric Distribution of Some Volatile Components of Mandarin Oils (1990–2001)

		Industrial Oils			Laboratory Oils	
		Cold-Pressed	Distilled	Commercial	Cold-Pressed	SFE
α-Pinene	R-(+)	45–57				
	S-(–)	55–43				
β-Pinene	R-(+)	96–98.8	96.1–97.7	29.3–97.8	98.3–98.8	
	S-(–)	4–1.2	3.9–2.3	70.7–2.2	1.7–1.2	
Sabinene	R-(+)	76.2–80.5	77.8–79.9	37.6–96.6	79.9–83.4	
	S-(–)	23.8–19.5	22.2–20.1	62.4–3.4	20.1–16.6	
Limonene	S-(–)	tr–2.6	1.7–2.2	0.7–5.0	1.5–1.9	
	R-(+)	100–97.4	98.3–97.8	99.3–95.0	98.5–98.1	
Linalool	R-(–)	13.1–19.8	16–20.4	3.5–26.5	12.7–19.3	4–6.5
	S-(+)	86.9–80.2	84–79.6	96.5–73.5	87.3–80.7	96–93.5
Terpinen-4-ol	S-(+)	10.0–19.2	25–30	12.4–38.9	10.5–17.5	
	R-(–)	90.0–80.8	75–70	87.6–61.1	89.5–82.5	
α-Terpineol	S-(–)	69.6–76.8	61.2–73.4	24.2–75.1	67.8–75.1	
	R-(+)	30.4–23.2	38.8–26.6	75.8–24.9	32.2–24.9	

Appendix to Table 7.3

- The results reported in Table 7.3 and in this appendix for the different categories of mandarin oils are taken from the following original papers:
  - Cold-pressed industrial oils: Mosandl et al. (1990); Hener et al. (1990a); Kreis et al. (1991); Mosandl (1995); Rocca et al. (1992); Dugo et al. (1992a,b, 1994a, 2001); Bicchi et al. (1994); Mondello et al. (1997, 1998b).
  - Industrial distilled oils: Dugo et al. (1994a, 2001); Mondello et al. (1996, 1998b).
  - Commercial oils: Mondello et al. (1998b); Dugo et al. (2001).
  - Cold-pressed laboratory oils: Mondello et al. (1998b); Dugo et al. (2001).
  - SFE-extracted oils: Casabianca and Graff (1996).
- Most of the results reported in Table 7.3 were obtained by MDGC, with the exception of those reported by Bicchi et al. (1994) obtained by direct analysis on chiral column; those by Dugo et al. (1992a,b), obtained by the coupling of conventional and chiral columns; those by Dugo et al. (1994a,b) and by Mondello et al. (1996) obtained by on line coupling HPLC-HRGC.
- Not included in the table are the results relative to a laboratory-extracted oil analyzed by Mosandl's research group (Mosandl et al., 1990, Herner et al., 1990a, Mosandl, 1995), which is defined, in the three articles, two times mandarin and once tangerine; oils distilled in laboratory by Kreis et al. (1991), which presented anomalous enantiomeric distribution of α-pinene (R-(+) = 66–100, S(–) = 34–tr) and of β-pinene (R(+) = 63–84, S(–) = 27–16); also, some of these oils presented only the R(+) isomer of α-pinene. All these oils were probably obtained from fruits different from the species *C. deliciosa* Ten. Also not included in the table are the results relative to cold-pressed oils obtained in laboratory from five cvs. from Uruguay (malvasio, ellendale, ortanique, commun, malaquina) (Dugo et al., 1994b) that presented for linalol the following range of enantiomeric distribution: R(–) = 12–30; S(+) = 88–70.
- The results reported in the table relative to industrial cold-pressed oils and to laboratory cold extracted oils are in agreement with each other and the enantiomeric distribution varies within narrow ranges. Distilled oils show a slight tendency to racemization for monoterpene alcohols, mainly α-terpineol and terpinen-4-ol.
- Mondello et al.(1998b) observed that the enantiomeric distributions of β-pinene, limonene and sabinene remain constant during the entire productive season; for α-terpineol, an irregular variation can be observed, while the enantiomeric excesses of (–)-terpinen-4-ol and (+)-linalol tend to increase during the productive season.

TABLE 7.4
Enantiomeric Distribution of Some Volatile Components of Mandarin Oils (1997–2009)

		1	2	3a	3b	4a	4b	5	6
α-Thujene	S-(+)*								
	R-(–)*								
α-Pinene	R-(+)	44.2–47.9	46.2			46.0–48.6	50.7–53.7	53.2–54.5	
	S-(–)	55.8–52.1	53.8			54.0–51.8	49.3–46.3	46.8–55.5	
Camphene	1S,4R-(–)								
	1R,4S-(+)								
β-Pinene	R-(+)	97.0–99.1		92.4–92.8	92.0–92.8	98.5–98.9	73.8–83.5	98.3–98.6	99.4–98.8
	S-(–)	3.0–0.9		7.6–7.2	8.0–7.2	1.5–1.1	26.2–16.5	1.7–1.4	1.6–1.2
Sabinene	R-(+)			83.5–84.1	83.1–84.1	77.6–83.3	98.6–98.8	80.3–80.6	79.8–80.6
	S-(–)			16.5–15.9	16.9–15.9	22.4–16.7	1.4–1.2	19.7–19.4	20.2–19.4
α-Phellandrene	R-(–)								
	S-(+)								
β-Phellandrene	R-(–)								
	S-(+)								
Limonene	S-(–)	1.6–2.9	2.0	2.1–2.4	1.7–2.3	1.4–2.0	1.6–1.9	1.7–2.4	2.1–2.9
	R-(+)	98.4–97.1	98.0	97.9–97.6	98.3–97.7	98.6–98.0	98.4–98.1	98.3–97.6	97.9–97.1
Camphor	1R,4R-(+)								
	1S,4S-(–)								
Linalol	R-(–)		17.2	45.6–49.3[a]	44.6–46.1[a]	16.2–22.7	14.4–20.2	17.2–17.5	17.7–21.0
	S-(+)		82.8	54.4–50.7[a]	55.4–53.9[a]	83.8–77.3	83.6–79.8	82.8–82.5	83.3–79.0
Citronellal	S-(–)		81.9						
	R-(+)		18.1						
Terpinen-4-ol	S-(+)			13.9–23.8	14.1–24.4	10.7–12.7	24.8–27.1	14.6–15.2	
	R-(–)			86.1–76.2	85.9–75.6	89.3–87.3	75.2–72.9	85.4–84.8	
α-Terpineol	S-(–)			73.9–75.9	74.1–76.8	69.1–74.1	68.3–71.9	73.6–73.8	
	R-(+)			26.1–24.1	25.9–23.2	30.9–25.9	31.7–28.1	26.4–26.2	

continued

TABLE 7.4 (continued)
Enantiomeric Distribution of Some Volatile Components of Mandarin Oils (1997–2009)

		7a	7b	8a	8b	8c	8d	8e	9
α-Thujene	S-(+)*	0.9	0.7	0.30–1.00	0.72	0.62	0.31–1.00	1.00–1.86	0.54–0.76
	R-(–)*	99.1	99.3	99.70–99.00	99.28	99.38	99.69–99.00	99.00–98.14	99.46–99.24
α-Pinene	R-(+)	44.1		41.74–45.45	42.83	43.21	42.40–44.45	34.80–48.29	
	S-(–)	55.9		58.26–54.55	57.17	56.79	57.60–55.55	65.20–51.71	
Camphene	1S,4R-(–)	31.8	46.7	51.60–55.28	51.62	51.36	51.42–54.96	41.87–72.60	44.72–48.40
	1R,4S-(+)	68.2	53.3	48.40–44.72	48.38	48.64	48.58–45.04	58.13–27.40	55.28–51.60
β-Pinene	R-(+)	96.5	96.7	94.76–98.48	96.41	85.95	94.56–98.87	91.92–98.58	87.76–98.75
	S-(–)	3.5	3.3	5.24–1.52	3.59	14.05	5.44–1.13	8.08–1.42	12.24–1.25
Sabinene	R-(+)	78.6	78.7	71.28–81.51	81.64	70.30	77.58–81.75	60.50–85.50	71.28–81.51
	S-(–)	21.4	21.3	28.72–18.49	18.36	29.70	22.42–18.25	39.50–14.50	28.72–18.49
α-Phellandrene	R-(–)	53.6	53.5	48.09–54.63	50.90	52.81	48.14–53.37	41.82–53.61	44.32–55.00
	S-(+)	46.4	46.5	51.91–45.37	49.10	47.19	51.86–46.63	58.18–46.39	55.68–45.00
β-Phellandrene	R-(–)	1.8	2.7	1.23–2.96	1.78	1.66	1.24–1.83	93.91–98.52	0.41–2.74
	S-(+)	98.2	97.3	98.77–97.04	98.22	98.34	98.76–98.17	6.09–1.48	99.59–97.26
Limonene	S-(–)	1.7	1.9	1.56–1.73	1.92	1.47	1.46–2.09	1.50–2.10	1.56–2.12
	R-(+)	98.3	98.1	98.44–98.27	98.08	98.53	98.54–97.91	98.50–97.90	98.44–97.82
Camphor	1R,4R-(+)		23.4						17.04–36.50
	1S,4S-(–)		76.6						82.96–63.50
Linalol	R-(–)	22.8	15.3	13.24–15.89	14.19	14.28	12.98–19.67	29.61–68.56	13.24–20.40
	S-(+)	77.2	84.7	86.76–84.11	85.81	85.72	87.02–80.33	70.39–31.44	86.76–79.60
Citronellal	S-(–)		6.1	4.89–8.78	9.20	4.62			3.94–8.90
	R-(+)		93.9	95.11–91.22	90.80	95.38			96.06–91.10
Terpinen-4-ol	S-(+)	12.5	12.1	10.56–13.13	14.57	12.58	14.57–18.17	22.51–33.05	9.48–18.26
	R-(–)	87.5	87.9	89.44–86.87	85.43	87.42	85.43–81.83	77.49–66.95	90.52–81.74
α-Terpineol	S-(–)	72.9	72.5	69.26–74.19	71.88	72.82	66.06–75.20	47.15–71.00	67.71–76.54
	R-(+)	27.1	27.5	30.74–25.81	28.12	27.18	33.94–24.80	52.85–29.00	32.29–23.46

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil; [a] see text.

Appendix to Table 7.4

1. Faulhaber et al. (1997). Range of the values of 10 Italian oil laboratory extracted by pentane:diethyl ether (1:1) and 6 commercial samples correspondent with genuine oils; double oven MDGC; precolumn: glass capillary column (30 m × 0.23 mm × 0.5 μm) coated with OV-215; main column: glass capillary column (25 m × 0.23 mm × 0.2 μm) coated with 2,3-di-*O*-methyl-6-*O*-*tert*-butylsilyl-β-CD (45%) and OV-1701 (55%).
2. Feger et al. (2003). One commercial sample from Italy; capillary column (10 m × 0.25 mm × 0.5 μm) coated with DB-1 connected to a capillary column (25 m × 0.25 mm) coated with di-*O*-methyl-*O*-pentyl-β-CD (50%) and OV-1701 (50%).
3. Catalfamo et al. (2004). Calabria, Italy; laboratory samples cold-pressed; (a) range of the values of 9 samples from fruits of the cv. Avana collected from October 2003 to January 2004; (b) range of the values of 7 samples from fruits of the cv. Tardivo di Ciaculli collected from January to April 2004; for the analytical method see point 4 of the appendix to Table 7.2.
4. Frizzo et al. (2004). Uruguay; laboratory samples; range of the values of 4 samples each cold-pressed from fruit of the cv. Cai (a) and Montenegrina (b) range of the values of 4 samples each hydrodistilled from fruits of the cv. Cai (c) and Montenegrina (d); double oven MDGC; precolumn: capillary column (30 m × 0.32 mm × 0.4–45 μm) coated with SE-52; main column: capillary column (25 m × 0.25 mm × 0.25 μm) coated with 2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-CD in PS-086.
5. Zimbalatti (2006). Sicily, Italy; 10 industrial samples cold-pressed produced in October 2003; the enantiomeric ratios of α-pinene and limonene was determined by a single oven MDGC; column 1: capillary column (25 m × 0.25 mm × 0.25 μm) coated with 2,3-di-*O*-ethyl-6-*O*-*tert*-butylsilyl-β-CD; column 2: capillary column (30 m × 0.25 mm × 0.25 μm) coated with 2,3-di-*O*-methyl-6-*O*-*tert*-butylmethylsilyl-β-CD; the enantiomeric ratios of all the component analyzed were determined by direct analysis on the column 1.
6. Bonaccorsi et al. (2009). Sicily, Italy; Torchi, FMC; 27 samples industrial cold-pressed from fruits of the cv. Tardivo di Ciaculli collected from October 2007 to January 2008; direct enantio-GC on capillary column (30 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.
7. Sciarrone et al. (2010). Sicily, Italy; several samples industrial extracted; (a) direct enantio-GC; (b) double oven MDGC.
8. Schipilliti et al. (2010). (a) 53 samples from Sicily, Italy industrial cold-pressed, (b,c) 2 Japanese samples industrial extracted, (d) 7 genuine commercial oils, (e) 5 adulterated commercial oils; direct enantio-GC on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.
9. Dugo P. et al. (2010b). Sicily, Italy; 124 samples extracted by Brown machine and Torchi during the 2008/2009 productive season; direct enantio-GC on capillary column (25 m × 0,25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.

(R-(+)/S-(–) = 59.86/40.2), and the third sample is from the value of limonene (S-(–)/R-(+) = 5.1/94.9) and from the high amount of δ-3-carene. These results were confirmed with the values of $\delta^{13}C_{PDB}$, obtained by online GC-IRMS of some components. An additional fourth sample has been recognized as adulterated according to the last results. The values of the adulterated oils have been rejected from the ones reported in Table 7.4.

Feger et al. (2003) investigated some commercial samples of Italian mandarin oils and established the enantiomeric ratios for α-pinene, limonene, linalol, and citronellal. The latter, to our knowledge, was never reported before in mandarin oil.

Catalfamo et al. (2004) analyzed different samples of mandarin oil using a MDGC system. The oils were extracted in laboratory from fruits collected in Reggio Calabria (Italy). Specifically *Avana* fruits, sampled every 15 days from October 2003 to January 2004, gave 9 samples of oil; and *Tardivo di Ciaculli*, sampled every 15 days from January to April 2004, gave 7 samples of oil. The results are, in general, borderline if compared to those reported in Table 7.3 for laboratory-extracted oils. But the most eclatant value is the one reported for linalol, which shows to be almost racemic, as can be seen in Table 7.4. This is probably due to an author's error for the calculation of the enantiomer excess. In fact, from the figure reporting the chromatogram of the chiral separation, it can be assumed that the enantiomeric ratio of the (–)-linalol and (+)-linalol is about 20:80, values also reported by other authors for genuine mandarin oils. No relevant differences could be detected for the two different cultivars investigated.

In another paper based on the use of MDGC, Frizzo et al. (2004) determined the chiral distribution of seven components (see Table 7.4) in the cultivars Motenegrina and Cai (Uruguay). The results obtained for the two cultivars are almost superimposable within the same technology of extraction (cold pressure or hydrodistillation). The distilled oils showed, for both cultivars, a trend to racemization for β-pinene and terpinen-4-ol. For one of the hydrodistilled oils from the Cai cultivar, an anomalous ratio of the sabinene (R-(+)/S-(–) = 82.1/17.9) has been reported, probably due to a spelling error. The forementioned value is not reported in the table.

Zimbalatti (2006) investigated some samples of cold-pressed genuine mandarin oils through the coupling of two chiral columns within the same oven by a mechanical valve. The data obtained fall within the ranges of cold-pressed industrial essential oils.

Bonaccorsi et al. (2009), reported the analysis of 27 samples of mandarin essential oils industrially produced in Sicily during the 2007–2008 season. Among the GC and HPLC analysis of the main constituents of this oils, the enantiomeric ratio of β-pinene, sabinene, limonene, and linalol was determined by direct enantioselective analysis. The results are in agreement with those reported for genuine mandarin oils with an exception of (–)-limonene, which presents a value slightly higher (2.9%), and (–)-linalol (21.0%), with a value exceeding the ones reported for genuine essential oils.

Sciarrone et al. (2010) investigated the chiral distribution of several cold-pressed mandarin oils produced in southern Italy. The aim of the work was to compare the two analytical techniques used in the study, which were monodimensional enantio-GC and multidimensional GC. The authors were able to separate 13 couples of enantiomers, 5 of them for the first time in this citrus oil (α-thujene, camphene, α-phellandrene, β-phellandrene, and camphor). The comparison between enantio-GC and MDGC highlights that α-pinene could not be successfully separated in MDGC, and vice-versa for camphor. Also, due to coelutions occurring at different degrees, some chiral couples showed different enantiomeric ratios for the two techniques in camphene, β-phellandrene, and linalol (see Figure 7.4). For this reason, the authors called attention to a "correction factor" that can be measured using GC-FID information. This factor allows for more reliable data when using monodimensional e-GC, where the possibility of overlapping peaks is quite consistent. The average values for both techniques are reported in Tables 7.4 and 7.7a,b.

A deep investigation of different types of mandarin oil has been carried out by Schipilliti et al. (2010). Data are reported in Tables 7.4 and 7.8a–e. As can be seen, the genuine samples coming from Sicily show an enantiomeric distribution compatible with literature data. Anomalous values

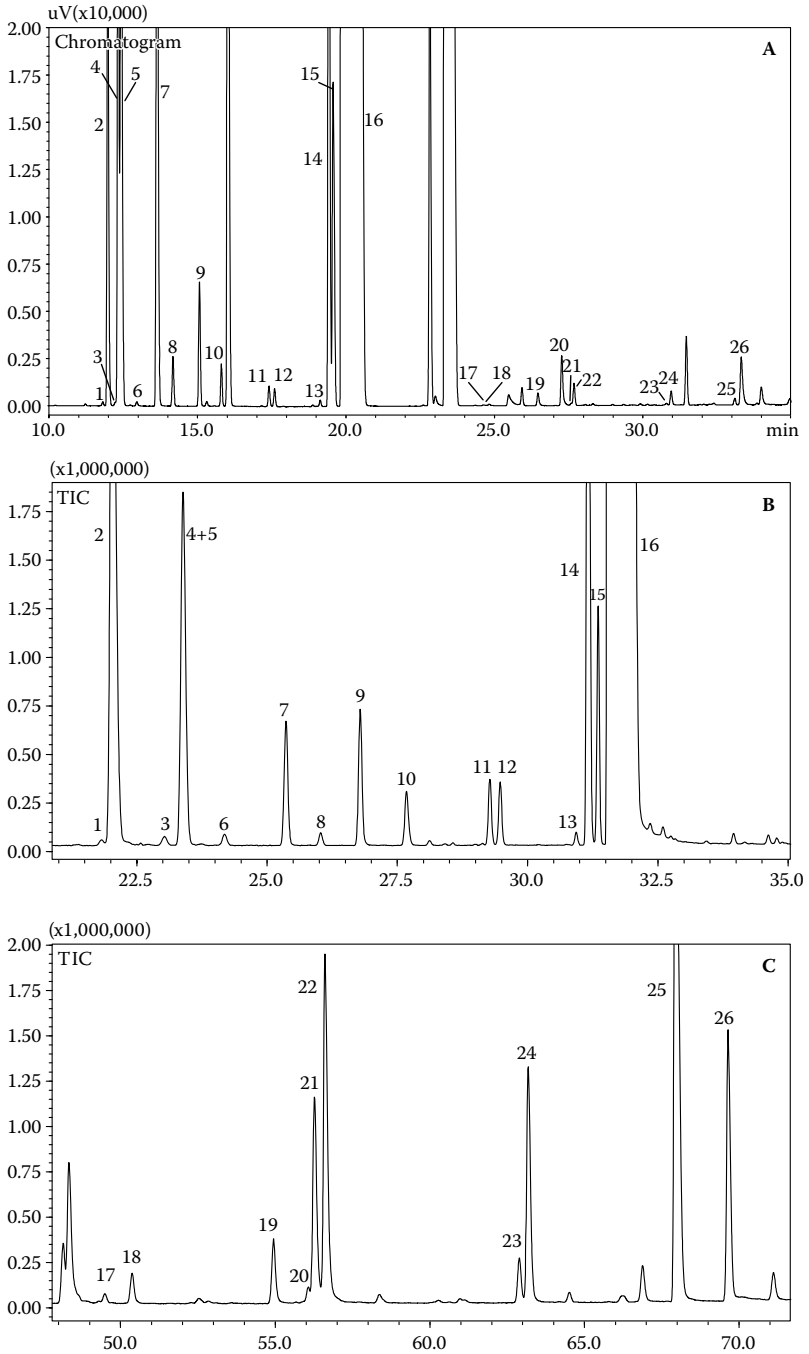

FIGURE 7.4 (A) Conventional enantio-GC-FID chromatogram of mandarin essential oil. (B) 20–35 min expansion and (C) 48–72 min expansion of the 2D enantio-chromatogram relative to the MDGC analysis. (**a**, **b**, and **c**) **1** (–)-α-thujene; **2** (+)-α-thujene; **3** (–)-camphene; **4** (+)-α-pinene; **5** (–)-α-pinene; **6** (+)-camphene; **7** (+)-β-pinene; **8** (–)-β-pinene; **9** (+)-sabinene; **10** (–)-sabinene; **11** (–)-α-phellandrene; **12** (+)-α-phellandrene; 13. (–)-β-phellandrene; **14** (–)-limonene; **15** (+)-β-phellandrene; **16** (+)-limonene; **17** (+)-camphor; **18** (–)-camphor; **19** (–)-linalol. (A) **20** (–)-citronellal; **21** (+)-citronellal; **22** (+)-linalol; **23** (+)-terpinen-4-ol; **24** (–)-terpinen-4-ol; **25** (–)-α-terpineol; **26** (+)-α-terpineol. (C) **20** (–)-citronellal; **21** (+)-citronellal; **22** (+)-linalol; 23. (+)-terpinen-4-ol; **24** (–)-terpinen-4-ol; **25** (–)-α-terpineol; **26** (+)-α-terpineol. (From Sciarrone, D., et al., *J. Chromatogr. A*. 1217, 1101–1105, 2010. Reproduced with permission of Elsevier.)

can be observed for β-pinene in Japanese samples, but the authors state that these samples did not report in the label information on the period of production or cultivar. More relevant deviations from the ranges were attributable to commercial samples reported in column 8e, in particular for camphene, linalol, and terpinen-4-ol. In many cases, the same chiral compound presented different enantiomeric ratios depending upon the single sample.

Dugo P. et al. (2010) analyzed 124 samples of genuine mandarin oils produced in Sicily from Calabrian and Sicilian fruits and extracted with Brown and Torchi machines. The results fall within the data reported in literature for genuine mandarin essential oils and are reported in column 9 of Table 7.4. In Figure 7.5, the enantiomeric excess of some components during the productive season is analyzed. The enantiomeric excess in many of the components analyzed, with some exception, shows constant values among the productive season: camphene and α-terpineol showed an enantiomeric excess for (–)-enantiomers, decreasing during the last part of the productive season; (+)-camphene enantiomeric excess showed a slight increase in the last part of the productive season; β-pinene and citronellal, in the limited number of samples produced in March, presented a rapid decrease of the enantiomeric excess of the dextrorotatory enantiomer. It is important to remember that in the past industrial samples produced in Sicily during the month of March were never analyzed.

7.4 TANGERINE (*CITRUS TANGERINA* HORT. EX TAN.) AND CLEMENTINE (*CITRUS CLEMENTINA* HORT. EX TAN.) OILS

7.4.1 2001–2009

Table 7.5 reports data on the enantiomeric composition of tangerine and clementine oils, found in literature from 2001 up until 2009.

Feger et al. (2003) utilized two columns in series (nonpolar with chiral) to determine the enantiomeric ratios of α-pinene, limonene, linalol, and citronellal in commercial Brazilian Murcott and Chinese tangerine oils. With the exception of limonene, all the other components show considerable differences between the sample coming from Brazil and the sample coming from China. However, the enantiomeric ratios of limonene are confirmed by successive paper by Dugo et al. (2005), who investigated the chiral composition of six samples of Mexican Dancy tangerine oils. For the first time, data relative to β-pinene and sabinene were reported. The authors concluded that the values obtained for β-pinene were similar to those of Mediterranean mandarin oils, while data obtained for limonene were similar to those of sweet orange oils. Figure 7.6 illustrates the chiral chromatogram of a cold-pressed Dancy tangerine oil.

Verzera et al. (1997) investigated clementine oils from Calabria (Italy) by direct enantio-GC. The chiral ratio of linalol of three samples of Monreal clementine and three samples of Comune clementine have been determined in detail. The authors have concluded that the enantiomeric ratio of linalol was very similar to those of mandarin genuine oils for the Oroval and Monreal cultivars, while the samples of Comune cultivar showed a similar ratio to the sweet orange oils. The following year, the same group also analyzed some clementine oils prepared in laboratory from the fruits of Nules and Comune cultivars from Uruguay. This time the enantiomeric ratio of some monoterpene hydrocarbons (β-pinene, sabinene, limonene) and of some monoterpene alcohols (linalol and α-terpineol) were analyzed by a double oven MDGC system. The data obtained demonstrated that the two cultivars presented a very similar ratio for all the components analyzed, with exclusion of the linalol ratio, which presented intermediate values in comparison to those obtained on the Italian cultivars. It must be noted that all the values for β-pinene, sabinene, limonene, and α-terpineol were very similar to those obtained for Italian sweet orange essential oils.

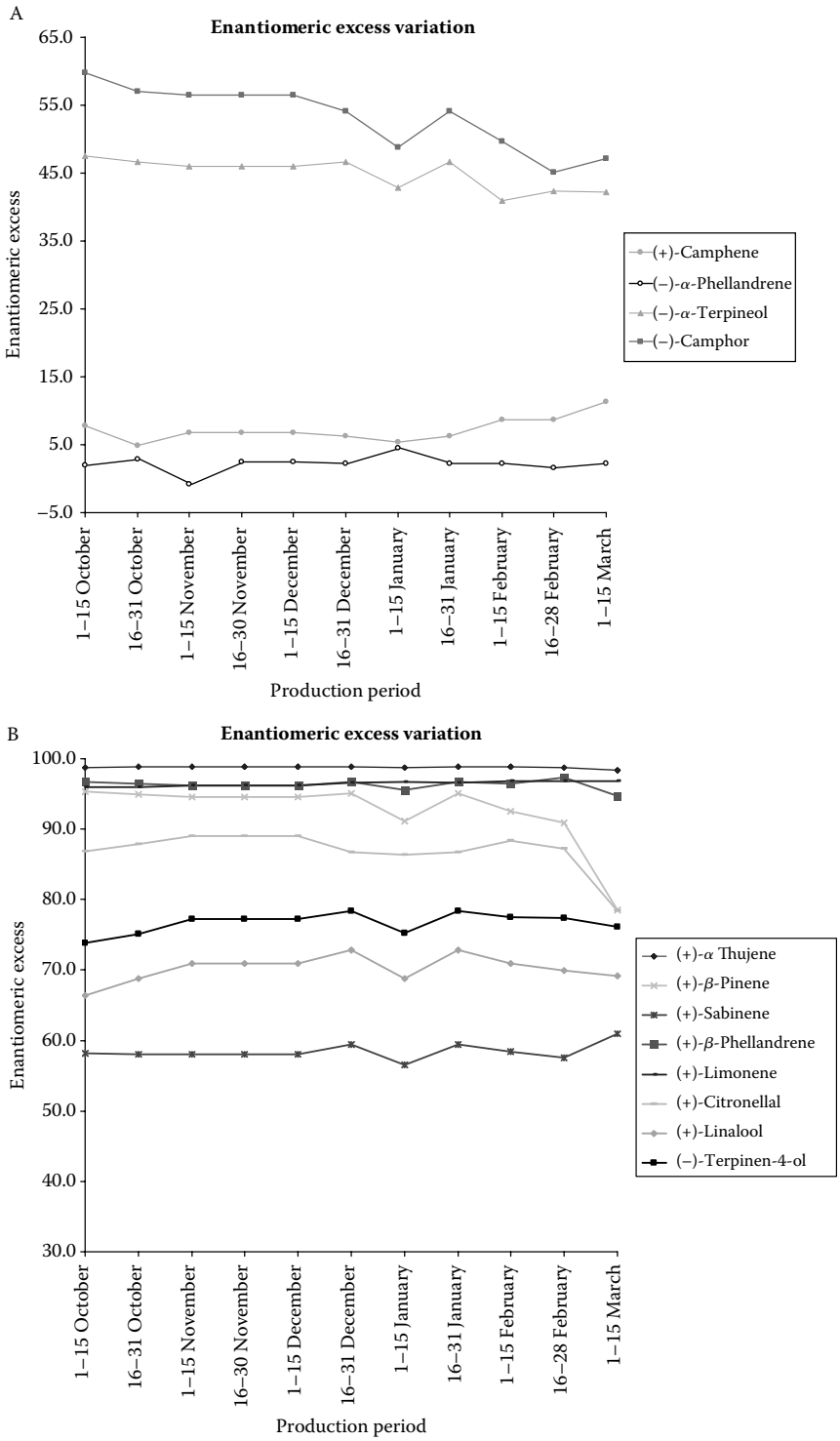

FIGURE 7.5 Enantiomeric excess variations during the production period in mandarin essential oil for (A) (+)-camphene, (–)-α-phellandrene, (–)-camphor, (–)-α-terpineol and (B) (+)-α-thujene, (+)-β-pinene, (+)-sabinene, (+)-β-phellandrene, (+)-limonene, (+)-citronellal, (+)-linalol, and (–)-terpinen-4-ol. (From Dugo, P., et al., *Flavour Fragr. J.* 2010c. In press.)

TABLE 7.5
Enantiomeric Distribution of Some Volatile Components of Tangerine and Clementine Oils (2001–2009)

		Tangerine Oils			Clementine Oils				
		1a	1b	2	1a	1b	1c	2a	2b
α-Pinene	R-(+)	100	76						
	S-(–)	0	24						
β-Pinene	R-(+)			97.6–98.3				58.1–61.1	60.1
	S-(–)			2.4–1.7				41.9–38.9	39.9
Sabinene	R-(+)			90.1–92.7				97.5–97.6	97.6
	S-(–)			9.9–7.3				2.5–2.4	2.4
Limonene	S-(–)	0.6	0.8	0.8				0.6	0.6
	R-(+)	99.4	99.2	99.2				99.4	99.4
Linalol	R-(–)	7.8	5.6		2.4–2.6	2.6–2.8	8.1–9.0	6.7	5.0–5.3
	S-(+)	92.2	94.4		97.6–97.4	97.4–97.2	91.9–91.0	93.3	95.0–94.7
Citronellal	S-(–)	8.6	5.8						
	R-(+)	91.4	94.2						
α-Terpineol	S-(–)							2.5–2.7	2.5–2.6
	R-(+)							97.5–97.3	97.5–97.4

Appendix to Table 7.5

Tangerine Oils

1. Feger et al. (2003). (a) Commercial Murcott tangerine oil from Brazil, (b) commercial tangerine oil from China; for analytical method see point 2 of appendix to Table 7.4.
2. Dupo P. et al. (2005). Mexico; 6 Dancy tangerine industrial cold-pressed oils; direct enantio-GC on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.

Clementine Oils

1. Verzera et al. (1997). Calabria, Italy; laboratory cold-pressed oils; (a) 3 samples of Oroval clementine oils, (b) 3 samples of Monreal clementine oils, (c) 3 samples of Comune clementine oils extracted from fruits picked from December 1995 to January 2006; direct enantio-GC on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.
2. Verzera et al. (1998). Uruguay; laboratory cold-pressed oils (a) 2 samples of Nules clementine oils, (b) 2 samples of Comune clementine oils extracted from fruits picked in April and May 1996; double oven MDGC; precolumn: capillary column (30 m × 0.32 mm × 0.40–045 μm) coated with SE-52; main column: capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.

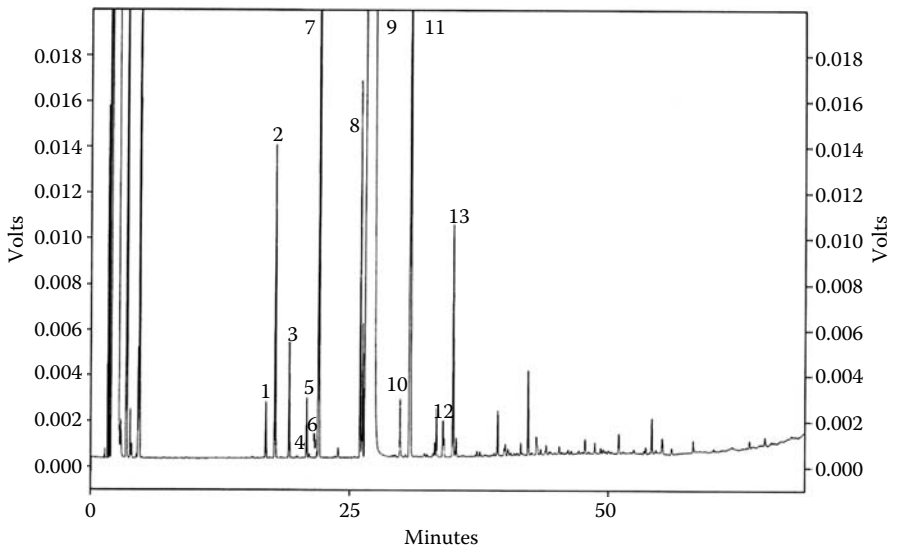

FIGURE 7.6 Conventional enantio-GC-FID chromatogram of a cold-pressed Dancy tangerine oil. Peak identification: 1. (±)-α-thujene; 2. (±)-α-pinene; 3. (+)-β-pinene; 4. (–)-β-pinene; 5. (+)-sabinene; 6. (–)-sabinene; 7. myrcene; 8. (–)-limonene; 9. (+)-limonene; 10. terpinolene; 11. γ-terpinene; 12. (–)-linalol; 13. (+)-linalol. (From Dugo, P., et al., *Flavour Fragr. J.* 20, 60–66, 2005. Reproduced with permission of Wiley-VCH Verlag GmbH.)

7.5 SWEET ORANGE (*CITRUS SINENSIS* [L.] OSB.) AND BITTER ORANGE (*CITRUS AURANTIUM* L.) OILS

7.5.1 1990–2001

Table 7.6 summarizes the results on the enantiomeric distribution of the components of sweet and bitter orange oils published after 1990, already revised by Mondello et al. (2002). The results are listed separately according to industrial and laboratory-extracted oils.

7.5.2 1999–2009

Table 7.7 reports the data relative to orange oils not revised by Mondello et al. (2002). Due to the low number of papers retrieved, bitter and sweet orange oils were put together on the same table.

As can be seen, the results obtained by Mitiku et al. (2001) on samples of Italian origin are not in agreement with those reported in Table 7.6 for bitter orange oils.

Gionfriddo et al. (2003) investigated both bitter and sweet orange oils. In particular, they harvested fruits of bitter orange every two weeks from December 2000 to March 2001, obtaining eight samples of essential oil. Plantations were located in Reggio Calabria (Italy). In the same location, they collected fruits from Ovale Calabrese and Valencia, which are sweet orange cultivars, from March to June 2001, obtaining in laboratory 10 and 9 samples, respectively. The results of this investigation show the separation of five chiral pairs for bitter orange oil. The enantiomeric ratios fall within the range reported in Table 7.6 for cold-pressed bitter orange. As regards sweet orange oil, it is only possible for linalol to be compared with previously reported data (see Table 7.6): the enantiomeric ratio for this compound is more or less similar to that reported for

TABLE 7.6
Enantiomeric Distribution of Some Volatile Components of Sweet Orange and Bitter Orange Oils (1990–2001)

		Sweet Orange					Bitter Orange
		Industrial	Laboratory Oils				Industrial
		Cold-Pressed and Commercial Oils	Cold-Pressed	Solvent	Distilled	SFE	Cold-Pressed and Commercial Oils
α-Pinene	R-(+)	100		100	100		92–95
	S-(–)	tr		tr	tr		8–5
β-Pinene	R-(+)	54–61		71–73	66		1.7–6
	S-(–)	46–39		29–27	34		98.3–94
Sabinene	R-(+)	94.6–97.9					44.4–54.5
	S-(–)	5.4–2.1					55.6–45.5
Limonene	S-(–)	tr–1.1		tr	tr		0.6
	R-(+)	100–98.9		100	100		99.4
Linalol	R-(–)	4–11	5–9		2.8–14	15	80.4–87.5
	S-(+)	96–89	95–91		97.2–86	85	19.6–12.5
Citronellal	S-(–)	36					
	R-(+)	64					
Carvone	S-(+)	59.27					
	R-(–)	40.73					
Terpinen-4-ol	S-(+)						65.3–67.6
	R-(–)						34.7–32.4
α-Terpineol	S-(–)						6.8–11.6
	R-(+)						93.2–88.4
α-Copaene	S-(+)	15–16					
	R-(–)	85–84					
δ-Cadinene	S-(+)	90					
	R-(–)	10					
(*E*)-Nerolidol	S-(+)						>80
	R-(–)						<20

Appendix to Table 7.6

Sweet Orange Oil

- The results reported in Table 7.6 and in this appendix, for the different categories of sweet orange oils, are taken from the following original papers:
  - Cold-pressed industrial and commercial oils: Mosandl et al. (1990); Hener et al. (1990a); Takeoka et al. (1990); König et al. (1990, 1994); Bernreuther and Schreier (1991); Rocca et al. (1992); Werkhoff et al. (1993); Dugo et al. (1994b, 2001); Mosandl (1995); Mondello et al. (1996,1997).
  - Laboratory cold-pressed oil: Dugo et al. (1994b), Mondello et al. (1996).
  - Laboratory solvent-extracted olis: Mosandl et al. (1990); Hener et al. (1990a); Kreis et al. (1991); Mosandl (1995).
  - Laboratory-distilled oils: Kreis et al. (1991); Wang et al. (1995); Casabianca and Graff (1996).
  - SFE-extracted oils: Casabianca and Graff (1996).
- The results reported in the table were obtained by using the following analytical techniques:
  - MDGC: Mosandl et al. (1990); Hener et al. (1990a); Kreis et al. (1991); Bernreuther and Schreier (1991); Rocca et al. (1992); Casabianca and Graff (1996); Wang et al. (1995); Casabianca et al. (1995); Mosandl et al. (1995); Mondello et al. (1997); Dugo et al. (2001).
  - Direct analysis on chiral column: König et al. (1990); Takeoka et al. (1990); Werkhoff et al. (1993).
  - Preparative GC and analysis on chiral column: König et al. (1994).
  - HPLC/HRGC: Dugo et al. (1994b); Mondello et al. (1996).
- The results relative to the enantiomeric distribution of volatile components of sweet orange oil, in the period considered, are limited and often relative to few or only one component. From the original papers is also often difficult to understand if they refer to samples of secure origin or to commercial samples; for this reason the two categories are grouped together.

Bitter Orange Oil

- The results reported in this table and appendix for bitter orange oils, are taken from the following original papers: Mosandl et al. (1990); Hener et al. (1990a); Kreis et al. (1991); Dugo et al. (1994b); Mosandl (1995); Mondello et al. (1996, 1997).
- Most of the results reported in the table were obtained by MDGC; with the exception of those reported by Dugo et al. (1994b) and by Mondello et al. (1996) obtained by on line HPLC/HRGC.
- The results relative to the enantiomeric distribution of volatile components of bitter orange oil, in the period considered, are limited and often relative to few or only one component. From the original papers is also often difficult to understand if they refer to samples of secure origin or to commercial samples; for this reason the two categories are grouped together.
- Not included in the table are the results relative to linalol determined in some commercial oils (Dugo et al., 1994b) that presented the enantiomeric distribution: R(–) = 37–68; S(+) = 63–32 very different from what determined by Mondello et al. (1996, 1997) in samples of secure origin.

TABLE 7.7
Enantiomeric Distribution of Some Volatile Components of Sweet Orange Oil and Bitter Orange Oils (1999–2009)

		Sweet Orange					Bitter Orange						
		1a	**1b**	**2**	**3**	**4**	**1**	**2**	**3**	**4a**	**4b**	**4c**	**4d**
α-Thujene	S-(+)*					10.20–61.99				10.33	23.61	30.85	36.38
	R-(−)*					89.80–38.01				89.67	76.39	69.15	63.62
α-Pinene	R-(+)			100.0	99.6–99.7	90.09–99.45	81.94		79.7	89.65	96.95	97.37	97.39
	S-(−)			0.0	0.4–0.3	9.91–0.55	18.06		20.3	10.35	3.05	2.63	2.61
Camphene	1S,4R-(−)										45.00	35.79	47.60
	1R,4S-(+)										55.00	64.21	52.40
β-Pinene	R-(+)				66.1–77.8	10.55–70.25	80.61	1.3–3.2	0.8	2.30	6.06	7.91	7.02
	S-(−)				33.9–22.2	89.45–29.75	19.39	98.7–96.8	99.2	97.70	93.94	92.09	92.98
Sabinene	R-(+)	97.8–98.4	97.8–98.5			97.46–98.80	71.62	42.1–49.7		49.38	75.37	80.61	54.62
	S-(−)	2.2–1.6	2.2–1.5			2.54–1.20	28.38	57.9–50.3		50.62	24.63	19.39	45.38
α-Phellandrene	R-(−)									74.91			60.11
	S-(+)									25.09			39.89
β-Phellandrene	R-(−)					0.44–1.42				5.70	0.56	1.12	1.18
	S-(+)					99.56–98.58				94.30	99.64	98.88	99.82
Limonene	S-(−)	0.5–0.8	0.6–0.7	0.6	0.6–0.9	0.47–0.54	0.45	0.6–0.7	0.8	0.50	0.53	0.52	0.58
	R-(+)	99.5–99.2	99.4–99.3	99.4	99.4–99.1	99.53–99.46	99.55	99.4–99.3	99.2	99.50	99.47	99.48	99.42
Linalol	R-(−)	2.2–4.8	4.7–16.0	7.9	3.7–17.5	7.81–17.91		85.3–92.4	79.7	89.81	80.23	61.15	84.47
	S-(+)	97.8–95.2	95.3–84.0	92.1	96.3–82.5	92.19–82.09		14.7–7.6	20.3	10.19	19.77	38.85	15.53
Citronellal	S-(−)			37.0	12.2–68.7	37.38–52.65			>98				42.50
	R-(+)			63.0	87.8–31.3	62.62–47.35			<2				57.50
Linalyl acetate	R-(−)							99.2–99.4		99.36			
	S-(+)							0.8–0.6		0.64			
Terpinen-4-ol	S-(+)									71.52			67.45
	R-(−)									28.48			32.55
α-Terpineol	S-(−)				1.6–3.0	5.09–15.68			23.6	6.63	17.45	29.82	10.24
	R-(+)				98.4–97.0	94.91–84.32			78.4	93.37	82.55	70.18	89.76

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil.

Appendix to Table 7.7

Sweet Orange Oil

1. Gionfriddo et al. (2003). Calabria, Italy; laboratory samples cold-pressed; range of the values of 10 samples from fruits of the cv. Ovale Calbrese (a) and of 10 sample from fruits of the cv. Valencia late (b); for the analytical method see point 4 of the appendix to Table 7.2.
2. Feger et al. (2003). One commercial sample from Brazil; for the analytical method see point 2 of the appendix to Table 7.2.
3. Hara et al. (1999). Japan; range of the values of the 3 laboratory samples; double oven MDGC.
4. Ragonese (2009, Personal communication). Sicily, Italy; range of the values of 17 cold-pressed industrial samples; for analytical method see point 9 to the Table 7.4.

Bitter Orange Oil

1. Mitiku et al. (2001). Italy; one sample laboratory cold-pressed; for the analytical method see point 2 of the appendix to Table 7.2.
2. Gionfriddo et al. (2003). Calabria, Italy; ranges of the values of 8 samples cold-pressed from fruits collected from December 200 to March 2001; for the analytical method see point 4 of the appendix to Table 7.2.
3. Hara et al. (1999). Japan. One laboratory sample; double oven MDGC.
4. Dugo et al. (2010a). Sicily, Italy; (a) commercial oil, (b) green bigarade oil from Egypt, (c) red bigarade oil from Egypt, (d) distilled bigarade oil from Egypt; for analytical method see point 9 to the Table 7.4.

laboratory cold-pressed oils. Data for limonene and linalol are in agreement with those of industrial cold-pressed oils. The difference in the enantiomeric ratios of linalol in the two cultivars is interesting, in that it could be used as a marker for the differentiation of the cultivars Ovale and Valencia.

Feger et al. (2003) analyzed a series of commercial sweet orange oils produced in Brazil. Only four enantiomeric couples were separated, and the data obtained are perfectly in agreement with those reported in Table 7.6 for industrial and commercial oils.

Hara et al. (2009) determined the enantiomeric ratios of six components in Japanese bitter and sweet orange oils. Data suggest that these oils were obtained through distillation (mainly for the value of α-terpineol). However, there are scarce data available in literature to make a comparison. Also, the range for citronellal in sweet orange oils is quite wide, therefore more information is needed for understanding this consistent variation.

Dugo et al. (2010a) analyzed a commercial sample of bigarade, with results very similar to those of Gionfriddo et al. (2003). The same authors also reported on three other samples of bigarade oil from green and red fruits and one distilled. Worthy of mention is the enantiomeric ratio of citronellal, which tends to racemization in the distilled oil, and the values of R-(–)-linalol for the red bigarade, which is lower (61.15 %) when compared to the values found for the other samples and also to the value reported by Hara et al. (1999).

Finally, Ragonese (2009, Personal communication) analyzed 17 samples of cold-pressed sweet orange oils. The chiral ratio of β-phellandrene and α-terpineol have been reported for the first time in these oils. Among the other compounds, it must be noted that β-pinene showed a very big variation through the different samples and α-pinene, sabinene, limonene, and citronellal confirmed the chiral ratios reported previously for cold-pressed and laboratory oils in Table 7.6.

7.6 KEY (*CITRUS AURANTIFOLIA* [CHRISTM.] SWING.) AND PERSIAN (*CITRUS LATIFOLIA* TAN.) OILS

7.6.1 1990–2001

Table 7.8 summarizes the results on the enantiomeric distribution of the components of Key lime and Persian lime oils published after 1990, already revised by Mondello et al. (2002). The results are listed separately according to industrial, commercial, and laboratory-extracted oils.

7.6.2 1999–2009

Only three papers in the last few years report some data on lime oil, as can be seen in Table 7.9. Two of these are relative to a few samples of Persian lime, laboratory extracted from Japanese fruits and in agreement with industrial Persian lime oils previously investigated. The third paper more extensively reported results relative to an industrial Persian lime oil from Mexico, two commercial Persian oils (one of these decolored), as well as distilled oil and Key lime type A and B from Mexico. In the last paper, for the first time the enantiomeric ratios of camphene and α- and β-phellandrene were determined. For cold-pressed oils, the results obtained for those components considered already are generally in good agreement with data reported in literature. Results obtained by Dugo et al. (2010b) for different samples of Key lime oil type A and B are in good agreement with each other, and the same consideration can be drawn for Persian lime oils. An exception is the value of the enantiomeric ratio of α-thujene in sample 1d, where the percentage of R(–) enantiomer is about double in comparison with values of cold-pressed oils of the same botanical origin. Also, in one of the samples of Persian lime (3c), a higher amount of S(–)-limonene than that in the other samples of the same botanical origin is present. The main differences between cold-pressed Key and Persian lime oils are encountered in the enantiomeric distribution of β-pinene (as already mentioned in literature). A slight racemization of α-thujene, β-pinene, linalol, and limonene can be observed

TABLE 7.8
Enantiomeric Distribution of Some Components of Lime Oils (1990–2001)

		Industrial Key Lime Oils				Persian Lime Oils	
						Industrial	Laboratory
		Type A	Type B	Distilled	Commercial	Cold-Pressed	Solvent
α-Pinene	R-(+)				23–25		30–34
	S-(–)				77–75		70–66
β-Pinene	R-(+)	3.4–3.5	3.5	3.2–4.0	2–4	9.1–10.3	8–10
	S-(–)	96.6–96.5	96.5	96.8–96.0	98–96	90.9–89.7	92–90
Sabinene	R-(+)	15.1–15.2	15.3			18.2–23.4	
	S-(–)	84.9–84.8	84.7			81.8–76.6	
Limonene	S-(–)	2.6–2.9	1.8	5.5–8.7	2	0.4–2.7	2
	R-(+)	97.4–97.1	98.2	94.5–91.3	98	99.6–97.3	98
Linalol	R-(–)	70.2–71.5	70.0	49.8–50.0		54.4–69.3	
	S-(+)	29.8–28.5	30.0	50.2–50.0		45.6–30.7	
Terpinen-4-ol	S-(+)	29.2–29.5	29.5	42.3–45.0		18.6–24.9	
	R-(–)	70.8–70.5	70.5	57.7–55.0		81.4–75.1	
α-Terpineol	S-(–)	84.0–85.5	82.8	53.3–56.8		74.5–80.8	
	R-(+)	16.0–14.5	17.2	46.7–43.2		25.5–19.2	

Appendix to Table 7.8

- The results reported in Table 7.8 and in this appendix, for the different categories of lime oils, are taken from the following original papers:
  - Cold-pressed Key lime type A and B and Persian lime, distilled Key lime oils: Mondello et al. (1998c); Dugo et al. (2001).
  - Laboratory solvent-extracted oils and commercial oils: Mosandl et al. (1990), Hener et al. (1990 a,b), Mosandl (1995).
- All the results reported in the table were obtained by MDGC.
- Laboratory-extracted oils were declared in the original articles to be extracted from Key lime fruits; the value of the enantiomeric ratio of β-pinene could indicate that they were extracted from fruits of Persian lime and were reported as it is in the table.
- Not considered among the commercial oils in the table are the results relative to two samples analyzed by Hener et al. (1990a,b) due to the value of the enantiomeric distribution of R(+)/S(–)α-pinene (76/24 and 43/57, respectively) which indicates adulteration, as per our opinion.

for distilled Key lime oil (4c). Differently, literature (see Table 7.8) reports for monoterpene alcohol values of enantiomeric ratios close to racemic value and a slightly higher racemization for limonene. It is possible that sample 4e of Table 7.9 was obtained under experimental conditions that permit to better maintain the natural composition of the oils. The chromatograms relative to the conventional chiral analysis of (a) Key lime oil type A, and heart-cut analysis of (b) Key lime oil type A, (c) Key lime oil type B, (d) Persian lime oil, and (e) distilled lime oil are reported in Figure 7.7.

7.7 GRAPEFRUIT (*CITRUS PARADISI* MACF.) OIL

7.7.1 1990–2009

Table 7.10 summarizes the results on the enantiomeric distribution of the components of grapefruit oil (white and pink), listed separately for commercial and laboratory-extracted oils from 1990 to 2009 because this oil has not been reviewed in the previous edition of this book.

TABLE 7.9
Enantiomeric Distribution of Some Components of Persian and Key Lime Oils (1999–2009)

		Persian Lime						Key Lime				
		1	**2a**	**2b**	**3a**	**3b**	**3c**	**1a**	**1b**	**1c**	**1d**	**1e**
α-Thujene	S-(+)*				0.89	1.00	1.56	1.43	1.74	1.41	3.93	19.72
	R-(–)*				99.11	99.00	98.44	98.57	98.26	98.59	97.07	80.28
α-Pinene	R-(+)	32.2	28.54	28.21	29.96	29.23	32.56	21.83	22.55	22.27	22.72	27.42
	S-(–)	67.8	71.46	71.79	70.04	70.77	67.44	78.17	77.45	77.73	77.28	72.58
Camphene	1S,4R-(–)				87.71+	88.96+			87.29+	92.81+		88.99+
	1R,4S-(+)				12.29+	11.04+			12.71+	7.19+		11.01+
β-Pinene	R-(+)	9.6	8.69	6.87	9.09	8.31	9.50	3.05	3.53	3.23	3.60	6.52
	S-(–)	90.4	91.31	93.13	90.91	91.69	90.50	96.95	96.47	96.77	96.40	93.48
Sabinene	R-(+)		19.29	18.67	18.04	17.56	20.35	14.88	14.38	14.62	14.61	21.40
	S-(–)		80.71	81.33	91.96	82.44	79.65	85.12	85.62	85.38	85.39	78.60
α-Phellandrene	R-(–)				57.31	56.44	49.06	54.59	55.44	53.26	58.94	54.85+
	S-(+)				42.69	43.56	50.94	45.41	44.56	46.74	41.06	45.15+
β-Phellandrene	R-(–)				30.46	37.77	27.89	44.17	46.34	45.13	45.10	39.50
	S-(+)				69.54	62.33	72.11	55.83	53.66	54.87	54.90	60.50
Limonene	S-(–)	2.2	2.88	2.43	2.28	2.34	4.07	2.44	2.47	2.48	2.47	3.31
	R-(+)	97.8	97.12	97.57	97.72	97.66	95.93	97.56	97.53	97.52	97.53	96.69
Linalol	R-(–)	70.0			64.25	67.68	52.34	73.09	71.57	67.22	68.38	54.09
	S-(+)	30.0			35.75	32.32	47.66	26.91	28.43	32.78	31.62	45.91
Citronellal	S-(–)	87.0			78.68	80.49			77.04	81.04	73.41	76.61+
	R-(+)	13.0			21.32	19.51			22.96	18.96	26.59	23.39+
Terpinen-4-ol	S-(+)				23.72	17.90	29.52	29.94	33.03	28.08	35.35	36.56
	R-(–)				76.28	82.10	70.48	70.06	66.97	71.92	64.65	63.44
α-Terpineol	S-(–)	79.4			79.10	82.56	77.26	87.50	87.56	82.86	87.58	80.03
	R-(+)	20.6			20.90	17.44	22.74	12.50	12.44	17.14	12.42	19.97

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil;+ MDGC value.

Appendix to Table 7.9

Persian Lime Oils

1. Hara et al. (1999). Japan; one laboratory sample; double oven MDGC.
2. Mitiku et al. (2001). One sample each laboratory extracted from fruits of Tahiti lime from Japan (a) and from Kenya (b). For analytical method see point 2 of the appendix to Table 7.2.
3. Dugo et al. (2010b). Industrial Persian lime oils; (a) Persian lime oil form Mexico, (b) commercial Persian lime oil, (c) decolorated commercial Persian lime oil; for analytical methods see point 9 of Table 7.4.

Key Lime Oils

1. Dugo et al. (2010b). Industrial Key lime oils; (a,b) two samples of Key lime oil type A from Mexico; (c,d) two samples of Key lime oil type B from Mexico; (e) one sample of distilled Key lime oil from Egypt; for analytical method see point 9 to appendix to Table 7.4.

The first three papers refer to the studies carried out by Mosandl's group (Herner et al., 1990a; Mosandl et al., 1990, 1995) that deal with the use of heart-cutting techniques. The studies featured two independent ovens, using a Carbowax 20M column as precolumn and a permethylated β-cyclodextrin as (chiral) main column. The authors were able to stereodifferentiate α-pinene, β-pinene, and limonene in grapefruit essential oils among other essential oils, concluding that the R(+)-limonene of high enatiomeric excess values characterize the *Rutaceae* family while the S(–)-limonene characterize the *Gramineae* family. Worthy of attention was also the excess of

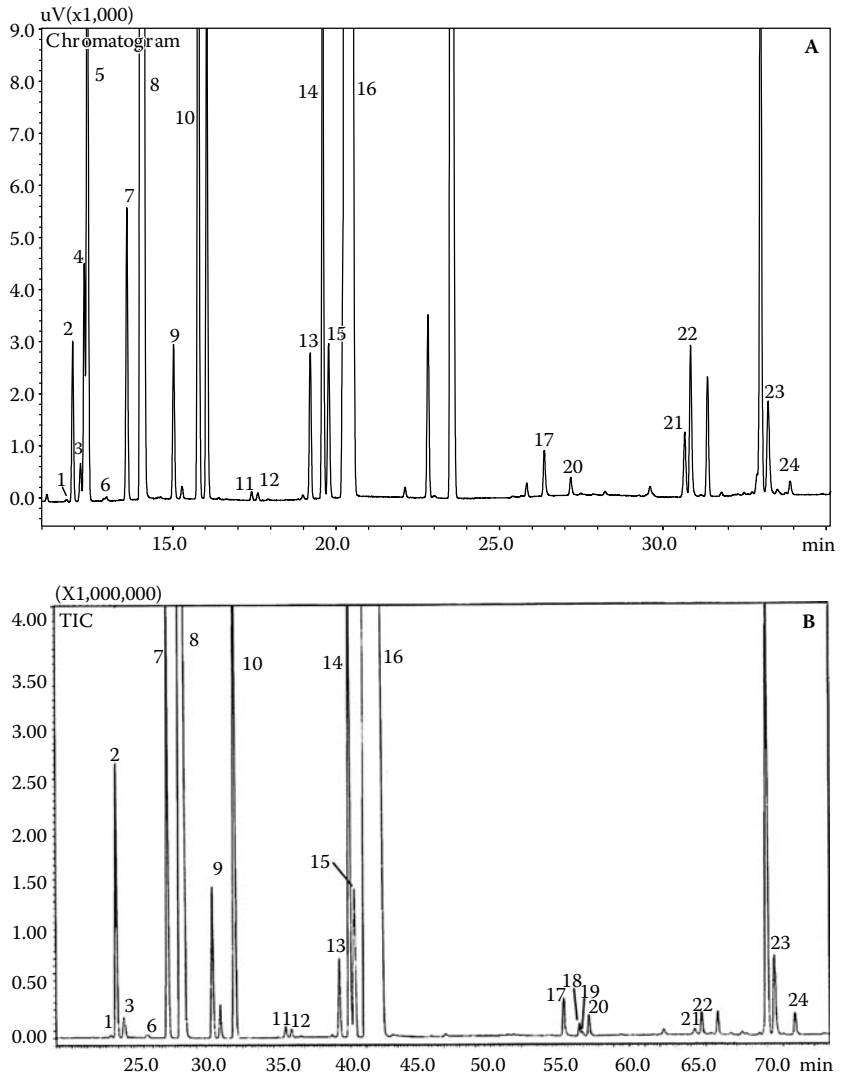

FIGURE 7.7 Direct enantio-GC result for (A) Key lime oil type A and heart-cut results for (B) Key lime oil type A, (C) Key lime oil type B; (D) Persian lime oil; (E) distilled lime oil. Peak identification: 1. (–)-α-thujene; 2. (+)-α-thujene; 3. (–)-camphene; 4. (+)-α-pinene; 5. (–)-α-pinene; 6. (+)-camphene; 7. (+)-β-pinene; 8. (–)-β-pinene; 9. (+)-sabinene; 10. (–)-sabinene; 11. (–)-α-phellandrene; 12. (+)-α-phellandrene; 13. (–)-β-phellandrene; 14. (–)-limonene; 15. (+)-β-phellandrene; 16. (+)-limonene; 17. (–)-linalol; 18. (–)-citronellal; 19. (+)-citronellal; 20. (+)-linalol; 21. (+)-terpinen-4-ol; 22. (–)-terpinen-4-ol; 23. (–)-α-terpineol; 24. (+)-α-terpineol. (From Dugo, G., et al., *J. Agric. Food Chem.* 2010b. In press.)

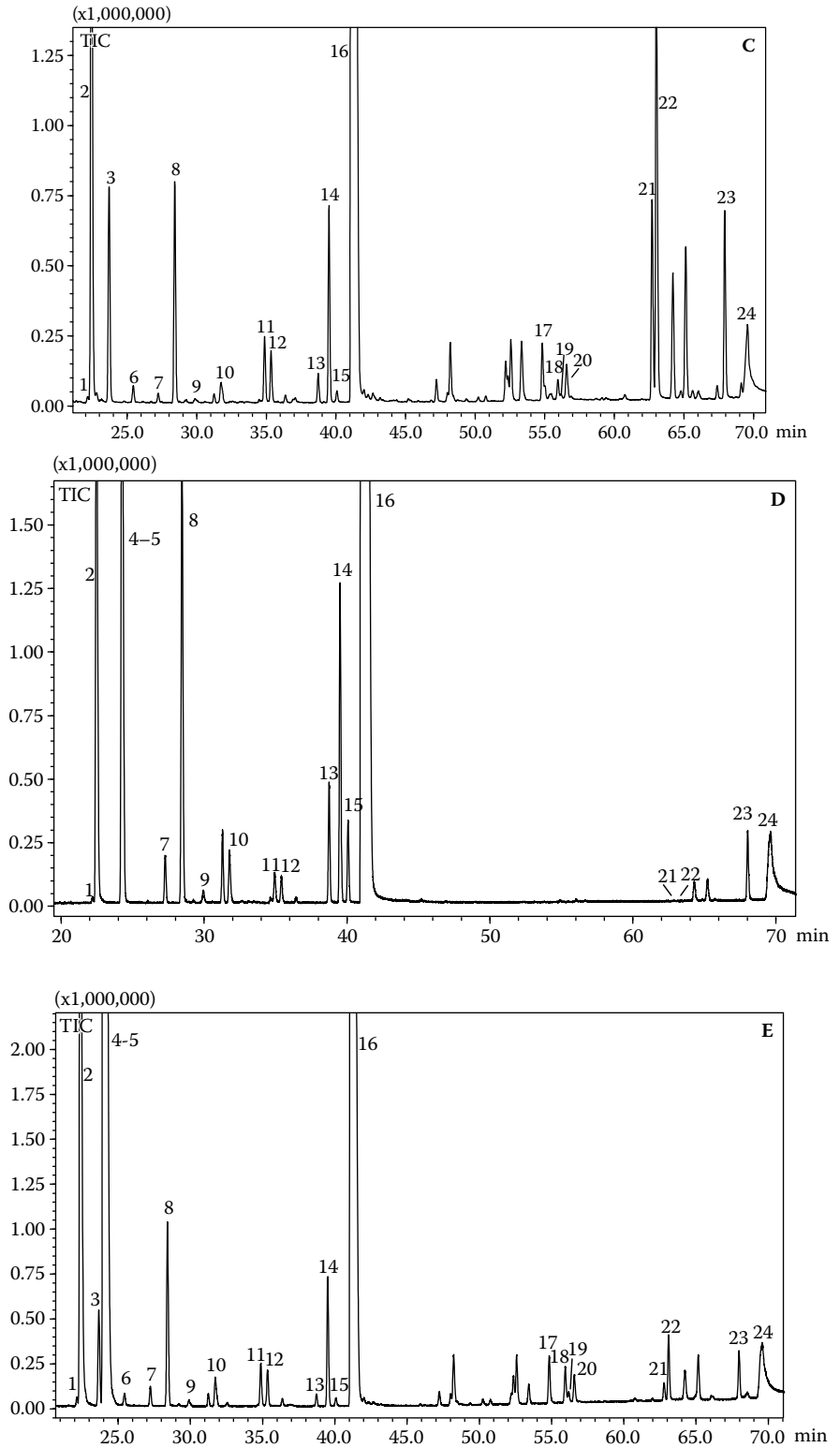

FIGURE 7.7 Continued

TABLE 7.10
Enantiomeric Distribution of Some Volatile Components of Grapefruit Oils (1990–2009)

		Commercial Oils					Laboratory Oils					
		1a	**2**	**3a**	**4**	**5**	**1b**	**3b**	**3c**	**6**	**7**	**8**
α-Pinene	S-(+)	100		100	100	93.90	99	99	100		99.6–99.7	99.15–99.50
	R-(–)	tr		tr	tr	6.10	1	1	tr		0.4–0.3	0.85–0.50
β-Pinene	R-(+)	64		62	60	80.64	63	63	87		62.0–76.8	63.79–72.44
	S-(–)	34		38	40	19.36	37	37	13		38.0–23.2	36.21–27.56
Sabinene	R-(+)											98.37–98.47
	S-(–)											1.63–1.53
Limonene	S-(–)	tr		tr	0.5	1.00	tr	tr	tr		0.8–1.0	0.45–0.54
	R-(+)	100		100	99.5	99.00	100	100	100		99.2–99.0	99.55–99.46
Linalol	R-(–)					67.86				32–43	23.4–25.5	
	S-(+)					32.14				68–57	76.6–74.5	
Citronellal	S-(–)										16.6–21.4	
	R-(+)										83.4–78.6	
α-Terpineol	S-(–)										1.2–3.3	
	R-(+)										99.8–96.7	
Carvone	S-(+)		65.21									
	R-(–)		34.79									

Appendix to Table 7.10

1. Herner et al. (1990a); Mosandl et al. (1990); Mosandl et al. (1995). (a) one commercial sample; (b) one laboratory sample cold extracted; double oven MDGC; precolumn: glass capillary column (60 m × 0.32 mm × 0.25 μm) coated with Carbowax 20M; main column: glass capillary column (47 m × 0.23 mm) coated with 2,3,6-tri-methyl-β-CD (10%) in OV-1701 (90%).
2. König et al. (1990). One commercial sample; direct analysis on chiral capillary column (2,3,6-tri-*O*-pentyl-α-CD).
3. Kreis et al. (1991). (a) one commercial sample of pink grapefruit oil, (b) one laboratory sample cold extracted, (c) one laboratory sample hydrodistilled; for the analytical conditions see point 1 of this appendix.
4. Rocca et al. (1992). One commercial sample; double oven MDGC.
5. Coleman et al. (1998). One commercial sample; SPME-chiral GC.
6. Casabianca and Graff (1996). Three laboratory sample SFE extracted; double oven MDGC.
7. Hara et al. (1999). Two laboratory samples; double oven MDGC.
8. Mitiku et al. (2001). Kenya; three samples cold-pressed from Red Blush grapefruit Marsh grapefruit and of unknown origin; for analytical method see point 2 of appendix to Table 7.2.

the R-(+)-α-pinene in the grapefruit oils, which was similar to that of the bitter orange oils but opposite to the one obtained in lemon essential oils, revealing possible adulteration of lemon oils with grapefruits terpenes. König et al. (1990) reported the enantiomeric ratio for carvone. To our knowledge, this is the only paper reporting the chiral separation of carvone in grapefruit oils. However, the sample was of commercial origin and there is no confirmation on the natural ratio of this compound—only that the sample of orange oil analyzed by the same authors showed a similar ratio to the one found in the grapefruit oil (see Tables 7.6 and 7.10).

Kreis et al. (1991) used a fully automated MDGC system (Siemens Sichromat 2) with a "live switching" coupling piece ("live-T-piece") to determine the enantiomeric distribution of α-pinene, β-pinene, and limonene in different citrus oils. The authors confirmed that the trends for all the compounds were similar to those reported by the other papers published by the same group of Professor Mosandl. One year later Rocca et al. (1992), using MDGC, reported the chiral ratio of

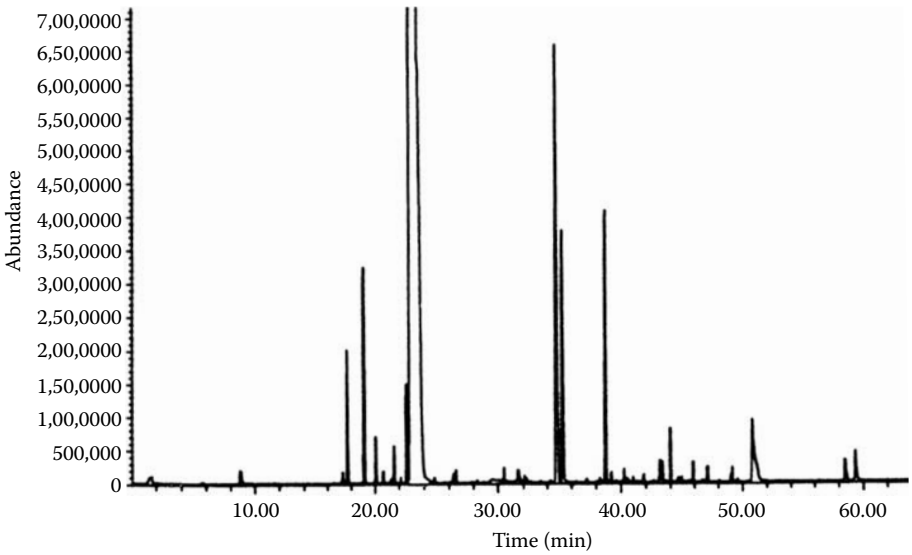

FIGURE 7.8 AutoSPME-chiral-GC-MSD total ion chromatogram of the headspace of grapefruit essential oil. (From Coleman, W.M., et al., *J. Chromatogr. Sci.* 36, 575–578, 1998. Reproduced with permission of Preston Publication, LTD.)

α-pinene, β-pinene, and limonene in one commercial sample of grapefruit oil: the results obtained perfectly matched the values already reported by the two German groups of Mosandl and König for this oil. Casabianca and Graff (1996) have investigated three samples of grapefruit oils extracted by SFE from fruits collected in Israel. They have reported only the chiral separation of linalol as compared to other citrus oils. Coleman et al. (1998) analyzed one sample of commercial grapefruit oil by means of autoSPME-chiral-GC-MSD (Figure 7.8). The results obtained presented a lower value of the enantiomeric excess of R(+)-α-pinene together with an higher amount of the R(+)-β-pinene, as well as a reversed ratio for linalol compared to the data reported in literature for laboratory-extracted oils.

Hara et al. (1999) used chiral GC to determine three monoterpene hydrocarbons and three oxygenated terpenoid constituents of two laboratory-extracted oils. The values of α-pinene, β-pinene, limonene, and linalol were in accordance with the ones previously reported for laboratory oils. Moreover, the authors presented data for the chiral separations of α-terpineol and citronellal that, to our knowledge, were never reported for this oil. Finally, Mitiku et al. (2001) studied the essential oils of two cultivars of grapefruit, Red Blush and Marsh, and one sample of unknown botanic origin harvested in Japan. The enantiomeric ratio of sabinene, to our knowledge, was never reported before in grapefruit oil. The other three enantiomeric couples determined present values compatible with those previously reported.

7.8 BERGAMOT (*CITRUS BERGAMIA*) OIL

7.8.1 1990–2001

Table 7.11 reports data previously published on the enantiomeric distribution of volatiles in bergamot oil, already revised by Mondello et al. (2002). Beyond industrial and laboratory oils, data relative to residues of the extraction process and extracts of flavored commercial products (e.g., tea) are reported.

TABLE 7.11
Enantiomeric Distribution of Some Volatile Components of Bergamot Oils (1990–2001)

		Industrial Oils				Oils Recovered from Cold Extraction Residues			Laboratory Oils		Extracts from Commercial Products	
		Cold-Pressed	Commercial									
		Genuine	Genuine	Adulterated	Bergapten Free[a]	Cold treated[b]	Distilled[c]	Distilled[d]	Cold Extracted[e]	Distilled	f	g
α-Pinene	R-(+)	26–34	29.6–32.5	25.4–34.0			30.2					
	S-(–)	74–66	70.4–67.5	74.6–66.0			69.8					
β-Pinene	R-(+)	6.8–9.5	8.0–9.0	4.1–7.4	8.1–9.2	7.4–8.0	8.2	8.9				
	S-(–)	93.2–90.5	92.0–91.0	95.9–92.6	91.9–90.8	92.6–92.0	91.8	91.1				
Sabinene	R-(+)	14.1–18.8			15.1–16.0	14.5–15.0	15.2	15.9				
	S-(–)	85.9–81.2			84.9–84.0	85.5–85.0	84.8	84.1				
Limonene	S-(–)	1.9 to <3	2.4–2.5	1.8–15.0	2.1–2.2	2.1–2.3	1.5–2.3	2.0			2	
	R-(+)	98.1 to >97	97.6–97.5	98.2–85.0	97.9–97.8	97.9–97.7	98.5–97.7	98.0			98	
Linalol	R-(–)	99.0–100	99.0–100	54.9–87.8	99.5–100	99.5–100	91.5–98.7	81.6	99–100	68.8–78[h]	100	50
	S-(+)	1.0–0	1.0–0	45.1–12.2	0.5–0	0.5–0	8.5–1.3	18.4	1–0	32.2–22[h]	0	50
Linalyl acetate	R-(–)	>99–100	>99–100	40–98	99.7–100	99.7–100	99.0–99.1	98.9	99–100	99–100	99.5	50
	S-(+)	<1–0	<1–0	60–2	0.3–0	0.3–0	1.0–0.9	1.1	1–0	1–0	0.5	50
Terpinen-4-ol	S-(+)	9.7–26.3			18.4–21.5	12.3–18.7	27.1	31.8				
	R-(–)	90.3–73.7			81.6–78.5	87.7–81.3	72.9	68.2				
α-Terpineol	S-(–)	17.5–69.4			31.4–52.1	4.9–41.8	11.2	26.6				
	R-(+)	82.5–30.6			68.6–47.9	95.1–58.2	88.8	73.4				
(*E*)-Nerolidol	S-(+)	>80										
	R-(–)	<20										

Notes: [a] Alkali treated and distilled; [b] Torchiati, ricicli, pulizia dischi; [c] Distilled at reduced pressure; [d] Distilled at atmospheric pressure; [e] Cold-pressed and solvent extracted; [f] Pharmaceutical; [g] Tea; [h] Casabianca and Graff (1996) determined in three samples extracted by steam distillation values of R(–)-linalol ranging from 99.9% to 100%.

Appendix to Table 7.11

- The results reported in Table 7.10 and in this appendix, for the different categories of bergamot oils, are taken from the following original papers:
  - Cold-pressed industrial oils: Cotroneo et al. (1992); Casabianca et al. (1995); Verzera et al. (1996); König et al. (1997); Mosandl and Juchelka (1997a,b); Dellacassa et al. (1997b); Mondello et al. (1997,1998d); Dugo et al. (2001).
  - Commercial oils (genuine): Bernreuther and Schreier (1991); Ravid et al. (1994); Juchelka and Mosandl (1996a).
  - Commercial oils (adulterated): Mosandl et al.(1990); Hener et al. (1990a); Mosandl and Schubert (1990); Schubert and Mosandl (1991); Weinrich and Nitz (1992); Bernreuther and Schreier (1991); Casabianca and Graff (1994,1996); Casabianca et al. (1995); Juchelka and Mosandl (1996a); König et al. (1997).
  - Bergaptene free oils: König et al. (1997); Mondello et al. (1998b).
  - Recovered oils: Juchelka and Mosandl (1996a); König et al. (1997); Mondello et al. (1998b).
  - Laboratory oils (cold extracted): Schubert and Mosandl (1991); Weinrich and Nitz (1992); König et al. (1997); Dellacassa et al. (1997b).
  - Laboratory oils (distilled): Bernreuther and Schreier (1991); Weinrich and Nitz (1992); König et al. (1997).
  - Extracts from commercial products: Casabianca and Graff (1994); Casabianca et al. (1995).
- Most of the results summarized in the table were obtained by MDGC, with the exception of those reported by Cotroneo et al. (1992); Ravid et al. (1994); Verzera et al. (1996); König et al. (1997); Dellacassa et al. (1997b) all obtained by direct analysis on chiral column.

7.8.2 2001–2009

Table 7.12 reports data on the enantiomeric distribution of bergamot oil published after 2001, with the exception of the results of column 1, which are relative to 1997 but not revised by Mondello et al. (2002).

Casabianca and Chau (1997) investigated samples of bergamot oil of various origin. These were solvent extracted, cold-pressed, and extracted by supercritical fluid (SFE) laboratory oils; industrial oils from Sicily and Ivory Coast; and commercial oils. Linalol and linalyl acetate presented values falling within the ranges reported previously for those types of oil, with the exception of one commercial sample considered adulterated by the authors. This last sample has values of the (–)-linalol and (–)-linalyl acetate definitely lower than the genuine samples reported previously.

Solvent-extracted oils were obtained partly from fresh fruits and partly from dry fruits, but the chiral composition was not distorted by the aging process of the fruits. To our knowledge, Casabianca and Chau (1997) were the first to determine the enantiomeric ratio for α-thujene, citronellal, and α-terpinyl acetate in bergamot oil.

TABLE 7.12
Enantiomeric Distribution of Some Volatile Components of Bergamot Oils (1997–2009)

		1a	1b	2a	2b	2c	2d	3	4	5
α-Thujene	S-(+)*	0.5–1.0	0.5–1.0							
	R-(–)*	99.5–99.0	99.5–99.0							
α-Pinene	R-(+)	29.3–38.4	26.3–35.0	32.2	34.3	34.2	32.5		32.8	30.52
	S-(–)	70.7–61.6	73.7–65.0	67.8	65.7	65.8	67.5		67.2	69.48
Camphene	1S,4R-(–)									
	1R,4S-(+)									
β-Pinene	R-(+)	7.3–8.6	6.2–8.6	7.1	7.3	8.0	7.7		8.5	7.40
	S-(–)	92.7–91.4	93.8–91.4	92.9	92.7	92.0	92.3		91.5	92.60
Sabinene	R-(+)	15.0–18.6	13.8–15.0							17.85
	S-(–)	85.0–81.4	86.2–85.0							82.15
α-Phellandrene	R-(–)									
	S-(+)									
β-Phellandrene	R-(–)									
	S-(+)									
Limonene	S(–)	1.7–2.2	1.7–2.7	1.9	1.8	1.5	2.0		1.7	1.70
	R(+)	98.3–97.8	98.3–97.3	98.1	98.2	98.5	98.0		98.3	98.30
Linalol	R(–)	99.2–99.5	99.2–99.5	>99.0	78.1	61.9	56.3	99.4	99.8	
	S(+)	0.8–0.5	0.8–0.5	<1.0	21.9	38.1	43.7	0.6	0.2	
Citronellal	S-(–)								>98	
	R-(+)								n.d.	
Linalyl acetate	R-(–)	99.7–100	99.8	>99.0	>99.0	>99.0	>99.0	99.7		
	S-(+)	0.3–0	0.2	<1.0	<1.0	<1.0	<1.0	0.3		
Terpinen-4-ol	S-(+)									
	R-(–)									
α-Terpineol	S-(–)	23.0–36.4			28.2	45.5	48.7		29.5	
	R-(+)	77.0–63.6			71.8	54.5	51.3		70.5	
Citronellol	S-(–)	12–20								
	R-(+)	88–80								
α-Terpinyl acetate	S-(–)	36–44								
	R-(+)	64–56								

TABLE 7.12 (continued)
Enantiomeric Distribution of Some Volatile Components of Bergamot Oils (1997–2009)

		6	7a	7b	7c	8a	8b	8c	9
α-Thujene	S-(+)*					0.5–1.0	0.7–1.0	0.5–1.3	
	R-(–)*					99.5–99.0	99.3–99.0	99.5–98.7	
α-Pinene	R-(+)					31.0–33.6	32.8–32.9	33.9–36.1	
	S-(–)					69.0–66.4	67.2–67.1	66.1–63.9	
Camphene	1S,4R-(–)					85.7–90.1+	86.7–92.7+	88.0–89.4+	
	1R,4S-(+)					14.3–9.9+	13.3–7.3+	12.0–10.6+	
β-Pinene	R-(+)	7.6–12.9	9.5		9.5	8.2–10.3	9.3–9.5	7.6–7.9	8.7–9.5
	S-(–)	92.4–87.1	90.5		90.5	91.8–89.7	90.7–90.5	92.4–92.1	91.3–90.5
Sabinene	R-(+)	15.4–21.1	14.6		14.6	15.8–17.4	16.8–17.0	16.4–19.8	13.7–16.3
	S-(–)	86.6–78.9	85.4		85.4	84.2–82.6	83.2–83.0	83.6–80.2	86.3–83.7
α-Phellandrene	R-(–)					52.0–54.7	52.8–53.1	43.1–46.6	
	S-(+)					48.0–45.3	47.2–46.9	56.9–53.4	
β-Phellandrene	R-(–)					26.3–36.0	24.1–25.5	33.8–36.9	
	S-(+)					73.7–64.0	75.9–74.5	66.2–63.1	
Limonene	S(–)	1.4–2.7	1.8		1.8	1.5–1.7	1.6	1.2–1.4	1.9–2.1
	R(+)	98.6–97.3	98.2		98.2	98.5–98.3	98.4	98.8–98.6	98.1–97.9
Linalol	R(–)	99.3–99.7	99.4	99.4	99.5	99.5–99.7	97.8–98.9	98.4–99.6	99.3–99.5
	S(+)	0.7–0.3	0.6	0.6	0.5	0.5–0.3	2.2–1.1	1.6–0.4	0.7–0.5
Citronellal	S-(–)								
	R-(+)								
Linalyl acetate	R-(–)	99.1–99.6	99.7	99.7	99.7	99.8–99.9	99.8	99.0–99.8	99.6–99.7
	S-(+)	0.9–0.4	0.3	0.3	0.3	0.2–0.1	0.2	1.0–0.2	0.4–0.3
Terpinen-4-ol	S-(+)					22.4–31.5	29.3–30.4	23.7–25.0	44.7–67.7[a]
	R-(–)					77.6–68.5	70.7–69.6	76.3–75.0	55.3–32.3[a]
α-Terpineol	S-(–)					14.2–55.4	20.5–31.9	14.0–27.2	56.3–68.5
	R-(+)					85.8–44.6	79.5–68.1	86.0–72.8	43.7–31.5
Citronellol	S-(–)								
	R-(+)								
α-Terpinyl acetate	S-(–)								
	R-(+)								

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil; +MDGC value; n.d., not determined; [a] see text.

Appendix to Table 7.12

1. Casabianca and Chau (1997). (a) Range of the values of industrial cold-pressed oils from Sicily (3 samples) and from Ivory Coast (2 samples); (b) range of the values of cold-pressed, solvent extracted, SFE (at 40°C and 80°C) laboratory oils (7 samples); direct enantio-GC on capillary column (25 m × 0.22 mm × 0.25 μm) coated with 6-dimethyl-*tert-O*-butyl silyl-2,3-dimethyl-β-CD (50%) and OV-1701 (50%) for the analysis of monoterpene hydrocarbons, coated with 6-dimethyl-*tert-O*-butyl silyl-2,3-diethyl-β-CD (30%) and PS-086 (70%) for the analysis of linalol and linalyl acetate, coated with Lipodex E for the analysis of citronellol, α-terpineol, and α-terpenyl acetate.
2. Kiwanuka et al. (2000). Italy; laboratory samples; one sample solvent (pentane/diethylether) extracted (a), one sample steam distilled at pH 5.3 (b), one sample steam distilled at pH 2.5 (c), one sample steam distilled from the whole fruits (d); double oven MDGC; precolumn: capillary column (30 m × 0.53 mm × 0.25 μm) coated with DBX-5; main column: capillary column (25 m × 0.25 mm) coated with 2,3,6-tri-*O*-methyl-β-CD.

continued

TABLE 7.12 (continued)
Enantiomeric Distribution of Some Volatile Components of Bergamot Oils (1997–2009)

3. Verzera et al. (2000a). Sicily, Italy; 8 samples laboratory cold-pressed from fruits of the cvs. Castagnaro, Fantastico, Femminello, PCF; direct enantio-GC on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-2,3-di-*O*-*tert*-butylmethylsilyl-β-CD (30%) and PS-086 (70%).
4. Hara et al. (1999). Japan; one laboratory oil; double oven MDGC.
5. Mitiku et al. (2001). Italy; one sample laboratory cold-pressed; for analytical method see point 2 of the appendix to Table 7.2.
6. Gionfriddo et al. (2003). Calabria, Italy; range of the values of 25 laboratory samples cold extracted from fruits of the cvs. Femminello, Castagnaro, Fantastico collected from December 2000 to March 2001; for the analytical method see point 4 of the appendix to Table 7.2.
7. Costa et al. (2010). Calabria, Italy; industrial oils; one sample cold-pressed (a); two samples terpeneless (b); two samples bergapten free (c); the two terpeneless oils and the two bergapten-free oils presented very similar values; direct enantio-GC analysis on capillary column (25 m × 0.25 mm × 0.25 μm) coated with diethyl-*tert*-butylsilyl-β-CD.
8. Sciarrone (2009, Personal communication). (a) 17 Italian cold-pressed industrial oils, (b) 3 Italian Peratoner oils, (c) 2 cold-pressed industrial oils from Ivory Coast; direct enantio-GC analysis as in point 7 of this appendix.
9. Mangiola et al. (2009). Calabria, Italy; pelatrice; 6 industrial samples produced from November 2007 to March 2008; double oven MDGC; precolumn: capillary column (30 m × 0.25 mm × 0.25 μm) coated with RTX-5; main column: capillary column (25 m × 0.20 mm × 0.18 μm) coated with diethyl-*tert*-butylsilyl-β-CD.

Kiwanuka et al. (2000) reported the chiral distribution of bergamot oils extracted in laboratory from fruits coming from Italy by means of solvent extraction or distillation at different values of pH of the peels and of the whole fruits. Each extraction was repeated 3 times, for a total of 12 samples. Enantiomeric ratios of linalol and linalyl acetate accord with data obtained from laboratory-distilled and cold-extracted oils (see Table 7.11). It is also the first time that values for this type of oils (laboratory distilled) are reported as concerns limonene, α-pinene, β-pinene, and α-terpineol. The paper shows that samples obtained by means of distillation, either of the peels or of the whole fruits, undergo a fluctuation of the enantiomeric ratio for linalol and α-terpineol. The racemization of linalol is attributed to the equilibrium existing between enantiomers in acidic medium due to hydration/dehydration phenomena. Also, part of linalol is formed from the hydrolysis of linalyl acetate during distillation, through the intermediate linalyl carbocation that leads to the formation of α-terpineol as well.

In 2000, Verzera et al. studied the chiral distribution of linalol and linalyl acetate in a series of samples belonging to different cultivars, namely Castagnaro, Femminello, PCF (a new clone of Femminello), and Fantastico. The results obtained were not only in agreement with data for genuine oils but were also identical to each other, demonstrating that the type of cultivar (at least those investigated in this study) does not affect the enantiomeric ratios of linalol and linalyl acetate. Hara et al. (1999) used a MDGC approach for the analysis of one sample of bergamot oil of Japanese origin cold-pressed in laboratory. The authors determined α-pinene, β-pinene, limonene, α-terpineol, and citronellal, with the results that all the chiral enantiomers had very similar values to the ones reported in Table 7.11 for cold-pressed bergamot oil. It is worthy of attention that the authors reported for the first time the chiral ratio of citronellal with an enantiomer excess very high for S(–)-citronellal (>98%). Two years later, Mitiku et al. (2001) analyzed a sample of Italian bergamot oil. The chiral couples of α-pinene, β-pinene, sabinene, and limonene gave results in accordance with the ones reported for cold-pressed bergamot oil in Table 7.11. A similar investigation was later carried out by Gionfriddo et al. (2003) on 25 samples of laboratory-extracted essential oils obtained from fruits collected in Reggio Calabria every two weeks starting from December 2000. The fruits belonged to the cultivars Femminello, Castagnaro, and Fantastico, and these were subjected to manual rasping of the peel in order to obtain the emulsion oil/water, then centrifuged and dehydrated with sodium sulfate. Linalol and linalyl acetate showed an enantiomeric distribution similar to that obtained by Verzera et al. (2000). For the other molecules, the enantiomeric ratio of

β-pinene, sabinene, and limonene are comparable with the previous data published by Casabianca and Chau (1997), although the results of Gionfriddo et al. (2003) varies in a range slightly wider of those of Casabianca and Chau (1997).

Recently, Costa et al. (2010) have carried out a deep investigation of the chemical composition of processed bergamot oil, which means terpeneless oils both with and without waxes, bergapten-free, and furocoumarin-free (colorless) oils. The enantiomeric analysis of these samples led to the separation of five enantiomeric couples, the values of which have been compared to a genuine sample of industrial cold-pressed bergamot oil. All the data are reported in columns 7a–c of Table 7.12. The main finding of the study was that the enantiomeric distribution is not affected by the technological process carried out (mainly distillation and fractionation), since all the values of chiral ratios fell within the ranges present in literature for cold-pressed bergamot oils. This outcome is also true when the technology used for processing bergamot oil is properly and skillfully utilized.

Columns 8a–c report data by Sciarrone (2009, Personal communication), obtained by means of conventional chiral GC from the samples of Italian cold-pressed bergamot oil, produced from January 2008 up to March 2009 (17 samples); Italian bergamot Peratoner (three samples); and cold-pressed essential oils from the Ivory Coast (two samples). For the first time, the enantiomeric composition of camphene, α- and β-phellandrene has been determined in bergamot oil. The analytical data obtained have been supported by the use of an MDGC system for establishing the elution order of chiral pairs. The enantio-GC profile of a cold-pressed bergamot oil is illustrated in Figure 7.9. One of the outcomes denies what previously determined by Casabianca and Chau (1997), as regards the elution order of the two enantiomers of α-thujene. All the other data are in agreement with previous works.

Finally, Mangiola et al. (2009) reported the chiral ratio of β-pinene, sabinene, limonene, linalyl acetate, terpinen-4-ol, and α-terpineol for six samples, taken from six lots of 1 metric ton using a pelatrice machine (Speciale) during the 2007–2008 season. The results for β-pinene, sabinene, limonene, linalol, and linalyl acetate fits very well with the data published for genuine cold-pressed

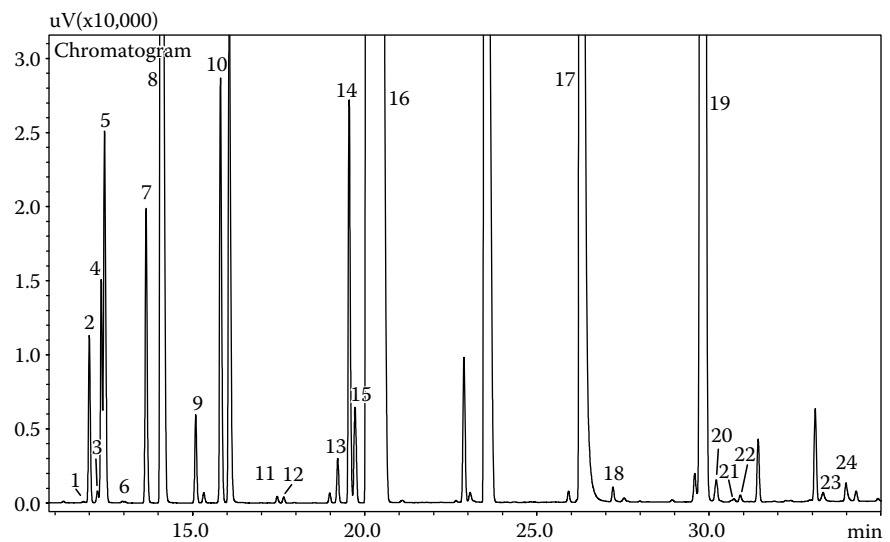

FIGURE 7.9 Conventional enantio-GC-FID chromatogram of a cold-pressed bergamot oil. Peak identification: 1. (–)-α-thujene; 2. (+)-α-thujene; 3. (–)-camphene; 4. (+)-α-pinene; 5. (–)-α-pinene; 6. (+)-camphene; 7. (+)-β-pinene; 8. (–)-β-pinene; 9. (+)-sabinene; 10. (–)-sabinene; 11. (–)-α-phellandrene; 12. (+)-α-phellandrene; 13. (–)-β-phellandrene; 14. (–)-limonene; 15. (+)-β-phellandrene; 16. (+)-limonene; 17. (–)-linalol. 18. (+)-linalol; 19. (–)-linalyl acetate; 20. (+)-linalyl acetate; 21. (+)-terpinen-4-ol; 22. (–)-terpinen-4-ol; 23. (–)-α-terpineol; 24. (+)-α-terpineol. (From Sciarrone, D., 2009. Personal communication.)

bergamot oil, while terpinene-4-ol presents values that are not consistent with data found by other groups. It is possible that data reported by Mangiola et al. for terpinen-4-ol are due to a printing or calculation error—in fact, in the chromatogram reported in the paper the ratio S(+)/R(–) terpinen-4-ol seems to be around 25/75, in agreement with literature data. Moreover, the values of R-(+)-α-terpineol, from 31.5% to 43.7%, follow the range determined for the cold-pressed bergamot oils. However, it must be noted that there is not a similar increase of R(+)-α-terpineol during the productive season reported earlier by Mondello et al. (1998d), with an increase of R(+)-α-terpineol from 17.5% to 69.4%.

7.9 NEROLI AND PETITGRAIN (*CITRUS AURANTIUM* L.) OILS

7.9.1 1990–2001

Tables 7.13 and 7.14 summarize data on the enantiomeric distribution of volatiles in neroli and petitgrain (bitter orange) oils, already revised by Mondello et al. (2002) for industrial and laboratory oils.

TABLE 7.13
Enantiomeric Distribution of Some Volatile Components of Neroli Oils (1990–2001)

		Industrial Oils			Laboratory Oils		
		Authentic	**Commercial**	**Artificial**	**Distilled[a]**	**Solvent[a]**	**SFE**
α-Pinene	R-(+)	11.8–13.6	10.5–23	52–54	2.2–12.3	8.7–13.7	
	S-(–)	88.2–86.4	89.5–77	48–46	97.8–87.7	91.3–86.3	
β-Pinene	R-(+)	0.3–0.5	0.1–4	4–5	<0.1–0.8	<0.1–0.7	
	S-(–)	99.7–99.5	99.9–96	96–95	>99.9–99.2	>99.9–99.3	
Limonene	S-(–)	2.8–3.1	1.7–4.2	8	1.9–6.9	1.5–2.6	
	R-(+)	97.2–96.9	98.3–95.8	92	98.1–93.1	98.5–97.4	
Linalol	R-(–)	71.0–71.4	63.1–78.7		70.8–81.5	72.0–90.6	78.6
	S-(+)	29.0–28.6	36.9–21.3		29.2–18.5	28.0–9.4	21.4
Linalyl acetate	R-(–)	96.0–96.2	62.8–97.9		95.4–98.2	96–98.2	95
	S-(+)	4.0–3.8	37.2–2.1		4.6–1.8	4–1.8	5
Terpinen-4-ol	S-(+)	36.0–37.6	35.1–58.8				
	R-(–)	64.0–62.4	64.9–41.2				
α-Terpineol	S-(–)	29.9–30.6	28.1–39.8				
	R-(+)	70.1–69.4	71.9–60.2				
(*E*)-Nerolidol	R-(–)	1.2–1.4	<1–33.4		0.4–1.8	0.9–1.1	
	S-(+)	98.8–98.6	>99–66.6		99.6–98.2	99.1–98.9	

Notes: [a] Extracted from fresh or dried blossoms.

Appendix to Table 7.13

- The results reported in Table 7.13 and in this appendix, for the different categories of neroli oils, are taken from the following original papers:
  - Authentic oils: Juchelka et al. (1996).
  - Commercial oils: Hener et al. (1990a, b); Mosandl et al. (1990); Mosandl (1995); Ravid et al. (1995); Juchelka et al. (1996).
  - Artificial oils: Hener et al. (1990a).
  - Laboratory oils: Juchelka et al. (1996); Casabianca and Graff (1996).
- All the results reported in the table were obtained by MDGC, with the exception of those reported by Ravid et al. (1995) obtained by direct analysis on chiral column.

TABLE 7.14
Enantiomeric Distribution of Some Volatile Components of Bitter Orange Petitgrain Oils (1990–2001)

		Industrial Oils		Laboratory Oils		
		Authentic	**Commercial**	**Distilled[a]**	**Solvent[a]**	**SFE**
α-Pinene	R-(+)	19.9	11.6–18.0	6.7–12.0	7.6–34.7	
	S-(–)	80.1	88.4–82.0	93.3–88.0	92.4–65.3	
β-Pinene	R-(+)	0.5	0.2–8.0	<0.1–1.1	0.5–1.1	
	S-(–)	99.5	99.8–92.0	>99.9–98.9	99.5–98.9	
Limonene	S-(–)	3.7	9.2–44.0	29.2–39.2	23.9–46.7	
	R-(+)	96.3	90.8–56.0	70.8–60.8	76.1–53.3	
Linalol	R-(–)	70.1	53.4–80.9	66.4–90.2	98.6 to >99.9	100
	S-(+)	29.9	46.6–19.1	33.6–9.8	1.4 to <0.1	0
Linalyl acetate	S-(+)	1.6	0.7–25.3	0.9–6.6	0.8–1.5	0
	R-(–)	98.4	99.3–74.7	99.1–93.4	99.2–98.5	100
Terpinen-4-ol	S-(+)	46.0	43.0–49.0	47.9–67.4		
	R-(–)	54.0	57.0–51.0	52.1–32.6		
α-Terpineol	S-(–)	26.4	27.0–43.1	27.5–28.4		
	R-(+)	73.6	73.0–56.9	72.5–71.6		

Notes: [a] Extracted from fresh or dried leaves.

Appendix to Table 7.14

- The results reported in Table 7.14 and in this appendix, for the different categories of bitter orange petitgrain oils, are taken from the following original papers:
  - Authentic oils: Juchelka et al. (1996).
  - Commercial oils: Hener et al. (1990a,b); Mosandl et al. (1990); Mosandl (1995); Bernreuther and Schreier (1991); Ravid et al. (1995).
  - Laboratory oils: Juchelka et al. (1996); Casabianca and Graff (1996).
- Most of the results reported in the table were obtained by MDGC, with the exception of those reported by Bernreuther and Schreier (1991) and by Ravid et al. (1995) obtained by direct analysis on chiral column.

7.9.2 2001–2009

Just a few data (Table 7.15) have been published in the last years on the enantiomeric distribution of neroli oil. Braun and Regensburg (2001) investigated nine commercial oils by conventional capillary GC and capillary enantio-GC. They found out that only one sample was genuine, while two resulted as adulterated, based on the values of enantiomeric ratios of linalol. Also, six samples resulted adulterated when the peak area percentiles and the limit prescribed by European Pharmacopoeia NT 2000 for linalol and linalyl acetate were considered. These limits (18%–42% for linalol and 3%–16% for linalyl acetate) seem to be rather narrow when compared to the ranges found for these components in literature (see Chapter 6). On this basis, we can conclude that some or all the samples are genuine. Dugo et al. (2010a) reported values of the enantiomeric distribution of five neroli oils from Egypt declared as genuine by the producer. The authors reported the enantiomeric distribution of α-thujene, α- and β-phellandrene, and sabinene for the first time in neroli oil. Enantiomeric ratio of limonene, linalol, terpinen-4-ol, and α-terpineol, already reported for genuine neroli oils, show values in good agreement with these oils, while the enantiomeric ratio of β-pinene is slightly different, similar to that previously reported for some commercial oils (see Table 7.13). Taking into consideration the limited availability of data on authentic neroli oil, it is

TABLE 7.15
Enantiomeric Distribution of Some Volatile Components of Neroli and Petitgrain Oils (Bitter Orange, Lime, Mandarin) Oils (2001–2009)

		Neroli Oils				Petitgrain Oils			
						Bitter Orange	Lime	Lime	Mandarin
					Egypt	Egypt	Mexico	Egypt	Egypt
		1a	1b	1c	2a	2b	3a	3b	4
α-Thujene	S-(+)*				30.98–59.02[a]	40.11	12.62		1.30
	R-(–)*				69.02–40.98[a]	59.89	87.38		98.70
α-Pinene	R-(+)				32.96–41.37[a]	28.97	78.08	77.80	31.61
	S-(–)				67.04–58.63[a]	71.03	21.92	22.20	68.39
Camphene	1S,4R-(–)						28.52	72.19	35.76
	1R,4S-(+)						71.48	27.81	64.24
β-Pinene	R-(+)				1.72–2.08	1.81	31.05	5.89	98.55
	S-(–)				98.28–97.92	98.19	68.95	94.11	1.45
Sabinene	R-(+)				76.72–81.93		85.68	31.32	79.12
	S-(–)				23.28–18.07		14.32	68.68	20.88
α-Phellandrene	R-(–)				14.37–44.55[a]	4.88	16.66		79.19
	S-(+)				85.63–55.45[a]	95.12	83.34		20.81
β-Phellandrene	R-(–)				27.75–53.74[a]	21.07	30.05	26.67	6.04
	S-(+)				72.25–46.26[a]	78.93	69.95	70.33	93.96
Limonene	S-(–)				1.65–2.60	6.65	2.43	0.75	10.74
	R-(+)				98.35–97.40	93.35	97.57	99.25	89.26
Linalol	R-(–)	78.9	69.3–84.0	53.9–59.1	78.25–78.55	85.99	58.80	70.71	24.83
	S-(+)	21.1	30.7–16.0	46.1–40.9	21.75–21.45	14.01	41.20	29.29	75.17
Citronellal	S-(–)				45.09–47.14[a]	71.46	53.43	89.25	86.86
	R-(+)				54.91–52.86[a]	28.54	46.57	10.75	13.14
Linalyl acetate	R-(–)				98.95–99.47	99.76			98.54
	S-(+)				1.05–0.53	0.24			1.46

Terpinen-4-ol	S-(+)	60.00–62.74	57.54	95.87	88.29	31.48
	R-(–)	40.00–37.26	42.46	4.13	11.71	68.52
α-Terpineol	S-(–)	28.77–29.21	25.88	77.62	80.40	80.93
	R-(+)	71.23–70.79	74.12	22.38	19.60	19.07

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil; [a]The wide ranges of variability are commented in text.

Appendix to Table 7.15

1. Braun and Regensburg (2001). (a) One genuine commercial oil, (b) six commercial oils according to the European Phamacopeia for their content of linalol and linalyl acetate must be considered adulterated, (c) two commercial samples adulterated for the enantiomeric ratio of linalol; direct enantio-GC on chiral column.
2. Dugo et al. (2010a). (a) one sample of neroli oil from Egypt produced in 2008 and four samples of the same origin produced in 2009, (b) one sample of bitter orange petitgrain oil from Egypt; for analytical method see point 9 of Table 7.4.
3. Dugo et al. (2010b). (a) one sample of lime petitgrain oil from Mexico, (b) one sample of lime petitgrain from Egypt; for analytical methods see point 9 of Table 7.4.
4. Sciarrone (2009, personal communication) one sample of mandarin petitgrain oil from Egypt; for analytical methods see point 9 of Table 7.4.

very difficult to hypothesize whether the value presented by β-pinene is due to the characteristics of Egyptian oils or to contaminations. The wide ranges in variability of the enantiomeric ratios of α-thujene, α-pinene, and α- and β-phellandrene could be due to the low amount of these components, as well as possible chromatographic coelutions. The exact determination of these enantiomeric ratio requires the use of MDGC. Figure 7.10 reports a chiral chromatogram of an Egyptian neroli oil. Table 7.15 reports the results for a sample of bigarade petitgrain from Egypt, with the enantiomeric ratios for α-thujene and α- and β-phellandrene determined for the first time. Some of the results are different from the ones reported previously in literature as genuine samples (see Table 7.14). All the considerations expressed above for the neroli oils are also valid here.

7.10 OTHER PETITGRAIN OILS

Table 7.15 also report results of an industrial sample of lime petitgrain from Mexico and of an industrial mandarin petitgrain from Egypt. Literature does not report data on these two kinds of oils, so no comments can be expressed on them.

7.11 MINOR *CITRUS* SPECIES

Table 7.16 reports enantiomeric information on various minor citrus species and some hybrids published in the last decades. Information on the analyzed oils can be found in the appendix. The results reported are, for the main part, difficult to comment on because no literature data can be found for most of these oils. However, some consideration on a few oils now follows. Verzera et al. (2000) studied the chiral distribution of some samples of *Citrus unshiu*, known as Satsuma mandarin, and *Citrus reticulata* Blanco cv. Nova, known as Nova mandarin, both coming from Uruguay. The Satsuma mandarin originated in China, from where it was introduced in Japan and into the Western countries. Nova mandarin is a hybrid of *Citrus clementina* cv. Fina and Orlando tangelo (Duncan grapefruit × Dancy tangerine). Authors utilized MDGC to determine the enantiomeric ratios of five chiral compounds in essential oils extracted in laboratory by cold pressure and centrifugation. Big differences arise from comparison of the two oils with the exception of limonene, which, of course, cannot be considered a marker for differentiating the two species.

In a wide investigation of mandarin oils and their hybrids, Mitiku et al. (2002) analyzed the essential oils obtained in laboratory from fruits of *Citrus unshiu* collected in Japan. Also, in this case, oils were extracted in laboratory from fresh fruits. Direct injection on a chiral stationary phase was used for the assessment of the enantiomeric ratios of seven pairs. The results obtained are more or less similar to those by Verzera et al. (2000b), although not superimposable. This could be attributed to either the geographical origin of the fruits utilized or to the incorrect identification of the species under investigation. Figure 7.11 shows the enantio-GC analysis of Ponkan mandarin, Satsuma mandarin, and Minneola, a mandarin hybrid.

Commercial Sweetie oils (*C. grandis* Osbeck × *C. paradisi* Macf.) coming from Israel were analyzed by Feger et al. (2001). The species is a hybrid between pummelo and white grapefruit and is also known as "Oroblanco." Authors produced results for three enantiomeric couples, arriving at the conclusion that the values obtained are superimposable to those of grapefruit. Therefore, in this case, chiral GC cannot be a good means to distinguish between grapefruit and its hybrid.

Citrus medica L. cv. Diamante (citron oil) was investigated by Gabriele et al. (2009), who established the enantiomeric ratios for five molecules. In particular, the oils obtained were extracted in laboratory, partly by manual squeezing of the peels and partly with the aid of a syringe directly applied to the utricles. Also, the fruits were divided into categories of small green, big green, and yellow. In total, nine samples were obtained, but no relevant differences could be attributed to the nature of fruits.

TABLE 7.16
Enantiomeric Distribution of Some Volatile Components of Minor Citrus and Hybrids

		1a	2a	2b	2c	3a	3b	3c			3d	3e	3f	3g	3h	3i	3j
α-Pinene	R-(+)	100				99.6	30.1	60.6	92.2	65.5	61.8	67.1	98.1	64.1	79.1	70.1	71.3
	S-(–)	tr				0.4	69.9	39.4	7.8	34.5	38.2	32.9	1.9	35.9	20.9	29.9	28.7
β-Pinene	R-(+)	95	95.1	0.7	11.2	86.5	6.9	57.8	4.3	81.0	62.0	93.4	63.1	78.4	1.0	96.3	93.0
	S-(–)	5	4.9	99.3	88.8	13.5	93.1	42.2	95.7	19.0	38.0	6.6	36.9	21.6	99.0	3.7	7.0
Sabinene	R-(+)		73.2	21.2	23.4												
	S-(–)		26.8	78.8	76.6												
Limonene	S-(–)	tr	1.5	0.7	1.1	0.8	0.2	1.3	1.0	2.2	1.2	1.0	0.8	1.4	0.8	1.7	0.9
	R-(+)	100	98.5	99.3	98.9	99.2	99.8	98.7	99.0	97.8	98.8	99.0	99.2	98.6	99.2	98.3	99.1
Linalol	R-(–)		65.7	50.4	52.4	57.9	50.0	49.9	59.1	36.2	70.6	47.6	1.9	2.7	12.7	5.8	82.5
	S-(+)		34.3	49.6	47.6	42.1	50.0	50.1	40.9	63.8	29.4	52.4	98.1	97.3	87.3	94.2	17.5
Citronellal	S-(–)						89.1	30.3	91.9		70	22.0		31.1	63.2	8.2	23.4
	R-(+)						10.9	69.7	8.1		30	78.0		68.9	36.8	91.8	76.6
Terpinen-4-ol	S-(+)		11.2	70.5	30.1												
	R-(–)		88.8	29.5	69.9												
α-Terpineol	S-(–)		75.0	44.7	60.3	17.9	79.2	53.8	19.9	46.2	49.9	46.0	4.2	47.3	17.0	40.7	40.5
	R-(+)		25.0	55.3	39.7	82.1	20.8	46.2	80.1	53.8	50.1	54.0	95.8	52.7	83.0	59.3	59.5

continued

TABLE 7.16 (continued)
Enantiomeric Distribution of Some Volatile Components of Minor Citrus and Hybrids

		3k	3l	3m	3n	3o	3p	3q	4a	4b	5	6a	6b	6c	6d	6e
α-Pinene	R-(+)	72.2	70.8	55.9	99.7	99.7	99.3	69.4				64.86	53.18	61.36	73.26	97.89
	S-(–)	27.8	29.2	44.1	0.3	0.3	0.7	30.6				35.14	46.82	38.64	26.74	2.11
β-Pinene	R-(+)	98.5	97.7	98.3	58.5	66.2	56.1	0.7	95.8–95.9	63.3–69.7	100	100.00	85.39	52.15	100.00	56.63
	S-(–)	1.5	2.3	1.7	41.5	33.8	43.9	99.3	4.2–4.1	36.7–30.3	0	0.00	14.61	47.85	0.00	43.37
Sabinene	R-(+)								92.5–92.6	97.2–97.3		90.00	94.90	60.81	89.11	98.05
	S-(–)								7.5–7.4	2.8–2.7		10.00	5.10	39.19	10.99	1.95
Limonene	S-(–)	2.7	0.9	1.5	0.7	0.6	0.9	2.0	0.8	0.6	0.6	1.20	2.70	0.80	0.64	0.55
	R-(+)	97.3	99.1	98.5	99.3	99.4	99.1	98.0	99.2	99.4	99.4	98.80	97.30	99.20	99.36	99.45
Linalol	R-(–)	5.2	55.2	97.5	7.5	58.8	7.5	59.7	72.6–72.9	3.7–4.0	36					
	S-(+)	94.8	44.8	2.5	92.5	41.2	92.5	40.3	27.4–27.1	96.3–96.0	64					
Citronellal	S-(–)	1.5			6.3	70.1	2.0	8.2								
	R-(+)	98.5			93.7	29.9	98.0	91.8								
Terpinen-4-ol	S-(+)															
	R-(–)															
α-Terpineol	S-(–)	31.0			1.5	1.2	1.4	27.8	33.1–33.2	4.5–5.6						
	R-(+)	69.0			98.5	98.8	98.6	72.2	66.9–66.8	95.5–94.4						

		6f	6g	6h	6i	6j	6k	6l	6m	6n	6o	6p	6q	6r
α-Pinene	R-(+)	53.07	85.02	63.41	84.26	69.31	94.55	89.16	69.57	50.15	69.09–74.91	77.04	74.10	74.40
	S-(–)	46.93	14.98	36.59	15.74	30.69	5.45	10.84	30.43	49.85	30.91–25.09	22.96	25.90	25.60
β-Pinene	R-(+)	100.00	1.03	79.02	0.00	95.59	3.95	1.45	21.40	95.42	74.55–93.62	87.77	89.27	73.43
	S-(–)	0.00	98.97	20.98	100.00	4.41	96.05	98.55	78.60	4.58	25.45–6.38	12.23	10.73	26.57
Sabinene	R(+)	73.57	39.67	81.82	50.00	100.00	62.69	48.75	48.51	77.59	87.76–88.05	100.00	90.12	90.08
	S-(–)	26.43	60.33	18.18	50.00	0.00	37.31	51.25	51.49	22.41	12.24–11.95	0.00	9.88	9.92
Limonene	S-(–)	1.17	0.53	0.87	6.32	0.73	0.45	0.46	0.63	1.27	0.64–0.67	0.69	0.71	0.67
	R(+)	98.83	99.47	99.13	93.68	99.27	99.55	99.54	99.37	98.73	99.36–99.33	99.31	99.29	99.33
Linalol	R-(–)													
	S-(+)													
Citronellal	S-(–)													
	R-(+)													
Terpinen-4-ol	S-(+)													
	R-(–)													
α-Terpineol	S-(–)													
	R-(+)													

continued

TABLE 7.16 (continued)
Enantiomeric Distribution of Some Volatile Components of Minor Citrus and Hybrids

		6s	6t	6u	6v	6w	7a	7b	8a	8b	8c	8d	8e	8f	8g	9
α-Pinene	R-(+)	99.44	99.13	47.68	99.54	98.80			0.9	0.6	25.6	25.9	23.0	25.1	30.9	
	S-(–)	0.56	0.87	52.32	0.46	1.20			99.1	99.4	74.4	74.1	77.0	74.9	69.1	
β-Pinene	R-(+)		44.93	16.87	100.00	12.14	95.9	63.3	85.4	84.1	74.6	79.0	87.8	67.5*	93.6	90.6–94.4
	S-(–)		55.07	83.13	0.00	87.86	4.1	36.7	14.6	15.9	25.4	21.0	12.2	32.5*	6.4	9.4–5.6
Sabinene	R-(+)	97.75	97.88	30.41	100.00	91.09	92.5	97.3	97.9	97.8	90.1	90.1	100.0	88.0	87.8	63.5–67.9
	S-(–)	2.25	2.12	69.59	0.00	8.91	7.5	2.7	2.1	2.2	9.9	9.9	0.0	12.0	12.2	36.5–32.1
Limonene	S-(–)	0.47	0.49	1.12	0.52	0.87	0.8	0.6	0.8	6.3	0.6	0.5	0.7	0.6	0.7	2.1–2.4
	R-(+)	99.53	99.51	98.88	99.48	99.13	99.2	99.4	99.2	93.7	99.4	99.5	99.3	99.4	99.3	97.9–97.6
Linalol	R-(–)								1.2	7.6	5.8	27.7	5.9	87.5	90.0	41.0–47.3
	S-(+)								98.8	92.4	94.2	72.3	94.1	12.5	10.0	59.0–52.7
Citronellal	S-(–)								86.6	12.9	10.6	10.6**	7.7	19.4	8.8	
	R-(+)								13.4	87.1	89.4	89.4**	92.3	80.6	91.2	
Terpinen-4-ol	S-(+)															
	R-(–)															
α-Terpineol	S-(–)								7.8	14.6	38.1	37.3	37.9	36.7	43.4	75.2–77.4
	R-(+)								92.2	85.4	61.9	62.7	62.1	63.3	56.6	24.8–22.6

Notes: * In the original paper 67.5/22.6; ** in the original paper 0.6/90.4.

Appendix to Table 7.16

1. Mosandl et al. (1990), Herner et al. 1990a, Mosandl 1995. MDGC; *Fortunella margarita.*
2. Starrantino et al. (1997). MDGC; three hybrids from a cross between Femminello Siracusano lemon and Pera del Commendatore (probably a natural hybrid of pummelo).
3. Hara et al. (1999). Japan;. MDGC; (a) *Fortunella crassifolia* (Kumquat), (b) *Citrus medica* (Citron), (c) *C. grandis* (Pummelo), (d) *C. hassaku*, (e) *C. natsudaidai* (Natsudaidai), (f) *C. tankau*, (g) *C. iyo*, (h) *C. tachibana* (Tachibana) (i) *C. reticolata*, (j) *C. unshiu* (Satsuma), (k) *C. sudachi* (Sudachi), (l) *C. sphaerocarpa* (Kabosu), (m) *C. junos* (Yuzo), (n) *C. reticulata* × *C. sinensis*, (o) *C. unshiu* × *C. grandis*, (p) Kiomi × *C. reticulata*, (q) *C. trifoliata*.
4. Verzera et al. (2000b). Uruguay; MDGC; (a) *C. unshiu* (Satsuma), (b) *C. reticulata* (Nova).
5. Feger et al. (2001). Israel; Sweete orange or Oroblanco (pummelo × white grapefruit); for analytical method see point 2 of the appendix to Table 7.4.

6. Mitiku et al. (2001). (a) *C. junos* (Yuzu), (b) *C. yuko* (Yuko), (c) *C. wilsonii* (Ichang lemon), (d) *C. sphaerocarpa* (Kabosu), from Japan; (e) *C. jambhiri* Lush. (Rough lemon), (f) Coastal lemon, from Kenia; (g) *C. grandis* forma Hirado-butan; (h) *C. grandis* forma Tosa (Tosa-butan), Japan; (i) Kiyooka daida; (j) *C. natsudaidai* (Natsudaidai), (k) *C. aurantium* var. cyathifera (Daidai), (l) *C. aurantium* var. Kabusu (Kabusu), (m) *C. neo-aurantium* (Konejime), (n) *C. ujukitu* (Ujukitsu), (o) *C. unshiu* (Satsuma), from Japan; (p) Sabine tangerine, (q) *C. unshiu* × *C. nobilis* (Kara), (r) *C. paradisi* × *C. tangerine* (Minneola), from Kenya; (s) *C. tangerine* × *C. paradisi* (Orlando), (t) *C.reticulata* × *C. sinensis* (Temple), from Ethiopia; (u) *C. tachibana* Ten. (Tachibana); (v) *Fortunella japonica* (Marukinkan), (w) *C. ozu* (Ozu). For the analytical method see point 2 of the appendix to Table 7.2.
7. Lorenzo et al. (2002). Laboratory oils; (a) one sample of Satsuma (*C. unshiu* Marcov.) oil; (b) one sample of Nova (*C. reticulata* Blanco) oil; for analytical method see point 4 of appendix to Table 7.4.
8. Mitiku et al. (2002). Laboratory oils cold-pressed; (a) Temple tangor (*C. reticulata* × *C. sinensis*) from Ethiophia, (b) Orlando tangelo (Duncan grapefruit × Dancy tangerine) from Ethiopia, (c) Minneola (Duncan grapefruit × Dancy tangerine) from Kenya, (d) Kara (*C. unshiu* × *C. nobilis*) from Kenya, (e) Ponkam (*C. reticulata* Blanco cv. F-2426) from Japan, (f) Satsuma (*C. unshiu* Marcov. forma *Miyagawa-wase*) from Japan; (g) Satsuma (*C. unshiu* Marcov. forma Imamura) from Japan; enantiomeric ratio of α-pinene, β-pinene and limonene were determined coupling on line a conventional capillary column (60 m × 0.25 mm × 0.25 μm) coated with Thermon 600T and a chiral capillary column (30 m × 0.25 mm × 0.25 μm) coated with 2,3,6-tri-*O*-methyl-β-CD; enantiomeric ratio of sabinene was determined by direct analysis on the chiral column; enantiomeric ratios of linalol, α-terpineol, and citronellal were determined by coupling on line a conventional capillary column (30 m × 0.25 mm × 0.25 μm) coated with DB-5 and a chiral capillary column (30 m × 0.25 mm × 0.25 μm) coated with 2,3-di-*O*-acetyl-6-*tert*-butyldimethylsilyl-β-CD; for analysis of Satsuma oils was used a double oven MDGC.
9. Gabriele et al. (2009). Diamante citron (*C. medica* L. cv. Diamante); laboratory cold-extracted oils from green fruits of big and small size; direct enantio GC on capillary column (25 m × 0.25 × 0.25 mm) coated with diethyl-*tert*-butylsilyl-β-CD.

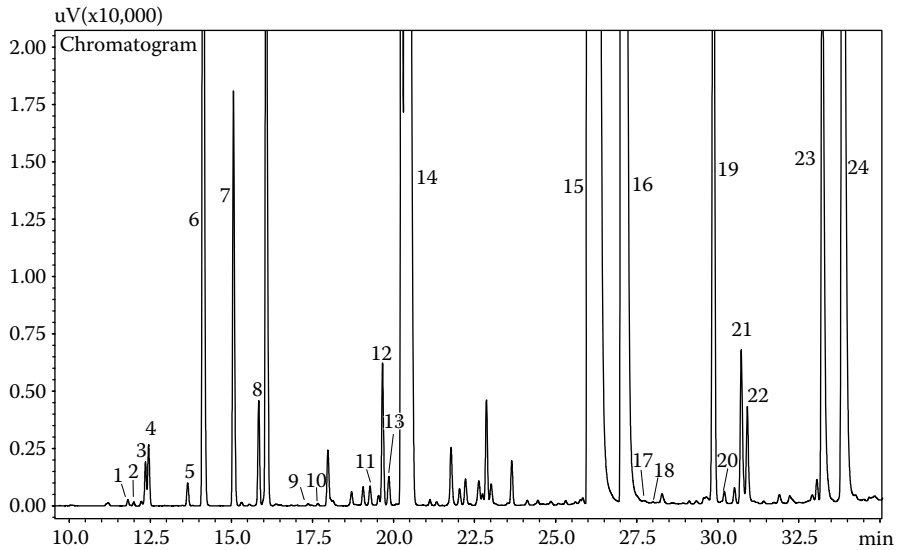

FIGURE 7.10 Conventional enantio-GC-FID chromatogram of a neroli oil from Egypt. 1. (–)-α-Thujene; 2. (+)-α-thujene; 3. (+)-α-pinene; 4. (–)-α-pinene; 5. (+)-β-pinene; 6. (–)-β-pinene; 7. (+)-sabinene; 8. (–)-sabinene; 9. (–)-α-phellandrene; 10. (+)-α-phellandrene; 11. (–)-β-phellandrene; 12. (–)-limonene; 13. (+)-β-phellandrene; 14. (+)-limonene; 15. (–)-linalol; 16. (+)-linalol; 17. (–)-citronellal; 18. (+)-citronellal; 19. (–)-linalyl acetate; 20. (+)-linalyl acetate; 21. (+)-terpinen-4-ol; 22. (–)-terpinen-4-ol; 23. (–)-α-terpineol; 24. (+)-α-terpineol. (From Dugo, G., et al., *J. Essent. Oil Res.* 2010a. In press.)

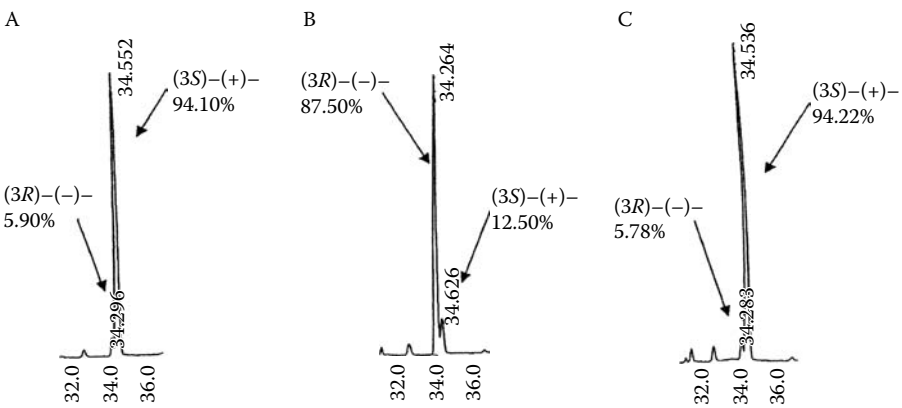

FIGURE 7.11 Difference between cold-pressed oils of (A) mandarin (Ponkan); (B) Satsuma mandarin (Miyagawa-wase); and (C) mandarin hybrid (Minneola) in enantioselective linalol analysis. (From Mitiku, A.B., et al., *J. Essent. Oil Res.* 14, 196–202, 2002. Reproduced with permission.)

7.12 FINAL REMARKS

The results reported in this chapter can be useful to evaluate the purity of citrus essential oils. When the enantiomeric ratios of specified components have been widely studied and present narrow ranges of variability in a citrus cold-pressed oils, they can be used for purity assessment. An example is given in bergamot oil by the enantiomeric ratios of linalol and linalyl acetate. Their determination

TABLE 7.17
Ranges of Variation of Some Components in Citrus Cold-Pressed Essential Oils and Their Enantiomeric Ratio

		Lemon	Bergamot	Key Lime	Persian Lime	Grapefruit	Bitter Orange	Sweet Orange	Mandarin	Tangerine	Clementine
	%	0.27–0.54	0.15–0.49	0.27–0.43	0.46–0.60		tr–0.02	tr–0.03	tr–1.06		
α–Thujene	S-(+)*	0.7–1.2	0.5–1.3	1.4–3.9	0.9–1.6		10.3–30.8	10.2–62.0	0.3–1.0		
	R-(–)*	99.3–98.8	99.5–98.7	98.6–96.1	99.1–98.4		89.7–69.2	89.8–38.0	99.7–99.0		
	%	0.88–4.40	0.46–1.84	1.91–2.70	1.78–2.25	0.48–1.7	0.29–0.89	0.36–1.4	1.55–5.24		
α-Pinene	R-(+)	25.5–38.0	26.0–38.4	21.8–22.7	29.2–32.6	99–100	89.6–97.4	90.1–99.4	41.7–54.5		
	S-(–)	74.5–62.0	74.0–61.6	78.2–77.3	70.8–67.4	1–0	10.4–2.6	9.9–0.6	58.3–45.5		
	%	tr–0.13	tr–0.11	0.07–0.12	0.04–0.11		tr–0.01		tr–0.02		
Camphene	1S,4R-(–)	86.2–92.4	85.7–90.1	87.3–92.8	87.7–89.0		35.8–45.0		31.8–55.3		
	1R,4S-(+)	13.8–7.6	14.3–9.9	12.7–7.2	12.3–11.0		64.2–55.0		68.2–44.7		
	%	8.57–17.79	2.97–10.60	18.25–25.45	10.01–12.20	tr–0.26	0.10–1.28	tr–0.11	1.0–2.44	0.29–0.34	0.03–0.26
β-Pinene	R-(+)	4.2–7.0	6.8–10.3	3.0–3.6	8.3–10.3	60.0–76.8	1.3–7.9	10.6–70.2	86.0–98.8	97.6–98.3	58.1–61.1
	S-(–)	95.8–93.0	93.2–89.7	97.0–96.4	91.7–89.7	40.0–23.2	98.7–92.1	89.4–29.8	14.0–1.2	2.4–1.7	41.9–39.9
	%	1.13–2.79	0.51–1.69	2.31–3.28	0.91–2.07	0.28–1.12	0.09–0.45	0.24–0.80	0.10–0.59	0.14–0.18	0.28–1.26
Sabinene	R-(+)	12.4–15.5	13.7–19.8	14.4–15.3	17.6–23.4	98.4–98.5	42.1–80.6	94.6–98.8	70.3–81.7	90.1–92.7	97.5–97.6
	S-(–)	87.6–84.5	86.3–80.2	85.6–84.7	82.4–76.6	1.6–1.5	57.9–19.4	5.4–1.2	29.7–18.3	9.9–7.3	2.5–2.4
	%	tr-0.13	0.01–0.18	0.02–0.05	tr–0.05		tr–0.08		0.03–0.11		
α-Phellandrene	R-(–)	46.9–52.6	43.1–54.7	53.3–58.9	49.1–56.4		74.9		41.8–55.0		
	S-(+)	53.1–47.4	56.9–45.3	46.7–41.1	50.9–43.6		25.1		58.2–45.0		
	%	tr–0.48	0.02–0.21	n.q.	tr		tr	tr-0.20	tr		
β-Phellandrene	R-(–)	31.1–53.9	26.3–36.9	44.2–46.3	27.9–37.8		1.1–5.7	0.4–1.4	0.4–3.0		
	S-(+)	68.9–46.1	73.7–63.1	55.8–53.7	72.1–62.2		98.9–94.3	99.6–98.6	99.6–97.0		
	%	59.57–71.82	23.5–54.85	47.87–51.14	51.47–59.81	92.20–96.20	91.54–96.52	91.15–96.10	65.30–77.82	89.58–90.94	91.50–94.78
Limonene	S(–)	1.0–2.6	1.2 to <3.0	1.8–2.9	0.4–4.1	tr-1.0	0.5–0.7	tr–1.1	tr-2.6	0.8	0.6
	R(+)	99.0–97.4	98.8 to >97.0	98.2–97.1	99.6–95.9	100–99.0	99.5–99.3	100–98.9	100–97.4	99.2	99.4
	%	0.05–0.46	1.58–36.14	0.15–0.24	0.11–0.24	tr–0.16	0.06–0.37	0.17–0.80	0.02–0.31		0.21–1.52
Linalol	R(–)	49.5–74.5	99.0–100	67.2–73.1	52.3–69.3	23.4–25.5	61.1–92.4	2.2–17.9	13.0–21.0		2.4–9.0
	S(+)	50.5–25.5	1.0–0	32.8–26.9	47.7–30.7	76.6–74.5	38.9–7.6	97.8–82.1	87.0–79.0		97.6–91.0
	%	0.03–0.17	tr-0.06	tr-0.04	0.03–0.06	tr–0.20	tr–0.02		tr–0.05		
Citronellal	S-(–)	89.5–94.8	>99	77.0–81.0	78.7–80.5	16.6–21.4	36.0		4.6–9.2		
	R-(+)	10.5–5.2	n.q	23.0–19.0	21.3–19.5	83.4–78.6	64.0		95.4–90.8		

continued

TABLE 7.17 (continued)
Ranges of Variation of Some Components in Citrus Cold-Pressed Essential Oils and Their Enantiomeric Ratio

		Lemon	Bergamot	Key Lime	Persian Lime	Grapefruit	Bitter Orange	Sweet Orange	Mandarin	Tangerine	Clementine
	%		11.80–41.36				0.08–1.17				
Linalyl acetate	R-(–)		99.0–100				99.4–99.2				
	S-(+)		1.0–0				0.6–0.8				
	%	0.01–0.10	tr–0.29	0.04–0.70	0.04–0.17		tr–0.03		tr–0.08		
Terpinen-4-ol	S-(+)	12.0–32.5	9.7–31.5	28.1–35.3	17.9–29.5		65.3–71.5		9.5–19.2		
	R-(–)	88.0–67.5	90.3–68.5	71.9–64.7	82.1–70.5		34.7–28.5		90.5–81.8		
	%	0.05–0.84	0.03–0.13	0.21–0.80	0.16–0.37	tr–0.20	0.03–2.94	0.02–0.15	0.04–0.46		0.02–0.13
α-Terpineol	S-(–)	64.2–82.0	14.0–69.4	82.8–87.6	74.5–82.6	96.7–98.8	6.8–29.8	5.1–15.7	66.1–76.8		2.5–2.7
	R-(+)	35.8–18.0	86.0–30.6	17.2–12.4	25.5–17.4	3.3–1.2	93.2–70.2	94.9–84.3	33.9–23.2		97.5–97.3
	%		tr–0.29								
Citronellol	S-(–)		12.0–20.0								
	R-(+)		88.0–80.0								
	%		0.07–0.29								
α-Terpinyl	S-(–)		36.0–44.0								
acetate	R-(+)		64.0–56.0								
	%							tr–0.04			
α-Copaene	(+)							15.0–16.0			
	(–)							85.0–84.0			
	%							tr–0.05			
δ-Cadinene	(+)							90			
	(–)							10			
	%								tr–0.02		
Camphor	(+)								17.0–36.5		
	(–)								83.0–63.5		
	%					0.01–0.21		tr–0.20			
Carvone	S-(+)					65.2		59.3			
	R-(–)					34.8		40.7			
	%		tr-0.04				0.04–0.23				
(*E*)-Nerolidol	S-(+)		>80				>80				
	R-(–)		<20				<20				

Notes: * Correct enantiomer not confirmed, tentatively assigned according to Casabianca and Chau (1997) for bergamot oil; n.q., not quantified.

is, in fact, considered as one of the fundamentals of the purity evaluation of bergamot oil (unless the oil is additionally adulterated by linalol and linalyl acetate isolated from natural sources different from citrus, where these components have a strong excess of the levorotatory enantiomers, as they occur in bergamot oil). Limonene has been largely investigated in all citrus oils; its levorotatory enantiomer range, in all oils, is between 0% and 3%. Apparently, its enantiomeric ratio can be used for the purity evaluation of the oils, but it can only reveal adulteration if this was performed adding products different from citrus of natural or synthetic origin and containing high level of the levorotatory isomer of limonene. Adulteration of citrus oils by the determination of the enantiomeric ratio of limonene cannot be detected if different citrus species were used as adulterant.

To better evaluate the potentiality and limits of the use of enantiomeric ratios of volatiles determined in cold-pressed citrus oils, Table 7.17 summarizes the ranges of variability determined in genuine industrial oils (when available), singularly discussed in Tables 7.1 through 7.14 of this chapter. In the same table, the ranges of variability of the same components previously discussed in Chapter 1 are reported, expressed as relative peak area percent. For both types of data, the writers decided not to include values they considered anomalous. Clementine oils report the values of the enantiomeric ratios as determined by Verzera et al. (1997b, 1998b) in oils cold extracted in laboratory due to the absence of literature on industrial oils. For this same reason, the values of the enantiomeric ratio determined in grapefruits are relative to the commercial samples and laboratory-extracted oils reported in Table 7.10 (with the exception of the enantiomeric ratio of linalol reported by Coleman et al. [1998], which was inverted compared to the other results). The enantiomeric ratios determined in sweet orange also include those determined by Gionfriddo et al. (2003) in laboratory-extracted samples. The enantiomeric ratio of citronellal, reported in bergamot oil, was determined in a sample cold extracted in laboratory, analyzed by Hara et al. (1999). The enantiomeric ratios determined in tangerine were obtained by Dugo P. et al. (2005) in industrial samples of Dancy Tangerine. The relative peak area percent determined in cases of coelutions indicated by the authors of the original papers were not included in the table.

The simultaneous presence in the table of levels of concentration of the single components in the different oils and their enantiomeric distribution can be indicative of the validity of this analytical approach for the characterization of each citrus oil. Evidently, these values must be considered, along with those reported in Tables 7.1 through 7.14 of this chapter and those reported in Chapter 1, to be aware of the number of papers used to generate them. Ranges in variation of the enantiomeric distribution can be considered reliable only if determined in a proper number of determinations. The validity of the values not only depends on the number of original measures but also on the reliability of analytical procedure used for the determination. On-line multidimensional methods or off-line sample preparation, used to isolate the components of interest prior to the chiral analysis, can avoid peak coelution, mainly for minor components, which are likely to occur in direct enantioselective analyses carried out on the whole sample. In fact, chromatographic coelutions and the difficulty encountered in determining small peak areas can greatly affect the single values and the ranges of variation.

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8 The Oxygen Heterocyclic Components of Citrus Essential Oils

Paola Dugo, Marina Russo

CONTENTS

8.1 INTRODUCTION

Oxygen heterocyclic components, present in the nonvolatile fraction of cold-pressed citrus essential oils, are mainly represented by coumarins, psoralens, and polymethoxylated flavones, the structures of which are reported in Figure 8.1.

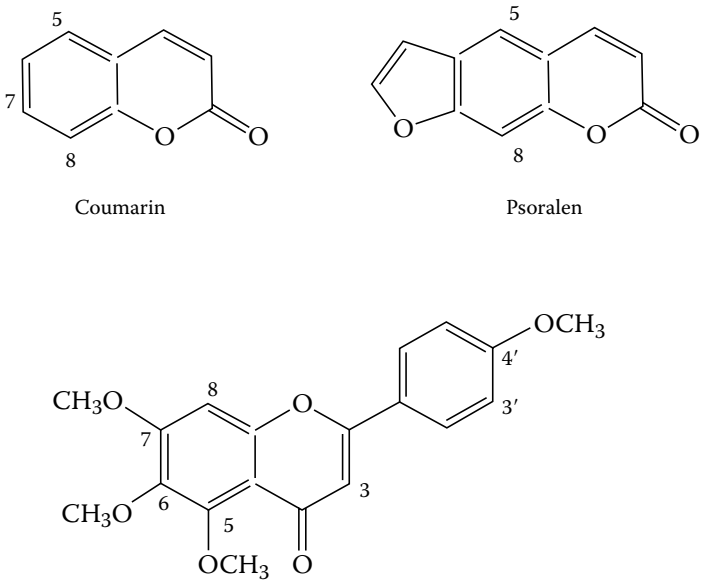

FIGURE 8.1 Structures of oxygen heterocyclic components present in citrus oils.

An important role in the characterization of citrus oils has been attributed to these components. In fact, qualitative and quantitative composition of the fraction is characteristic of each oil (Dugo et al., 1997a). Moreover, many pharmacological and toxicological activities have been demonstrated for most oils (Kleiner et al., 2008; Middleton et al., 2000; Row et al., 2006), though for others there are no data reported yet.

These components exhibit strong absorption in the ultraviolet region (with λ_{max} around 315 nm). This property was used in the past to detect the presence of less valuable distilled oils in cold-pressed oils through the acquisition of the UV spectrum of the diluted sample in the region 260–400 nm (McHale, 2002). This determination, called "CD" (Sale et al., 1953) is now of limited importance, due to the fact that UV absorbance enhancers with UV absorption characteristics resembling those of the oxygen heterocyclic components could be added to adulterated oils (McHale, 2002). In this case, more sophisticated analysis is required, such as chromatographic analysis of every single component of the fraction. More in details, due to their nonvolatile nature, liquid chromatography has been considered the ideal technique for their analysis. Both planar (TLC, OPLC) (Dugo et al., 1996a, 2000a) and column liquid chromatographic methods have been reported (Dugo et al., 2000b). However, other chromatographic methods, such as GC (Berahia et al., 1994; Chouchi and Barth, 1994) or SFC (Dugo et al., 1996b; Li et al., 2007), have been also used for the analysis of oxygen heterocyclic components in citrus products.

As regards detection methods, apart from UV and photodiode array detectors, fluorimetric detection can be also performed, with the advantage of gaining good selectivity and sensitivity. However, in the last few decades, the development of atmospheric pressure ionization (API) techniques as LC-MS interface has influenced the diffusion of this technique in the analytical laboratories, even in the field of natural products analysis.

Many applications using LC-MS equipped with both ESI and APCI interface report the analysis of oxygen heterocyclic components in citrus products (Dugo et al., 2000a; François et al., 2008; Frérot and Decorzant, 2004; Schmidt et al., 2006; Weber et al., 2006). In this way, it was possible to obtain online structural information and molecular weight useful in the identification of an unknown

or in the confirmation of a peak. In fact, it is worth mentioning that oxygen heterocyclic components, as with many other natural components, are often not available commercially as pure standards.

Isolation from real samples could be necessary for a complete structural characterization, for standard use in qualitative and quantitative determinations or for biological assays.

An other very informative technique, nuclear magnetic resonance (NMR), has been recently used in combination with high-performance liquid chromatography (HPLC) in the analysis of polymethoxylated flavones in residues from the molecular distillation of orange peel oil (Schmidt et al., 2006; Weber et al., 2006) and in the identification of the non-volatile components in lemon peel oil (Sommer et al., 2003).

Studies carried out on the characterization of the oxygen heterocyclic components in citrus essential oils before 2000, already revised by Dugo and McHale (2002), have reported the identification of a wide number of components, here summarized in a table for each oil for quick reference.

In the last decade, interest in the oxygen heterocyclic fraction, mainly for psoralens and their potential phototoxicity, has led to the publication of different papers dealing not only with the characterization of these components in citrus essential oils, but also in other products made with citrus, such as juices, teas, liquors, as well as cosmetic products.

The aim of this chapter is to give an up-to-date reference for the methods used to characterize oxygen heterocyclic components in citrus products, including steps on the procedures needed to isolate single components. Within this chapter, each citrus oil will be singularly treated. The most recent results on the composition of oxygen heterocyclic components of citrus oils will be reported in detail in tables and discussed. Then, a section will be dedicated to the determination of such components in products other than essential oils.

8.2 LEMON (*CITRUS LIMON* [L.] BURM) OIL

8.2.1 Before 1999

Table 8.1 reports a summary of the results published before 1999 on the qualitative and quantitative composition of the oxygen heterocyclic fraction of lemon oil, already revised by Dugo and McHale (2002). All the papers that reported only qualitative results have been summarized in one column. However, most of them reported identification in good agreement with each other, with the other papers reported in separated columns in the same table. Only one paper (Di Giacomo, 1990) reported the presence of umbelliferone and scopoletin. While Chouchi and Barth (1994) reported scoparone, this has never been confirmed by other authors. It is worth mentioning that Ziegler and Spiteller (1992) identified a high number of minor components of the fraction, cited as footnote to the table, never reported by other authors.

Papers reporting quantitative values are separately reported in the same table. As is seen, an overall agreement could be observed, with some exceptions that can reflect different origins of the oil, industrial process, or the freshness of the oil.

8.2.2 1999–2009

Table 8.2 summarizes qualitative and quantitative results reported in literature from 1999 to 2009 on the composition of the oxygen heterocyclic fraction of lemon essential oil. All the papers reported data obtained by HPLC analysis.

Bonaccorsi et al. (1999, 2000) developed a fast reversed-phase (RP)-HPLC method for the determination of oxygen heterocyclic components in citrus oils. They used a C18 30 × 4.6 mm i.d., 3 μm HPLC column and an optimized gradient program. The authors were able to obtain separation under the same experimental conditions of five different oils, one of which was lemon oil, as shown in Figure 8.2. The analysis time was less than 7 minutes, sufficient to obtain the level of resolution to characterize each essential oil for a rapid screening or fingerprint. A slightly slower method using a

TABLE 8.1
Qualitative and Quantitative (ppm) Results Reported in Literature for Oxygen Heterocyclic Compounds of Lemon Oil (before 1999)

Trivial Name	[1]*	[2]	[3]	[4]	[5]	[6]	[7]
Citropten	X	500–700	297–606	1200	1800	650	520–1420
5-Geranyloxy-7-methoxycoumarin	X	300	1290–1580	2000	1700	1600	1800–2500
5-Isopent-2′-enyloxy-7-methoxycoumarin	X				60	80	X
Herniarin							X
Umbelliferone	X						
Scoparone	X						
Scopoletin	X						
Bergamottin	X	1000–1200	X	2700	2000	2200	1600–2910
Bergapten	X				100		
Bergaptol	X	X					
Oxypeucedanin	X				1000	1100	890–1570
Oxypeucedanin hydrate	X		X		300	260	X
Isoimperatorin	X					180	X
Byakangelicol	X				500	450	660–1230
Byakangelicin	X		X		100	70	X
Phellopterin	X					90	X
5-Geranyloxy-8-methoxypsoralen	X		X				
5-Methoxy-8-isopent-2′-enyloxypsoralen	X						
5-Isopent-2′-enyloxy-8-(2′,3′-epoxyisopentenyloxy)psoralen	X					220	190–370
Isopimpinellin	X						
8-Geranyloxypsoralen	X		X		1000	750	190–360
Imperatorin	X					60	X

Notes: [1] Stanley and Vannier, 1957; Stanley, 1963; D'Amore and Calapaj, 1965; Stanley and Jurd, 1971; Latz and Ernes, 1978; Fisher and Trama, 1979; Benincasa et al., 1990; Di Giacomo, 1990; Ziegler and Spiteller, 1992; Chouchi and Barth, 1994; [2] Cieri, 1969; [3] Madsen and Latz, 1970; [4] Calabrò and Currò, 1976; [5] Glandian et al., 1978; [6] McHale and Sheridan, 1988; [7] Dugo et al., 1998.

*Ziegler and Spiteller (1992) also identified: aurapten; 7-(3′-isopent-2′-enyloxy)coumarin; 5-(2′,3′-epoxyisopentenyloxy)-7-methoxycoumarin; 5-(2′,3′-dihydroxyisopentenyloxy)-7-methoxycoumarin; 8-(6′,7′-epoxygeranyloxy)psoralen; heraclenin; heraclenol; pabulenol/gosferenol; 5-methoxy-8-geranyloxypsoralen; cnidicin; 5-isopentenyloxy-8-(2′,3′-dihydroxyisopentenyloxy)psoralen; neobyakangelicol.

"conventional" C18 HPLC column (150 × 4.6 mm i.d., 5 μm) was also developed (Bonaccorsi et al., 2000). This method gave higher resolution of all the components in about 16 minutes. Peak identification was carried out in comparison with literature data. The compounds usually accepted as being present in lemon oil were reported.

Again in 1999, Verzera et al. carried out extensive research on the influence of the extraction technology on cold-pressed lemon essential oil composition. They analyzed, by normal-phase (NP)-HPLC, a total of 364 samples of lemon oil produced in Italy during the 1997–1998 season using four different industrial technologies—Pelatrice, Sfumatrice, FMC, and Torchi. It was found that the technology does not influence the qualitative composition of the oils, but quantitative differences have been detected. Pelatrice oils presented the highest amount of total coumarins and psoralens (11801 mg/kg as average), followed by FMC (11267 mg/kg), and Sfumatrice (9648 mg/kg) oils. The authors attributed this finding to the efficacy of Pelatrice machines in breaking the utricles of the fruits to release the essential oils.

TABLE 8.2
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Lemon Oil (1999–2009)

	[1]	[2] (mg/kg)				[3]	[4]	[5]	[6]	[7]	[8]
Trivial Name		**SFUM* 100**	**PEL* 59**	**FMC* 124**	**TORC* 81**						
Oxypeucedanin hydrate							X	131.91		t	249
Byakangelicin								97.89		t	706
Citropten	X	1360	1495	1473	659	X	X		X	X	950
Isopimpinellin										X	
Bergapten								15.69		t	
Byakangelicol		992	1640	1536	555		X	197.36	X	X	755
Oxypeucedanin	X	1556	2200	1909	863	X	X	18.86	X	X	1900
Isoimperatorin	X					X	X	42.46	X		173
Imperatorin	X					X	X	16.24			326
Phellopterin	X					X	X	155.95	X		X
5-Isopent-2′-enyloxy-7-methoxycoumarin	X					X	X			X	X
5-(Isopent-2′-enyloxy)-8-(2′,3′-epoxy)-isopentyloxypsoralen	X	275	324	204	260	X	X		X	X	X
Cnidicin							X				82
8-Geranyloxypsoralen	X	399	437	454	440	X	X	807.15	X	X	916
Bergamottin	X	2635	2955	2877	2973	X	X	1253.37	X	X	4056
5-Geranyloxy-7-methoxycoumarin	X	2453	2711	2845	2656	X	X		X	X	2319
Heraclenin								27.46			
Xanthotoxin										t	
5-Geranyloxy-8-methoxypsoralen									X	X	
5-Methoxy-8-isopent-2′-enyloxypsoralen										X	
5-(2′,3′-Epoxy-isopentyloxy)psoralen										X	
5-Isopent-2′-enyloxy-8-(2′,3′-dihydroxy-isopentyloxy)psoralen							X				
5-(2′,3′-Epoxy-isopentyloxy)-8-(isopent-2′-enyloxy) psoralen							X				
5-(2′,3′-Dihydroxy-isopentyloxy)-8-(isopent-2′-enyloxy) psoralen							X				

Notes: [1] Bonaccorsi et al., 1999; [2] Verzera et al., 1999; [3] Bonaccorsi et al., 2000; [4] Sommer et al., 2003; [5] Frérot and Decorzant, 2004; [6] Dugo et al., 2004a; [7] François et al., 2008; [8] Dugo, P. et al., 2009.

t, tentative identification.

*Lemon oil obtained using "Sfumatrice" (SFUM), "Pelatrice" (PEL), "FMC," and "Torchi" (TORC) machines, with number of analyzed samples.

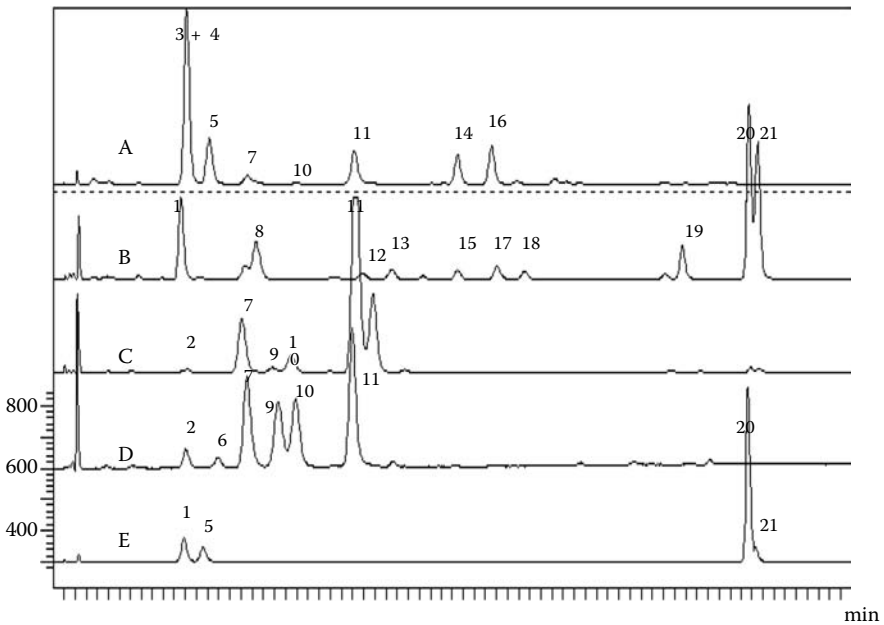

FIGURE 8.2 HPLC chromatograms obtained under identical conditions for five citrus essential oils: (A) bitter orange, (B) lemon, (C) mandarin, (D) sweet orange, and (E) bergamot. (1) Citropten, (2) sinensetin, (3) meranzin, (4) isomeranzin, (5) bergapten, (6) 3,3′,4′,5,6,7-hexamethoxyflavone, (7) nobiletin, (8) oxypeucedanin, (9) tetra-*O*-methylscutellarein, (10) 3,3′,4′,5,6,7,8,-heptamethoxyflavone, (11) tangeretin, (12) imperatorin, (13) phellopterin, (14) osthol, (15) isoimperatorin, (16) epoxybergamottin, (17) 5-isopent-2′-enyloxy-7-methoxycoumarin, (18) 5-isopent-2′-enyloxy-8-(2′,3′-epoxyisopentyloxy)psoralen, (19) 8-geranyloxycoumarin, (20) bergamottin, (21) 5-geranyloxy-7-methoxycoumarin. Solvent A: acetonitrile; solvent B: water; gradient program: 0–0.5 min: 70%B; 0.5–4.5 min: 70–20%B; 4.5–6.5 min: 20%B. Flow rate 1.5 mL/min; column 30 × 4.6 mm i.d., 3 μm P-E C18. Citrus oils were diluted 1:100 in acetonitrile. (Reprinted with permission from Bonaccorsi, I., et al., *J. Agric. Food Chem*. 47, 4237–4239. Copyright 1999 American Chemical Society.)

Torchi oils presented a lower value of total oxygen heterocyclic components (8388 mg/kg on average). Lower values obtained for oxypeucedanin and byakangelicol could be attributed to the extraction technology. In fact, the epoxy ring present in the side chain of these two components is prone to hydrolysis during the extraction process to give the corresponding diols, poorly soluble in the oil. It is well known that extraction with Torchi allows for a contact of the oil with the aqueous acid juice. It is more difficult to find an explanation for the lower amount of citropten in Torchi oils. Gathering samples according to the month of production and technology, the results of coumarins and psoralens were quite constant during the whole production season. In comparison with the previous results, obtained by the same research group for lemon oil produced during the 1995 season (Dugo et al., 1998), the quantitative values were in the same order of intensity, but higher amounts of 5-geranyloxy-7-methoxycoumarin, 8-geranyloxypsoralen, and oxypeucedanin were detected by Verzera et al.

In 2003, Sommers et al. reported an interesting application of HPLC-NMR to the identification of 16 components (coumarins and psoralens) in the nonvolatile residue of a Sfumatrice lemon peel oil from Italy. Even for low concentration components, ^{1}H-NMR spectra were obtained with less than 7000 scans on a 400 MHz NMR system. The authors pointed out that coumarins and psoralens are relatively easy to identify by ^{1}H-NMR spectroscopy, since they show characteristic spin system patterns. The 5- and 8-mono substitutions in psoralens were easily differentiated on the basis of the pattern of the signal position in the aromatic region of the ^{1}H-NMR spectrum. The substitution pattern of 5,8-disubstituted psoralens and 5,7-disubstituted coumarins were

established using DPFGSE-NOE experiments. As for the differentiation between the epoxides and their respective hydrates, the information about the molecular mass and mass fragments obtained by mass spectrometry was absolutely essential. It has to be underlined that components containing an epoxy ring in their side chain, such as oxypeucedanin or bykangelicol, resulted unstable in aqueous acidic solution in the "loop collection mode" used to perform the NMR experiment. Ring opening occurred with formation of the corresponding hydrates, detected as major components of the fraction. Opposite to this behavior, epoxy-components were found as major components when the rapid measurement in the "on-flow" mode was used. The structure of 5-(2′,3′-epoxy-isopentyloxy)-8-isopentenyloxypsoralen was reported for the first time in literature.

Frérot and Decorzant (2004) developed an RP-HPLC method for the analysis of 15 furocoumarins that could be present in citrus oils. These oils present the biggest potential contribution to furocoumarin content in fragranced products (Lawrence, 1982). Recently, the European Cosmetic Directive 76/768/EEC was modified, introducing a limit on the presence and use of furocoumarins in cosmetic products. This makes necessary the availability of a method for the quantification of as many furocoumaris as possible. A gradient program that used water:acetonitrile:THF (85:10:5) and acetonitrile:methanol:THF (65:30:5) has been developed by Frérot and Decorzant (2004). The presence of THF was necessary to separate critical pairs such as isopimpinellin-bergapten. Figure 8.3 shows the HPLC separation of 15 standard furocoumarins. Different detection systems were evaluated in the quantification of the 15 target analytes (UV-diode array, fluorescence, MS equipped with APCI source). The results of the fluorescence detector were not very useful, while both the UV and MS detectors gave very good results. However, the authors concluded that UV at 310 nm was the best detection method in terms of convenience, sensitivity, linearity, and reproducibility. The authors analyzed a sample of commercial Californian lemon oil. Peak identification resulted in agreement with literature data. The presence of bergapten, reported by these authors, was previously reported only in the Eureka variety of lemon oil from Ivory Coast (Chouchi and Barth, 1994; Glandian et al., 1978). Quantitative values resulted significantly different from those reported by other authors.

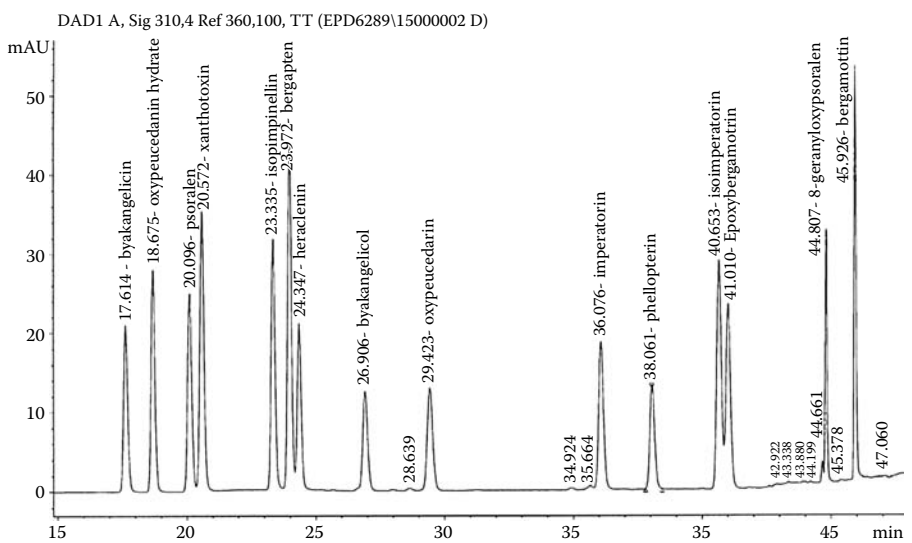

FIGURE 8.3 HPLC-DAD chromatogram of 15 furocoumarins at 310 nm (10 ppm each). HPLC conditions: solvent A: water:acetonitrile:THF, 85:10:5; solvent B: acetonitrile:methanol:THF, 65:30:5; gradient profile: 0–5 min: 0%B; 5–20 min: 0–32%B (linear); 20–24 min: 32%B; 24–38 min: 32–55%B (linear); 38–40 min: 55–90%B (linear). Flow rate: 0.3 mL/min; column: 150 × 2.1 mm i.d., 3 μm, 120A Interchim MS Uptisphere 3 ODB. (Reprinted with permission from Frerot, E., and Decorzant, E. *J. Agric. Food Chem.* 52, 6879–6886. Copyright 2004 American Chemical Society.)

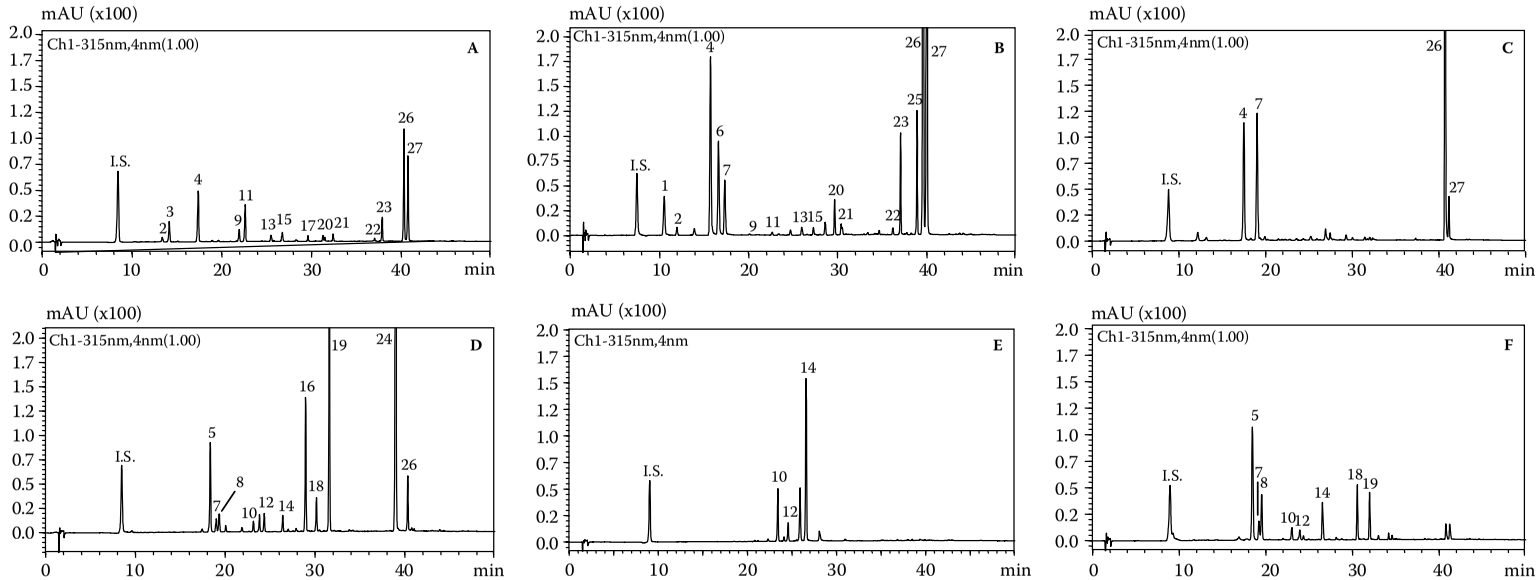

FIGURE 8.4 RP-HPLC chromatograms of citrus essential oils: (A) lemon oil; (B) lime oil; (C) bergamot oil; (D) grapefruit oil; (E) mandarin oil; (F) bitter orange oil. (1) Herniarin; (2) oxypeucedanin hydrate; (3) byakangelicin; (4) citropten; (5) meranzin; (6) isopimpinenllin; (7) bergapten; (8) isomeranzin; (9) byakangelicol; (10) nobiletin; (11) oxypeucedanin; (12) heptamethoxyflavone; (13) isoimperatorin; (14) tangeretin; (15) imperatorin; (16) epoxyaurapten; (17) phellopterin; (18) osthol; (19) epoxybergamottin; (20) 5-isopentenyloxy-7-methoxycoumarin; (21) 5-(isopent-2′-enyloxy)-8-(2′,3′-epoxy)isopentenyloxypsoralen; (22) cnidicin; (23) 8-geranyloxypsoralen; (24) aurapten; (25) 5-geranyloxy-8-methoxypsoralen; (26) bergamottin; (27) 5-geranyloxy-7-methoxycoumarin. Solvent A: water:acetonitrile:THF, 85:10:5; solvent B: acetonitrile:methanol:THF, 65:30:5; gradient program: 0–5 min: 0%B; 5–25 min: 0–40%B; 25–45 min: 40–90%B; 45–55 min: 90%B. Flow rate 1 mL/min; column 150 × 4.6 mm i.d., 2.7 µm Ascentis express C18. Citrus oils were diluted 1:50 in ethanol. (Reprinted with permission from Dugo, P., et al., *J. Agric. Food Chem.* 57, 6543–6551. Copyright 2009 American Chemical Society.)

Dugo P. et al. (2009) explored the composition of coumarins and psoralens in an Italian genuine sample of cold-pressed lemon oil. The authors used RP-HPLC coupled to UV-diode array detection. In this case, a C18 HPLC column packed with partially porous particles (150 × 4.6 mm i.d., 2.7 μm) was used. The same ternary mixtures of solvents used by Frérot and Decorzant (2004) were employed, with such a gradient program that allowed the baseline resolution of all the components of interest not only in lemon oil, but also in all the different citrus oils analyzed. Figure 8.4 reports the RP-HPLC chromatograms of six different citrus essential oils obtained under the same experimental conditions, acquired at 315 nm. Qualitative results were in good agreement with those reported in literature, while quantitative values were sometimes different from previously reported data. For example, a high value of bergamottin, as well as of oxypeucedanin, imperatorin, and byakangelicin, was found. However, only one sample was analyzed for each oil because, as stated by the authors, the aim of the work was the optimization and validation of the HPLC method rather than the analysis of a wide number of samples.

Dugo et al. (2004a) and François et al. (2006, 2008) reported the use of a multidimensional comprehensive HPLC system for the analysis of the nonvolatile fraction of lemon oil. More detailed information on this chromatographic technique can be found in the chapter of this book dedicated to advanced analytical techniques. In both papers, a NP × RP HPLC method was developed. Dugo et al. (2004a) used a combination of a microbore column in the first dimension (Si column, 300 × 1.0 mm i.d., 5 μm) and a monolithic column (30 × 4.6 mm i.d.) in the second dimension. A ten-port two-position valve equipped with two loops was used as interface. With such a system, they were able to separate 11 components located in the two-dimensional plane in characteristic positions on the basis of the specific classes of compounds.

François et al. (2008) presented a new interface for comprehensive two-dimensional HPLC, based on the use of an extra two-position ten-port switching valve, a detector, a pump, and an additional column placed in parallel with the column of the second dimension. This really more complex configuration gave the advantage of a significantly enlarged separation space in the second dimension. Peak identification, obtained by APCI-MS, is reported in Table 8.2.

Figures relative to the interfaces used by Dugo et al. (2004a) and François et al. (2008), as well as the two-dimensional chromatograms reported in the two papers for lemon oil, will be shown in the chapter on advanced analytical techniques of this book.

8.3 MANDARIN (*CITRUS DELICIOSA* TEN.) AND SWEET ORANGE (*CITRUS SINENSIS* [L.] OSB.) OILS

8.3.1 Before 1999

Tables 8.3 and 8.4 summarize the results published before 1999 on the qualitative and quantitative composition of the oxygen heterocyclic fraction of mandarin and sweet orange oils, respectively, already revised by Dugo and McHale (2002). Regarding qualitative data reported for sweet orange oil, citropten (D'Amore and Calapaj, 1965), aurapten (Bohme and Pietsch, 1938) and bergaptol (Fisher and Trama, 1979) were reported only once by different authors in earlier works and never confirmed by other authors. PMFs are usually the components identified in sweet orange oil. From a quantitative point of view, data were in an overall good agreement. For mandarin oil, citropten was reported only by D'Amore and Calapaj (1965), and successively by Di Giacomo et al. (1991), while Buiarelli et al. (1991) reported aurapten. As already seen with sweet orange oil, these components were not confirmed in recent studies, where only PMFs are reported. It is interesting to note that a large number of PMFs and hydroxylated PMFs have been reported by Iinuma et al. (1980) in the peel of *Citrus reticulata*. Gaydou et al. (1987) reported the presence of 3,3′,4′,5,6,7-hexamethoxyflavone in a sample of mandarin oil (*C. deliciosa* Ten.), even though at very low level (0.02 g/L). Quantitative data resulted in good agreement, except the high concentration of nobiletin reported by McHale and Sheridan (1989) and the highest value of heptamethoxyflavone reported by Gaydou et al. (1987) for a King mandarin oil (*C. Nobilis* Lour.).

TABLE 8.3
Qualitative and Quantitative (g/L) Results Found in Literature for Oxygen Heterocyclic Compounds of Mandarin Oil (before 1999)

Trivial Name	[1]	[2]	[3]	[4]	[5]
Citropten	X				
Aurapten	X				
Nobiletin	X	0.40–2.00	1.2	0.37	0.66
Tangeretin	X	1.90–2.80	2.0	2.14	2.18
5,7,4′-Trimethoxyflavone	X				
3′,4′,5,7,8-Pentamethoxyflavone	X				
4′,5,7,8-Tetramethoxyflavone	X				
5-Hydroxy-4′,7,8-trimethoxyflavone	X				
4′,5-Dihydroxy-7,8-dimethoxyflavone	X				
Sinensetin	X	0.07–0.20		0.02	0.01
5-Hydroxy-3′,4′,6,7-tetramethoxyflavone	X				
5-Hydroxy-3′,4′,6,7,8-pentamethoxyflavone	X				
4′,5,7-Trihydroxy-3′,6,8-trimethoxyflavone	X				
4′,5,6,7,8-Pentamethoxyflavone	X				
4′-Hydroxy-5,6,7,8-tetramethoxyflavone	X				
Tetra-*O*-methylscutellarein		0.00–0.50		0.05	0.04
Heptamethoxyflavone	X	0.20–2.70	0.5	0.37	0.50
3,5,6,7,3′,4′-Hexamethoxyflavone		0.00–0.02			

Notes: [1] D'Amore and Calapaj, 1965; Iiuma, 1980; Di Giacomo et al., 1990; Buiarelli et al., 1991; [2] Gaydou, et al., 1987; [3] McHale and Sheridan, 1989; [4] Dugo et al., 1994; [5] Dugo et al., 1996b.

8.3.2 1999–2009

Tables 8.5 and 8.6 report the results relative to the papers published after 1999 on the composition of the oxygen heterocyclic fraction of mandarin and sweet orange essential oils. Unlike the other citrus oils, sweet orange and mandarin oil, as well as tangerine and clementine oils (treated in a separate section), contain almost entirely polymethoxylated flavones in this fraction.

In agreement with the most recent data that reported the presence of six polymethoxylated flavones in sweet orange oil (sinensetin, nobiletin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, tetra-*O*-methylscutellarein, 3,3′,4′,5,6,7-hexamethoxyflavone, and tangeretin), and the same components with the exception of 3,3′,4′,5,6,7-hexamethoxyflavone in mandarin oil, Bonaccorsi et al. (1999, 2000) reported these components in Sicilian sweet orange and mandarin oils. The two oils can be differentiated thanks to the presence/absence of the peak corresponding to 3,3′,4′,5,6,7-hexamethoxyflavone in the chromatogram, and to the nobiletin/tetra-*O*-methylscutellarein ratio of about 2/1 for sweet orange oil and 14/1 for mandarin oil. Analyses were carried out using a fast (1999, 2000) and a conventional (2000) HPLC method, as was previously described in the section dedicated to lemon oil. With both the techniques, the PMFs were baseline resolved, as shown in Figure 8.2.

Dugo et al. (2000b) presented an HPLC-APCI-MS method for the identification of oxygen heterocyclic components in different genuine cold-pressed citrus oils. The analysis of sweet orange and mandarin oils was carried out under isocratic conditions using a C18 conventional HPLC column (250 × 4.6 mm, 5 μm) and a mobile phase consisting of THF:acetonitrile:methanol:water (15:5:22:58). MS data obtained in positive ionization mode are also reported in the paper. The authors stated that it was the first report of HPLC-APCI-MS data on oxygen heterocyclic components present in citrus oils.

TABLE 8.4
Qualitative and Quantitative (g/L) Results Found in Literature for Oxygen Heterocyclic Compounds of Sweet Orange Oil (before 1999)

Trivial Name	[1]	[2]			[3]	[4]	[5]	[6]
		Guinea	Israel	Brazil				
Citropten	X							
Aurapten	X							
Bergaptol	X							
Nobiletin	X	0.98	0.25	0.34	0.50–1.10	0.50	0.52	0.39
Tangeretin	X	0.84	0.36	0.68	0.50–1.00	0.40	0.48	0.60
5,8-Dihydroxy-3,7,3′,4′-tetramethoxyflavone	X							
Tetra-*O*-methylscutellarein	X	0.50	0.16	0.18	0.20–0.60	0.29	0.31	0.24
Heptamethoxyflavone	X	1.24	0.42	0.36	0.30–2.00	0.59	0.84	0.71
Sinensetin	X	0.16	0.05	0.04	0.10–0.30	0.10	0.09	0.06
3,5,6,7,3′,4′- Hexamethoxyflavone	X	0.20	0.05	0.04	0.00–0.04	0.08	0.13	0.07
Auranetin	X							
3,5,7,8,3′,4′-Hexamethoxyflavone	X							
5,7,8,4′-Tetramethoxyflavone	X							
5,7,8,3′,4′-Pentamethoxyflavone	X							
5-Hydroxy-3,7,8,3′,4′-Pentametoxyflavone	X							
5-Hydroxy-3,6,7,8,3′,4′-Hexametoxyflavone	X							

Notes: [1] Böhme and Pietsch, 1938; Swift, 1960; D'Amore and Calapaj, 1965; Tatum and Berry, 1972; Fisher and Trama, 1979; Bianchini and Gaydou, 1980; Gaydou et al., 1987; Di Giacomo et al., 1990; Buiarelli et al., 1991; [2] Bianchini and Gaydou, 1981; [3] Gaydou et al., 1987; [4] McHale and Sheridan, 1989; [5] Morin et al., 1991; [6] Dugo et al., 1994; [7] Dugo et al., 1996b.

Raman et al. (2005) have separated and isolated nobiletin and tangeretin from an extract (with hexane) of dried mandarin peel powder. Mandarin (*Citrus reticulata*) peels were obtained from fruits harvested in Texas. Separation was achieved using strong cation exchange resins. Purity of the isolated components was monitored by HPLC.

Bonaccorsi et al. (2009) have investigated the composition of PMFs of 27 mandarin essential oils industrially produced in Sicily in the 2007–2008 season using Torchi and FMC machines. They also reported data relative to the residue on evaporation, which ranged between 2.3% and 3.0% and CD value (0.45–1.23). Five PMFs were detected. The quantitative values for the three main components were reported and compared with those obtained for samples produced in the 1992–1993 season (Dugo et al., 1994). Average values were in good agreement with previous results. However, the heptamethoxyflavone maximum value was slightly higher than that reported in 1994.

Dugo P. et al. (2009) reported the presence of three PMFs in a sample of genuine Italian mandarin oil analyzed by HPLC with UV detection at 315 nm. These PMFs correspond to the components generally reported as the main constituents of the fraction. However, no quantitative data were reported.

Very recently, Schipilliti et al. (2009) have analyzed 53 genuine cold-pressed samples of mandarin oil produced during the 2008–2009 season. Nine samples of industrial origin (N1–N9) were declared as natural and five samples were declared to be of commercial origin (Co1–Co5). Quantitative results were calculated for nobiletin and tangeretin. Samples N1–N9 presented values comparable to those of the 53 genuine samples except for one of them, which contained slightly

TABLE 8.5
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Mandarin Essential Oil (1999–2009)

Trivial Name	[1–3]	[4]	[5]	[6]	[7]			[8]				
								BOE*			TORCHI*	
					Genuine	Industrial	Commercial	Green**	Yellow**	Red**	Green**	Yellow**
Sinensetin	X			X	X	X	X	X	X	X	X	X
Nobiletin	X	X	X	660–1400	1000–2200	784–1761	687–1440	903–2459	1933–2452	991–2032	864–1856	838–1479
Heptamethoxyflavone	X		X	290–920	X	X	X	976–1467	1021–1380	650–1175	666–1049	456–681
Tetra-*O*-methylscutellarein	X			X	X	X	X	X	X	X	X	X
Tangeretin	X	X	X	1450–3160	2200–3900	2091–3868	1653–4940	2284–3865	3086–3689	2224–4435	2625–4311	2126–2868
Isosinensetin								X	X	X	X	X
Demethylnobiletin								X	X	X	X	X
Demethyltangeretin								X	X	X	X	X

Notes: [1] Bonaccorsi et al., 1999; [2] Bonaccorsi et al., 2000; [3] Dugo et al., 2000b; [4] Raman et al., 2005; [5] Dugo, P. et al., 2009; [6] Bonaccorsi et al., 2009; [7] Schipilliti et al., 2009; [8] Dugo, G. et al., 2009.

*Mandarin oil obtained using Brown Extractor "BOE" and "Torchi" machines.

**Mandarin oil obtained during different period of the productive season: green (September and October), yellow (November), and red (from December to March).

TABLE 8.6
Qualitative Data Found in Literature for Oxygen Heterocyclic Compounds of Sweet Orange Essential Oil (1999–2009)

Trivial Name	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
Sinensetin	X	X	X	X	X	X	X	
3,5,6,7,3′,4′-Hexamethoxyflavone	X	X	X	X	X	X	X	
Nobiletin	X	X	X	X	X	X	X	X
3,5,6,7,8,3′,4′-Heptamethoxyflavone	X	X	X	X	X	X	X	X
Tetra-*O*-methylscutellarein	X	X	X	X		X	X	
Tangeretin	X	X	X	X	X	X	X	X
5,6,7,4′-Tetramethoxyflavone					X			X
5-Hydroxy-6,7,4′-trimethoxyfavone					X			
5-Hydroxy-6,7,8,4′-tetramethoxyflavone					X			
3-Hydroxy-5,6,7,4′-tetramethoxyflavone					X			
3-Hydroxy-5,6,7,8,4′-pentamethoxyflavone					X			
5-Hydroxy-3,7,8,3′,4′-pentametoxyflavone					X			
5-Hydroxy-6,7,8,3′,4′-pentametoxyflavone					X	X		
5-Hydroxy-3,6,7,8,3′,4′-hexametoxyflavone					X	X	X	
5-Hydroxy-3,7,3′,4′-tetramethoxyflavone					X			
5,6,7,4′-Tetramethoxyflavanone					X			
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavanone					X			
2′-Hydroxy-3,4,4′,5′,6′-pentamethoxychalcone					X			
2′-Hydroxy-3,4,3′,4′,5′,6′-hexamethoxychalcone					X			

Notes: [1] Bonaccorsi et al., 1999; [2] Bonaccorsi et al., 2000; [3] Dugo et al., 2000b; [4] Dugo et al., 2004b ; [5] Li et al., 2006; [6] Weber et al., 2006; [7] Schmidt et al., 2006; [8] Li et al., 2007.

lower amounts of tangeretin, and other two samples that presented lower nobiletin concentrations. Two commercial samples were characterized by lower amounts of tangeretin and nobiletin than the genuine oils, which could confirm the presence of distilled mandarin oils in these samples.

The same group, from the University of Messina (Italy) (Dugo G. et al., 2009), has recently characterized the oxygen heterocyclic fraction of mandarin oil produced in Sicily using HPLC-UV and HPLC-IT-TOF instrumentation. This technique allowed the confirmation of the presence of the five PMFs previously reported in mandarin oil. In addition, demethylnobiletin, demethyltangeretin, and isosinensetin, previously identified in green tangerine peel (Dandan et al., 2007), were reported for the first time in Italian mandarin oil. In the same work, the authors reported quantitative values for the three main components divided by extraction technology (BOE and Torchi) and period of extraction. Samples extracted with BOE show higher values of PMFs than the corresponding Torchi oils extracted in the same period, except for tangeretin in green oils.

A study carried out by Li et al. (2006), on a sample of an extract obtained by cold-pressed orange peel oil from Florida, identified hydroxylated polymethoxyflavones and methylated flavonoids, as reported in Table 8.6. Components were isolated using a combination of silica gel normal-phase chromatography, C18 HPLC, as well as chiral HPLC. Structures of the isolated components were elucidated using MS, UV, and different NMR techniques. Some of the hydroxylated PMFs and chalcones were found for the first time in sweet orange peel.

Weber et al. (2006) and Schmidt et al. (2006) studied PMFs in the residue from the molecular distillation of sweet orange cold-pressed peel oil, by liquid chromatography coupled to mass spectrometry (APCI interface) and to nuclear magnetic resonance. In addition to components

reported in Table 8.6, the authors identified proceranone, an acetylated tetranortriterpenoid with limonoid structure, for the first time in a citrus product.

In 2007, Li et al. presented a method for the isolation of PMFs from an orange peel extracted by supercritical fluid chromatography. Using a DAICEL AD chiral column and a mobile phase of 45% MeOH and 55% liquid CO_2, the authors were able to isolate four PMFs. Compared to separation under normal-phase or reversed-phase HPLC, SFC technology resulted in the best separation efficiency. Moreover, the capabilities of short time purification cycles and fully automated stacking injection provided ideal conditions for scalability of large quantity separation.

No new data on the quantitative content of PMFs in orange oils have been found in the literature.

Recently, Lawrence (2009) reviewed literature on oxygen heterocyclic components in orange oil.

8.4 BITTER ORANGE (*CITRUS AURANTIUM* L.) OIL

8.4.1 Before 1999

Table 8.7 reports a summary of the results, published before 1999, on the qualitative and quantitative composition of the oxygen heterocyclic fraction of bitter orange oil, already revised by Dugo and McHale (2002). Many components, such as aurapten, auraptenol, umbelliferone, citropten,

TABLE 8.7
Qualitative and Quantitative (ppm) Results Found in Literature for Oxygen Heterocyclic Compounds of Bitter Orange Oil (before 1999)

Trivial Name	[1]	[2]	[3]	[4]	
				Italian	Spanish
Aurapten	X				
Auraptenol	X				
Umbelliferone	X				
Meranzin	X		3000	7880–11720	3070–3320
Isomeranzin			1800	1540–2110	2080–2130
Osthol	X		620	1540–1840	3660–3710
Meranzin hydrate				100–700	180–390
Citropten	X				
Isoimperatorin	X				
Bergapten	X	690–730	1000	520–730	710–710
Bergaptol	X				
Epoxybergamottin	X		820	1880–3220	3040–3280
Epoxybergamottin hydrate	X			130–450	240–420
Auranetin	X				
Desmethylnobiletin	X				
Tangeretin	X		890	590–1560	950–990
Nobiletin	X		500	340–870	760–850
Sinensetin	t				
Heptamethoxyflavone	X		310	50–140	230–250
Tetra-*O*-methylscutellarein				100–180	50–80

Notes: [1] Komatsu et al., 1930; Boheme and Pietsch, 1938; Patnayak et al., 1942; Sarin and Seshadri, 1960; Stanley, 1963; Stanley et al., 1965; D'Amore and Calapaj, 1965; Stanley and Jurd, 1971; Fisher and Trama, 1979; McHale and Sheridan, 1983; Di Giacomo, 1990; [2] Shu et al., 1975; [3] McHale and Sheridan, 1989; [4] Dugo et al., 1996c.

t = tentative.

isoimperatorin, bergaptol, auranetin, desmethylnobiletin, and sinensetin, were identified by one or more authors in paper published up to 1965, and only in few recent cases were confirmed by other authors. Identification of aurapten in 1983 by McHale and Sheridan was attributed to contamination of the commercial sample with grapefruit oil.

Quantitative values were reported in a limited number of papers. Italian oils presented higher values than Spanish oils (Dugo et al., 1996c). Values presented by McHale and Sheridan (1989) seem similar to those of Spanish oils, but lower values were obtained for osthol, epoxybergamottin, and nobiletin.

8.4.2 1999–2009

The qualitative and quantitative results reported in the studies on bitter orange oil carried out since 1999 are presented in Table 8.8. As can be seen, only five papers have been found in the literature, and all of them have been cited in the previous sections relative to the other citrus oils. Specifically, Bonaccorsi et al. (1999, 2000), found eight components in a sample of bitter orange oil: three coumarins, two psoralens, and three PMFs. Meranzin and isomeranzin, which represent a critical pair in RP-HPLC, were coeluted in the fast HPLC analysis (7 minutes), as shown in Figure 8.2, while they were perfectly resolved in the conventional HPLC analysis that lasted about 16 minutes.

Using HPLC-APCI-MS with positive ionization, Dugo et al. (2000b) confirmed the presence of 11 components in a sample of bitter orange oil. The peaks identified were in agreement with previously reported data.

Frérot and Decorzant (2004) analyzed a commercial sample of bitter orange oil by RP-HPLC, using both UV-diode array and APCI-MS detection. In this case, the authors determined only psoralens. Bergapten and epoxybergamottin were the main components of the psoralen fraction, even

TABLE 8.8
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Bitter Orange Essential Oil (1999–2009)

Trivial Name	[1]	[2]	[3]	[4]	[5]
Meranzin	X	X	X		1923
Bergapten	X	X	X	1671.1 (315.1)*	388
Oxypeucedanin				457.8**	
Isomeranzin	X	X	X		1041
Meranzin hydrate			X		
Nobiletin	X	X	X		X
Heptamethoxyflavone	X	X	X		X
Tetra-*O*-methylscutellarein			X		
Tangeretin	X	X	X		X
Phellopterin				46.3**	
Osthol	X	X	X		X
Epoxybergamottin	X	X	X	814.2	592
Epoxybergamottin hydrate			X		
8-Geranyloxypsoralen				119.0	
Bergamottin				111.6	

Notes: [1] Bonaccorsi et al., 1999; [2] Bonaccorsi et al., 2000; [3] Dugo et al., 2000b; [4] Frérot and Decorzant, 2004; [5] Dugo, P. et al., 2009.

*The two values were obtained under different experimental conditions explained in the text.

**False positive.

though the values found in this work were substantially different from those reported by other authors. Mainly, the value obtained for bergapten was substantially higher than those reported in literature. In fact, the authors pointed out that bergapten coeluted with a coumarin, meranzin, or isomeranzin, and correct quantification, carried out under different conditions that excluded coelution, gave bergapten a value of 315.1 ppm. Moreover, the authors reported quantitative values for other psoralens usually not seen as constituents of bitter orange oil but of oxygen heterocyclic fraction of other citrus oils, such as lemon and lime (oxypeucedanin, phellopterin, and 8-geranyloxypsoralen). However, thanks to MS and UV-DAD detection, the authors were able to detect another false positive: a PMF with MW 372 (most probably tangeretin) was found at the retention time at which oxypeucedanin was expected. Also, phellopterin was commented by the authors as a false positive, demonstrating how important the method optimization is for avoiding coelutions and how the use of detectors is able to give structural information for each peak.

In 2009, Dugo P. et al. analyzed a sample of bitter orange oil using an optimized RP-HPLC method that allowed the baseline separation of coumarins, psoralens, and PMFs reported as present in citrus oils residue. In this case, eight components were detected, in agreement with literature data. Quantitative values were calculated for some of the components. Meranzin and epoxybergamottin resulted lower in comparison with literature data. These components contain an epoxy ring in the side chain. As already reported for lemon oil, an epoxy ring may undergo hydrolysis with formation of the corresponding diols poorly soluble in the essential oil.

8.5 KEY LIME (*CITRUS AURANTIFOLIA*, [CHRISTM.] SWING.) AND PERSIAN LIME (*CITRUS LATIFOLIA*, TAN.) OILS

8.5.1 Before 1999

Table 8.9 reports a summary of the results published before 1999 on the qualitative and quantitative composition of the oxygen heterocyclic fraction of lime oils, already revised by Dugo and McHale (2002). Results were reported for the two varieties of cold-pressed Key lime oil (type A and type B), as well as for cold-pressed Persian lime oil. Where quantitative values have been provided, the main components of the fraction were bergamottin and 5-geranyloxy-7-methoxycoumarin. However, there are quite significant differences in the quantitative values reported by various authors for every component. McHale and Sheridan (1989) and Dugo et al. (1997b) reported higher values of 5-geranyloxy-7-methoxycoumarin, isopimpinellin, and citropten in Key lime oils than in Persian oils, Dugo et al. (1997a) reported higher amounts of herniarin and bergapten in the latter oils. It must be mentioned that geographical origin, storage conditions, and age may influence the composition of oxygen heterocyclic fraction.

8.5.2 1999–2009

Only few papers in the last few years report some data on lime oil, as can be seen in Table 8.10. The first of these, authored by Buiarelli et al. (2002), refers to a sample of distilled oil, defined limette (distilled), obtained from a private company. No botanic name is specified. Although nonvolatile and usually not present in distilled oils, the authors examined this oil to uncover the possible presence of toxic coumarins and psoralens, which must be eliminated before using these oils in the food or cosmetic industry. Using HPLC with different detectors (UV, fluorescence, MS), they were able to identify five components in the distilled oil. Four of them were already reported in Key and Persian lime oils, but methyl umbelliferone was never reported before. The authors suspected that it could be herniarin, a component usually reported in lime oil. However, they excluded this identification on the basis of UV trials and MS spectrum.

Feger et al. (2006) separated nonvolatile constituents from three samples of cold-pressed Key lime oil type A from Mexico and three samples of Persian lime from Brazil by high speed countercurrent

TABLE 8.9
Qualitative and Quantitative (mg/100 g) Results Found in Literature for Oxygen Heterocyclic Compounds of Lime Oil (before 1999)

Trivial Name	[1]	[2]	[3]	[4]	[5]		[6]*		[7]		
		Key Lime			Key Lime	Persian Lime	Key Lime	Persian Lime	Key Lime Type A	Key Lime Type B	Persian Lime
Citropten	X	464	700–1700	203–842	440–670	280–340	291.1	310.1	581	484	443
5-Geranyloxy-7-methoxycoumarin	X	1725	2200–5200	690–4660	1770–1960	1490–1520			3703	4093	3111
5-Isopentenyloxy-7-methoxycoumarin					13–17	2–7			X	X	X
Herniarin					50–390	170–270			91	74	435
Psoralen								3.9			
Bergamottin	X	3025	2200–2500	X	1500–1600	1360–1420			3408	3154	3067
Bergapten	X		170–330	X	120–240	140–220	20.9	128.7	113	89	217
Bergaptol	X		60–100								
Oxypeucedanin					210–430	120–210			-	144	272
Oxypeucedanin hydrate	X	25		X					X	X	X
Isoimperatorin		33							X	X	X
Heraclenin					**	**					
Byakangelicol					66–100	8–16					
Byakangelicin				X							
Phellopterin	X	11		X							
5-Geranyloxy-8-methoxypsoralen	X	945		X	330–450	55–88			X	X	X
Cnidilin	X			X	60–110	8–15			31	24	7
Isobyakangelicol					27–46	5–9					
Isopimpinellin	X	508	0–100	X	300–570	100–210	22.0	53.7	356	331	217
Xanthotoxin								5.9			
8-Geranyloxypsoralen	X	105		X	160–350	58–100			X	X	X
Imperatorin	X	18		X							

[1] Caldwell and Jones, 1945; Latz and Madsen, 1969; Stanley and Jurd, 1971; Calvarano and Gallino, 1975; Latz and Ernes, 1978; [2] Stanley and Vannier, 1967; [3] Cieri, 1969; [4] Madsen and Latz, 1970; [5] McHale and Sheridan, 1989; [6] Nigg et al., 1993; [7] Dugo et al., 1997b.

* Results expressed as μg/g of rind fresh weight.

** Coeluted with byakangelicol.

TABLE 8.10
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Lime Oil (1999–2009)

Trivial Name	[1] Distilled	[2]	[3]	[4]
Herniarin		X		1258
Oxypeucedanin hydrate		X		784
Citropten	141	X		5725
Isopimpinellin		X		7422
Bergapten	X	X	140	2402
Byakangelicol				83
Oxypeucedanin		X		243
Isoimperatorin		X		384
Imperatorin		X		805
Phellopterin		X		
5-Isopentenyloxy-7-methoxycoumarin		X		X
5-(Isopent-2′-eniloxy)-8-(2′,3′-epoxy)-isopentenyloxypsoralen				X
Cnidilin		X		
Cnidicin				252
8-Geranyloxypsoralen		X		6722
Aurapten		X		
Bergamottin		X	950	45699
5-Geranyloxy-7methoxycoumarin	15	X		42000
Osthol		X		
6,7-Dimethoxy-5-geranyloxycoumarin		X		
5,7,8-Trimethoxycoumarin		X		
7-Isopentenyloxycoumarin		X		
5-Geranyloxy-8-methoxypsoralen		X		X
Xanthotoxin		X		
Heraclenin		X		
Heraclenol		X		
Pabulenol		X		
Isooxypeucedanin		X		
Oxypeucedanin methanolate		X		
Nobiletin		X		
3,3′,4′,5,6,7,8-Heptamethoxyflavone		X		
Tetra-*O*-methylscutellarein		X		
Neral oxypeucedaninyl acetal (diastereomer a)		X		
Neral oxypeucedaninyl acetal (diastereomer b)		X		
Geranial oxypeucedaninyl acetal (diastereomer a)		X		
Geranial oxypeucedaninyl acetal (diastereomer b)		X		
Methylumbelliferone	9.3			

Notes: [1] Buiarelli et al., 2002; [2] Feger et al., 2006; [3] Prosen and Kočar, 2008; [4] Dugo, P. et al., 2009.

chromatography. In addition to the isolation of main components, minor constituents were enriched and characterized by GC-MS and HPLC-UV. UV and MS data were reported for each identified component. In addition, NMR characterization was carried out for some components. The authors identified a very large number of components, including a series of minor trace components, such as osthol and aurapten (typical of grapefruit oil), and PMFs (typical of orange oil). The authors could not exclude that these components are related to contamination with other citrus oils during

industrial production, or to different geographical origin, source, and production methods. For this reason, they concluded that further investigation on laboratory-extracted oils should be necessary.

Prosen and Kočar (2008) used LC-UV and LC-MS/MS to detect bergapten and bergamottin in different commercial citrus oils. Values obtained for the lime oil are very low in comparison with those reported by other authors for samples of cold-pressed lime oils. However, no information is given on the type of sample analyzed, except that it was obtained by the cold pressing of the peel.

Dugo P. et al. (2009) reported quantitative values of a sample of cold-pressed lime oil. It was not specified if the sample was Key or Persian oil. However, comparing these results with those present in the literature, qualitative values were in agreement with previous identifications. Due to the high variability reported in literature for quantitative data, it is not easy to identify this sample as Key or Persian oil.

8.6 GRAPEFRUIT (*CITRUS PARADISI* MACF.) OIL

8.6.1 Before 1999

Table 8.11 reports a summary of the results published before 1999 on the qualitative and quantitative composition of the oxygen heterocyclic fraction of grapefruit oil, already revised by Dugo and

TABLE 8.11
Qualitative and Quantitative (ppm) Results Found in Literature for Oxygen Heterocyclic Compounds of Grapefruit Oil (before 1999)

Trivial Name	[1–5]	[2]	[3]	[4]
Citrusal (artefact)	X			
Aurapten	X		7200	11240
Meranzin	X		4900	5100
Isomeranzin			400*	810
Osthol	X		700	58
Meranzin hydrate	X			120
Citropten	X			
Marmin (synthetic)	X			
5-Geranyloxy-7-methoxycoumarin	X			
Epoxyaurapten	X		4300	9300
Bergamottin	X		2000	970
Aurantiumal [artifact]	X			
Bergapten	X	120–130		110
Bergaptol	X			
Epoxybergamottin	X		9500	11260
Epoxybergamottin hydrate	X			700
Byakangelicin	X			
Tangeretin	X		600	68
Nobiletin	X		*	460
Heptamethoxyflavone			570	370
Tetra-*O*-methylscutellarein				20

Notes: [1–5] Fisher and Nordby, 1965, 1966; Stanley and Vannier, 1967; Fisher et al., 1967; Stanley and Jurd, 1971; Latz and Ernes, 1978; Tatum and Berry, 1979; Fischer and Trama, 1979; [2] Shu et al., 1975; [3] McHale and Sheridan, 1989; [4] Dugo et al., 1997a.

* Nobiletin + isomeranzin.

McHale (2002). Regarding qualitative data in this case, some components reported in the less recent works were not confirmed later by other authors. This is the case for components such as citrusal, citropten, 5-geranyloxy-7-methoxycoumarin, and byakangelicin. Two sets of quantitative data were reported. Data were in reasonable agreement for components such as meranzin, osthol, epoxybergamottin, and the three major PMFs. Aurapten, epoxyaurapten, isomeranzin, and bergamottin differed significantly. Once again, these differences can be due to the different origins of the oils and the production technology.

8.6.2 1999–2009

Table 8.12 reports data published after 1999 and not previously revised by Dugo and McHale (2002). Results obtained during these ten years show an overall good agreement. Dugo et al. (2000b, 2004b) reported the identification of the same number of components already reported by the same authors in 1997 (Dugo et al., 1997a), with the exception of tetra-*O*-methylscutellarein, which was detected as a minor component in 1997. Also, meranzin hydrate was not reported in Dugo et al. (2004b). As already stated in this chapter, the presence/absence of this component could be correlated with the freshness of the oil or the extraction technology, due to the fact that it is formed from the hydration

TABLE 8.12
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Grapefruit Essential Oil (1999–2009)

Trivial Name	[1]	[2]	[3]	[4]	[5] (mg/kg)	[6]
Citropten	X			X		
Meranzin	X	X		X		1971
Meranzin hydrate	X			X		
Bergapten	X	X	513.2 (75.0)*	X	1–5	263
Isomeranzin	X	X		X		412
Oxypeucedanin			700.2**			
Nobiletin	X	X		X		X
3,3′,4′,5,6,7,8-Heptamethoxyflavone	X	X		X		X
Tetra-*O*-methyl-scutellarein				X		
Tangeretin	X	X		X		X
Epoxyaurapten	X	X		X		X
Osthol	X	X		X		X
Epoxybergamottin	X	X	1732.1	X		5317
Epoxybergamottin hydrate	X	X		X		
Aurapten	X	X		X		9271
Bergamottin	X	X	428.6	X	140	1791
Marmin				X		
Auraptenol				X		
Hydroxypentamethoxyflavone 1				X		
Hydroxypentamethoxyflavone 2				X		
Hydroxyhexamethoxyflavone				X		

Notes: [1] Dugo et al., 2000b; [2] Dugo et al., 2004b; [3] Frérot and Decorzant, 2004; [4] Feger et al., 2006; [5] Prosen and Kočar, 2008; [6] Dugo, P. et al., 2009.

* The two values were obtained under different experimental conditions as explained in the text.

** False positive.

of meranzin. Research was always carried out using HPLC under reversed-phase conditions. Dugo et al. (2000b) used a conventional HPLC column under gradient conditions and an APCI-MS as detector. In the paper of Dugo et al. (2004b), the authors used a C18 monolithic column (100 × 4.6 mm i.d.), under gradient conditions at a flow rate of 1.5 mL/minute. Figure 8.5 reports the HPLC-UV chromatogram obtained for the grapefruit oil. As can be seen, this column allowed to obtain a good separation of all the target analytes, with a substantial gain in terms of time of analysis and solvent consumption in respect to analogous analyses carried out using conventional columns. This is in agreement with the general considerations reported in literature for monolithic columns (Lubda et al., 2001; Tanaka et al., 2002). The same identification was obtained again by Dugo P. et al. in 2009. In this case, quantitative values for some components were also reported. Quantitative values presented significant differences for some components with those previously reported in literature, while other components presented values in good agreement, such as that of aurapten, which is also a main component of the fraction and almost exclusively found in grapefruit oil among the most common citrus oils.

Frérot and Decorzant (2004) determined furocoumarins in a grapefruit oil from Israel (*C. paradisi* Macf. or *C. decumana* L.) by HPLC using UV, fluorimetry, and MS. As described already for bitter orange oil, the amount of bergapten resulted higher than the real value due to the possible coelution of this component with the peak of a coumarin (probably meranzin or isomeranzin). This component was correctly quantified modifying the HPLC program. Its value is reported in brackets in the table. Oxypeucedanin was a false positive identification. LC-MS analysis confirmed the peak as a pentamethoxyflavone (probably tangeretin), in agreement with data from the UV spectrum. Bergamottin and epoxybergamottin were the main components of this fraction, in agreement with results reported by other authors.

Prosen and Kočar (2008) determined bergapten and bergamottin in a sample of commercial grapefruit oil using LC-MS-MS. These values resulted significantly lower than all the other values reported in literature for the same components.

As already described in the paragraph dedicated to lime oil, Feger et al. (2006) proposed a method that used high-speed countercurrent chromatography (HS-CCC) to separate the nonvolatile

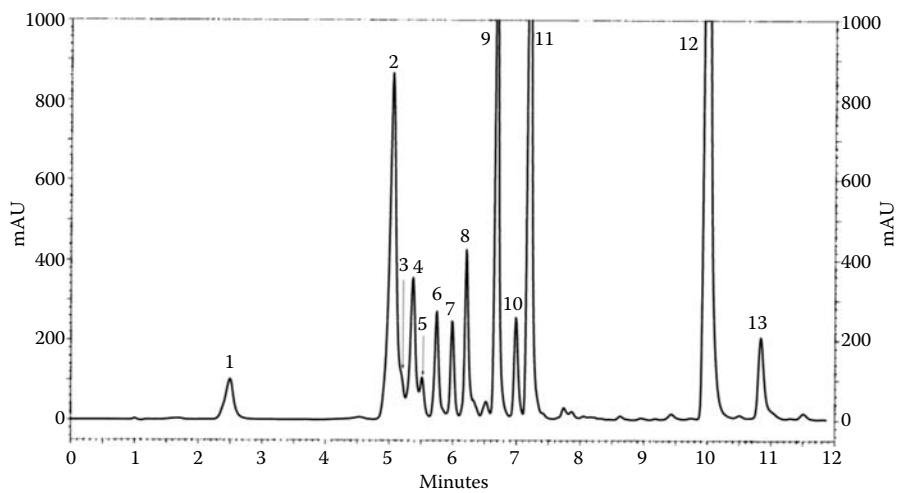

FIGURE 8.5 HPLC-UV chromatogram (315 nm) of a grapefruit essential oil using a monolithic column. (1) Coumarin (internal standard); (2) meranzin; (3) bergapten; (4) isomeranzin; (5) epoxybergamottin hydrate; (6) nobiletin; (7) 3,3′,4′,5,6,7,8-heptametoxyflavone; (8) tangeretin; (9) epoxyaurapten; (10) osthol; (11) epoxybergamottin; (12) aurapten; (13) bergamottin. Solvent A: water; solvent B: acetonitrile; gradient program: 0–3 min: 30%B; 3–4 min: 30–45%B; 4–5 min: 45–60%B; 5–10 min: 60–70%B; 10–15 min: 90%B. Flow rate: 1.5 mL/min. Column: Merk Chromolith 100 × 4.6 mm.

constituents in two samples of white grapefruit from Florida. HS-CCC represents a universal preparative chromatographic method that permits both normal and reversed-phase operations, with the advantages of no adsorption, which allows for a complete recovery of the chromatographed sample; simple technology (low-pressure method), and low cost of operation (technical grade solvents). The nonvolatile fraction of grapefruit oil presented auraptenol in addition to the components already reported in literature for this oil. This component was only reported once in orange oil in 1971 (Stanley and Jurd, 1971). Three trace constituents identified as hydroxypolymethoxyflavones were detected using GC-MS. These components have been reported before only in orange, tangerine, and mandarin peel oils. Citropten, reported as a trace constituent, was previously reported in papers published between 1965 and 1978, and never confirmed in more recent works, as well as marmin.

8.7 BERGAMOT (*CITRUS BERGAMIA*) OIL

8.7.1 1990–1999

Table 8.13 reports data previously published on qualitative and quantitative composition of the oxygen heterocyclic fraction of bergamot oil, already revised by Dugo and McHale (2002). Oxygen heterocyclic fraction of bergamot oil has been studied more extensively than other oils. This has been for reasons linked to the high value of this product used mainly in perfumery and cosmetics and, at the same time, to the photosensitizing activity of bergapten, present in this oil at a level higher than in other citrus oils.

Most of the data reported in literature regards the presence of two coumarins and two psoralens in the oxygen heterocyclic fraction of bergamot oil. Bergamottin being the main component. Only two works (Calvarano et al., 1995; Gionfriddo et al., 1997) reported the presence in bergamot oil of coumarins and psoralens usually found in lemon and lime oil, while an HPLC-APCI-MS study (Dugo et al., 1999) allowed the identification of two PMFs as trace constituents.

It is also worth mentioning that, due to the phototoxicity of this psoralen, bergapten-free oils obtained using different methods are available on the market. The procedures commonly used to remove bergapten from the oil involve either distillation or treatment with NaOH. Distillation drastically reduces the amount of all the four components of the oxygen heterocyclic fraction, while NaOH treatment affects only citropten and bergapten content.

8.7.2 1999–2009

Table 8.14 reports data published after 1999 and not previously revised by Dugo and McHale (2002). As can be seen, most of the data refer to the four oxygen heterocyclic components already reported in less recent papers.

Bonaccorsi et al. (1999, 2000) obtained an acceptable separation of the four components in less than 7 minutes using a fast HPLC method (see Figure 8.2). Using the same method for five different oils, the authors were able to differentiate each one even without obtaining a complete resolution of each single peak.

Di Giacomo and Di Giacomo (1999) reported the most recent data obtained at that time by HPLC on the content of coumarins and psoralens in bergamot oil. These data were obtained for Calabrian oils obtained from fruits of the Fantastico variety, which is today the most common in Calabria. They reported that the average content of bergapten could be considered to be below 0.25% of the whole bergamot oil. This finding should be taken into consideration when the amount of this oil is restricted due to problems linked to phototoxicity of bergapten. For the use of bergamot oil in perfumery, the IFRA regulation recommends a maximum value of 2%, calculated on the basis of an average content of 0.35% of bergapten in the oil. This value should be proportionally higher, considering a significantly lower amount of bergapten in the oil.

TABLE 8.13
Qualitative and Quantitative (mg/L) Results Found in Literature for Oxygen Heterocyclic Compounds of Bergamot Oil (before 1999)

Trivial Name	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
Bergapten	X	2400–3600	1620–3570	2800–3300	1500–3300	2110–4560	6000–8700	1560–4040
Citropten	X	2100–3200			1400–2400		5000–7500	1600–3000
Bergamottin	X	11000–19000			14000–22000			11400–27300
5-Geranyloxy-7-methoxycoumarin	X	400–600			1000–1500			
Bergaptol	X							
5-(2′-Isopentenoxy)7-methoxycoumarin	X							
5-Isopentenyloxy-8-methoxypsoralen*	X							
Oxypeucedanin								
Byacangelicol								
Oxypeucedanin hydrate								
Byakangelicin								
Tetra-*O*-methylscutellarein								
Sinensetin								

Trivial Name	[9]	[10]	[11]	[12]	[13]	[14]	[14] Bergapten Free	
							NaOH	Distilled
Bergapten	2300–3000	2700	1100–3200		1893–3310	1100–2900	0–91	0–41
Citropten		2100	1400–3500		1176–3207	1200–3500	0–52	0–47
Bergamottin		18300	10200–27500		6327–8263	10000–19400	11726–16250	0–3017
5-Geranyloxy-7-methoxycoumarin		1100	800–2200		534–1072	900–2700	1539–1975	0–349
Bergaptol								
5-(2′-isopentenoxy)7-methoxycoumarin				20–80	40–169			
5-Isopentenyloxy-8-methoxypsoralen*				tr–60	0–15			
Oxypeucedanin				440–640	180–475			
Byacangelicol				150–410	165–895			
Oxypeucedanin hydrate				120–190	22–59			
Byakangelicin				60–80	23–90			
Tetra-*O*-methylscutellarein						X		
Sinensetin						X		

Notes: [1] D'Amore and Calapaj, 1965; Stanley and Jurd, 1971; Di Giacomo and Calvarano, 1974; Latz and Ernes, 1978; Benincasa et al., 1990; Di Giacomo, 1990; [2] Cieri., 1969; [3] Porcaro and Shubiak, 1974; [4] Shu et al., 1975; [5] Calabrò and Currò, 1975; [6] Di Giacomo and Calvarano, 1978; [7] Vernin et al., 1979, [8] Calvarano et al., 1979; [9] Analytical Methods Committee, 1987; [10] McHale and Sheridan, 1989; [11] Mondello et al., 1993; [12] Calvarano et al., 1995; [13] Gionfriddo et al., 1997; [14] Dugo et al., 1999.

* Reported as 5-isopentenyloxy-7-methoxypsoralen in Calvarano et al., 1995 and Gionfriddo et al., 1997.

TABLE 8.14
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Bergamot Essential Oil (1999–2009)

Trivial Name	[1]	[2]	[3]	[4]	[5] Distilled	[6] Bergapten Free	[7] mg/kg	[8] mg/kg	[9]	[10]	[11]		
											PEL*** 16	TORCHI*** 1	Ivory Coast*** 2
Citropten	X	2300	X	X	230			1340–2124	2582	1927	1588–2708	1558	1838–2469
Bergapten	X	2500	X	X	0.16	8.0	80	1384–2089	2374	2070	1321–2354	929	1684–2118
Oxypeucedanin						53.5*							
Sinensetin				X									
Tetra-*O*-methylscutellarein				X									
Epoxybergamottin						70.3**							
Bergamottin	X	20300	X	X	13	16312.0	620	10969–14089	21419	21685	18417–23893	16701	11068–15663
5-Geranyloxy-7-methoxy-coumarin	X		X	X				878–1035	1120	1423	1195–1818	905	861–907
5-Methoxy-7-hydroxycoumarin					70								

Notes: [1] Bonaccorsi et al., 1999; [2] Di Giacomo and Di Giacomo, 1999; [3] Bonaccorsi et al., 2000; [4] Dugo et al., 2000b; [5] Buiarelli et al., 2002; [6] Frérot and Decorzant, 2004; [7] Prosen and Kočar, 2008; [8] Mangiola et al., 2009; [9] Dugo P. et al., 2009; [10] Costa et al., 2009; [11] Sciarrone, 2009. Personal communication.

*False positive.

**Overestimated, coeluted with a compound of MW 274; 17.8 ppm (MS detection), 11.9 ppm (fluorescence detection).

***Bergamot oil from Italy obtained using "Pelatrice" (PEL) and "Torchi" machines, and bergamot oil from Ivory Coast, with number of analyzed samples.

In 2000, Dugo et al. (2000b) reported HPLC-APCI-MS determination of oxygen heterocyclic fraction of bergamot oil. In addition to the four components usually reported in literature, they confirmed the presence of two PMFs that were reported by the same authors in a previous paper (Dugo et al., 1999). However, these components have never been reported more recently by other authors.

Buiarelli et al. (2002) determined the presence of oxygen heterocyclic components in a sample of distilled bergamot oil. Because of their nonvolatile nature, their presence in distilled oil is substantially reduced with respect to cold-pressed oil. However, because of problems linked to the possible toxicity of some cumarins (mainly bergapten), their presence in distilled oil should be assessed before using these products in the food or cosmetic industry. Data were obtained by HPLC-UV and spectrofluorimetry. The presence of bergapten resulted below 1 mg/L, while citropten was surprisingly higher (230 mg/L). The authors also identified bergamottin at a level of 13 mg/L and another coumarin (5-methoxy-7-hydroxycoumarin) at the level of 70 mg/L. This last component, never reported before in bergamot oil, seems an erroneous identification of 5-geranyloxy-7-methoxycoumarin, since this compound is eluting close to bergamottin under the reversed-phase conditions used by the authors. The presence of the hydroxyl group should have increased the polarity of the molecule and greatly reduced its retention.

Frérot and Decorzant (2004) determined psoralens in a sample of bergamot oil subjected to alkaline treatment to remove bergapten. They used three different detection systems—UV-DAD, MS, and fluorescence—in combination with RP-HPLC. Results obtained by UV-DAD are reported in Table 8.14. The amount of bergapten and bergamottin found with the three different detection systems was comparable. A peak identified as oxypeucedanin by HPLC-UV-DAD was not confirmed using MS and fluorescence detection. It corresponded to a peak with MW 342. In agreement with data reported in literature (Dugo et al., 2000b), this component could be identified as the PMF tetra-O-methylscutellarein, which MW is 342. Epoxybergamottin coeluted with a component with MW 274. Its amount was overestimated using HPLC-UV technique. However, this component, usually not detected in bergamot essential oil, was confirmed using the other detection systems.

Prosen and Kočar (2008) determined bergapten and bergamottin in a sample of commercial cold-pressed bergamot peel oil, using a LC-MS-MS technique. However, values reported by the authors resulted significantly lower than those reported in literature for untreated cold-pressed bergamot oils.

Most recently, Mangiola et al. (2009) presented a study on six samples of Calabrian cold-pressed bergamot oil produced in different periods of the 2007–2008 season. Each sample corresponded with lots of one metric ton, and was extracted using the so-called special machine. Analyses were carried out under normal-phase HPLC conditions, using UV-DAD as a detector. The values obtained were in agreement with literature data. It is noteworthy that bergapten content decreased with ripening of the fruits, from 2089 mg/kg for oil produced in November to 1384 mg/kg for oil obtained in March.

Other recent papers from the group of Messina (Costa et al., 2009; Dugo P. et al., 2009) reported the quantification of bergapten, citropten, bergamottin, and 5-geranyloxy-7-methoxycoumarin in genuine Calabrian bergamot oil obtained with Pelatrice machine. Only one sample was analyzed in each work, but data are in good agreement each other and with those reported in literature for the same components. In addition to the cold-pressed oil, Costa et al. (2009) analyzed some processed bergamot oil samples. Data obtained for these samples are reported in Table 8.15. Samples that were "terpeneless, colored," "terpeneless, colorless," and "furocoumarin free," were obtained by vacuum, steam, and fractional distillation. A "bergapten-free" sample was obtained through treatment with alkaline solution. Results were obtained by HPLC analysis with UV-DAD detection. Compared to results obtained for the cold-pressed bergamot oil, the terpeneless colored sample presented the highest amount of all the components and the specific presence of two additional ones, sinensetin and herniarin. Sinensetin was already reported in bergamot oil, while herniarin was previously reported only in commercial bergamot oils, supposedly adulterated with lime oil. However, this compound, found by the authors as trace level in genuine cold-pressed bergamot oil, increased in

TABLE 8.15
Content of Coumarins and Psoralens (g/L) Reported in Samples of Some Processes Bergamot Oils

Trivial Name	Terpeneless, Colored	Terpeneless, Colorless	Furocoumarins Free	Bergapten Free
Herniarin	0.251	–	–	–
Sinensetin	0.372	–	–	0.183
Citropten	6.134	–	Traces	Traces
Bergapten	4.215	–	Traces	Traces
Bergamottin	39.203	–	0.020	18.194
5-Geranyloxy-7-methoxycoumarin	2.827	–	Traces	1.299

amount in this processed sample owing to the concentration effect obtained by fractional distillation. In fact, this process was used to selectively remove the monoterpene hydrocarbons (more volatile) from the oil. The terpeneless, colorless oil, obtained by distillation, was completely deprived of all the nonvolatile components, pigments included. Similar conclusions were drawn for the furocoumarin free oil, where a distillation process was carried out. Bergapten-free oil presented trace amounts of citropten and bergapten, while bergamottin and 5-geranyloxy-7-methoxycoumarin were still present at a level comparable to that of cold-pressed oils. This result agrees with literature data (Dugo et al., 1999; Frérot and Decorzant, 2004). In fact, citropten and bergapten are more easily subjected to ring opening than bergamottin and 5-geranyloxy-7-methoxycoumarin, which present the steric hindrance of the geranyl chain.

Again in 2009, Sciarrone et al. analyzed 16 samples of Calabrian bergamot oil obtained using Pelatrice machine, one sample obtained with Torchi and two samples produced in Ivory Coast. As can be seen, the sample obtained with Torchi presented lower values for the four investigated components than samples produced using Pelatrice machine. The Ivory Coast samples presented values comparable to those of Italian samples for all the target analytes.

8.8 TANGERINE (*CITRUS TANGERINA* HORT. EX TAN.) AND CLEMENTINE (*CITRUS CLEMENTINA* HORT. EX TAN.) OILS

8.8.1 1997–2009

Table 8.16 reports data found in literature for different types of tangerine and clementine oils.

Among citrus fruits, apart from orange, mandarins are the most popular, with a broad spectrum of species, varieties, and hybrids resulting in peel oils of unusually variable composition (Feger et al., 2001, 2003; Lawrence, 2001). The species *C. clementina* Hort. ex Tan. and *C. tangerine* Hort. ex Tan. also make up part of this group (Ortiz, 2002).

Verzera et al. (1997a) carried out research on the composition of clementine oil laboratory extracted from the peel of fruits of *C. clementine* Hort. harvested in Calabria. Fruits of the cultivars Comune, Oroval and Monreal were used, and three different samples for each cultivar were obtained. Normal-phase HPLC analyses allowed the identification and quantification of six polymethoxylated flavones in the nonvolatile fraction of the oils (the same PMFs reported in sweet orange oil). Heptamethoxyflavone was the main component of the fraction and, among the three cultivars, Comune presented the highest content of PMFs. A comparison with quantitative values reported for mandarin and sweet orange oil (Dugo et al., 1994) revealed substantial quantitative differences among these oils.

Only one other paper reported the qualitative composition of clementine oil (*C. reticulata* Blanco var. Clementine), determined by HPLC-MS and HPLC-NMR (Schmidt et al., 2006). In addition to the six PMFs already reported by Verzera et al. (1997a), the authors also identified a

TABLE 8.16
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Clementine and Tangerine Oils (1997–2009)

Trivial Name	[1] Dancy Tangerine	Clementine Monreal	[2] Clementine Oroval	Clementine Comune	[3] Murcott Tangerine	[4] Dancy Tangerine	[5] Green Tangerine	[6] Clementine
Sinensetin	X	Traces	Traces	Traces	30–40	X	X	X
3,3′,4′,5,6,7-Hexametoxyflavone*		200	200	200	0–20			X
Nobiletin	X	900	900	900	800–1900	1790	X	X
Heptamethoxyflavone	X	2700	2900	5100	670–830	540	X	X
Tetra-*O*-methylscutellarein	X	500	400	600	60–130	X**	X	X
Tangeretin	X	1000	1000	1700	1100–3100	2910	X	X
Isosinensetin	X						X	
Tetra-*O*-methylisoscutellarein	X						X	
5-Demethylnobiletin	X						X	
5-Demethyltangeretin							X	
5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone								X
7-Hydroxy-3,5,6,3′,4′-pentamethoxyflavone	X							
7-Hydroxy-3,5,6,8,3′,4′-hexamethoxyflavone	X							

Notes: [1] Chen et al., 1997; [2] Verzera et al., 1997; [3] Feger et al., 2003; [4] Dugo et al., 2005; [5] Dandan et al., 2007; [6] Schmidt et al., 2006.
* Quercetogetin.
**Coeluted with nobiletin.

hydroxyl-hexamethoxyflavone, as reported in Table 8.16. The exact position of the hydroxyl group was assigned after isolation of the compound by preparative HPLC and ^{1}H-NMR analysis.

Four papers recently reported data on the composition of the nonvolatile fraction of different varieties of tangerine peel oil. Chronologically, the first (Chen et al., 1997) reported the isolation and characterization of ten PMFs from the solid residue of cold-pressed Dancy tangerine oil. This fraction is isolated from citrus oil by a process called winterizing (at –20°C). Preparative column chromatography, followed by prepHPLC using a C18 column, were used to isolate PMFs. Then, mass nuclear magnetic resonance and UV spectroscopy were used for their characterization. Of the 10 components identified, 2 resulted novel natural products.

A more recent paper reports on the composition of Murcott tangerine peel oil (Feger et al., 2003). This kind of tangerine is also known as Honey tangerine (mainly within the United States) or Smith tangerine. It is mainly produced in Brazil and used for fresh fruit consumption. Commercial oils from Brazil were analyzed by HPLC with UV detection. Six PMFs were identified, in agreement with components usually identified in sweet orange and mandarin oils. In Murcott tangerine, as well as in other tangerine oils, tangeretin represented the main component of the fraction. Significant differences were found in the analyzed oils. These may be due to oil production method, the year of production, the winterizing process, or time of storage between processing and sampling.

In 2005, Dugo et al. have reported data on the composition of six samples of Mexican Dancy tangerine (*C. tangerina* Hort. ex Tan.) of different geographical origin. Five PMFs were reported and some of them were quantified. Tangeretin was the main component of the fraction, followed by nobiletin. The sample from the mountain region showed the lowest values for all the PMFs, while the samples from the tropical region of Tabasco presented the highest amount of PMFs, followed by that of the semiarid region of Tamaulipas.

Dandan et al. (2007) characterized PMFs in green tangerine peel (dry powder) using different chromatographic and spectroscopic techniques. The HPLC-UV analysis allowed the separation of 14 components, 9 of which were positively identified on the basis of UV spectra and HPLC/MS/MS data. For four of them (tangeretin, nobiletin, 5-demethylnobiletin, and 5-demethyltangeretin) additional studies using NMR and IR were carried out. This last component is reported for the first time in citrus.

8.9 VARIOUS PRODUCTS MADE WITH CITRUS

Literature reports data on the presence of furocoumarins in other citrus products, such as juices, as well as in products such as liquors or teas, where citrus have been used as an ingredient. Due to the numerous biological activities attributed to oxygen heterocyclic components present in citrus products (Fukuda et al., 1997; Murakami et al., 1997; Tanaka et al., 1997), mainly furocoumarins, the evaluation of their content in food products could give useful information about a possible beneficial or toxic effect based on dietary habits.

Many studies suggested that aurapten is a promising cancer-preventive component contained in citrus fruits. Ogawa et al. (2000) determined aurapten in citrus fruit products, including juice and marmalade, using HPLC analysis. They also determined aurapten in the peel and juice sacs of various citrus plants, as well as hybrids between Citrus species and hybrids between Citrus species and *Citrus trifoliata* [L.] Raf. (ex *Poncirus trifoliata*). Table 8.17 reports results obtained for grapefruit juice. The authors stated that the process of making juice involves steps such as the removal of pulp and peel oil, which cause a decrease in aurapten content. Among citrus juices, grapefruit was studied by different authors more than other juices. When consumed with certain orally administered medications, grapefruit juice has been shown to increase their bioavailability (Bailey et al., 1989). Initially, naringin was considered responsible of this effect, but many studies have demonstrated that a series of furocoumarins were the primary responsible agents through their inhibition of an intestinal P-450 enzyme, cytochrome P-450 3A4 (CYP3A4) (Ameer and Weintraub, 1997; Ho et al., 2001). Both monomers and dimers of furocoumarins have been identified as inhibitors of CYP3A4 activity

TABLE 8.17
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Grapefruit Juice

	[1]	[2]		[3]				[4] mg/kg			[5]	[6]
Trivial Name		**Red**	**White**	**Raw Juice**	**Retentate**	**Supernatant**	**Pulp**	**Pulp**	**Peel**	**100% Natural**		
Bergapten								< LOD	1.92	0.001	0.3	
Isopimpinellin								< LOD	< LOD	0.002		
6,7-Epoxy-bergamottin				16.2	116.4	1.7	9.4					
6,7-Dihydroxy-bergamottin		0.22–4.12	0.27–7.66	89.0	628.1	57.1	139.1					1.62
Aurapten	1.1–1.4			6.0	105.6	1.0	8.7					
Bergamottin		1.56–6.19	1.75–7.26	45.0	892.7	4.5	89.1	2.96	10.18	0.098	4.4	0.11
Bergaptol												0.36
Psoralen								< LOD	< LOD	< LOD		0.62
Xanthoxin								< LOD	< LOD	< LOD		
OSE1 (MW 726; m/e 203, 337, 355, 373, 557)		0.02–1.22	0.02–1.10									
OSE2 (MW indeterminate; m/e 203, 337, 339, 355)		nd–0.17	0.01–0.15									
OSE3 (MW 708; m/e 203, 337, 357)		0.02–0.18	0.04–0.24									
OSE4 (MW indeterminate; m/e 203, 337, 339, 355)		nd–0.12	nd–0.05									
OSE5 (MW 708; m/e 203, 337, 355, 557)		0.01–0.21	0.02–0.16									
OSE6 (MW 708; m/e 203, 339, 355, 557)		0.04–0.47	0.08–0.34									

Notes: [1] Ogawa et al., 2000; [2] Widmer and Haun, 2005; [3] Manthey and Buslig, 2005; [4] Peroutka et al., 2007; [5] Prosen and Kočar, 2008; [6] Lin et al., 2009.
nd = non detected.

(Dresser et al., 2000; Kane and Lipsky, 2000). Head-to-tail furocoumarins dimers from grapefruit juice, called orthospiroesters (OSEs), have been considered to be the only significant active CYP3A4 inhibiting components in grapefruit juice (Fukada et al., 1997). However, their amount in grapefruit juice is significantly lower than monomers. A study was carried out in 2005 by Widmer and Haun on 58 commercial grapefruit juices collected over two seasons, using HPLC with DAD and MS detection. Samples were previously extracted with ethyl acetate. The authors identified furocoumarins, as well as a series of OSEs, on the basis of their characteristic UV (more prominents UV absorbance at 245 nm) and MS spectra. Table 8.17 reports results obtained for red and white juice samples.

Again in 2005, Manthey and Buslig have studied, by HPLC-MS, the distribution of furanocoumarins and their dimers in four fractions separated from freshly extracted Marsh white grapefruit juice (raw finished juice, centrifugal retentate, centrifuged supernatant, and coarse finisher pulp). Centrifugal retentate had the highest furocoumarins content, as well as a high content of furocoumarin dimers (467 mg/L). This fraction can represent a useful starting material for preparative-scale isolation of these compounds. A series of furocoumarins dimers were identified by HPLC-MS. On the basis of MS spectra, dimers of 6′,7′-dihydroxybergamottin, linked tail-to-tail and head-to-tail, and 6′,7′-epoxybergamottin, linked tail-to-tail, were detected. Moreover, the authors identified mixed heterodimers between 6′,7′-epoxybergamottin and bergamottin, and between 6′,7′-dihydroxybergamottin and bergaptol, which were not previously detected in grapefruit.

Determination of furocoumarins in grapefruit juices have been also reported recently in many papers (Lin et al., 2009; Prosen and Kočar, 2009; Peroutka et al., 2007). HPLC coupled to UV or MS was used. Samples were extracted with solvent (methanol or ethyl acetate) or by SPE (Prosen and Kočar, 2009).

Among other citrus juices, bergamot was also studied by different authors, and results are summarized in Table 8.18. Gattuso et al. (2007) determined the distribution of flavonoids and furocoumarins in bergamot juices prepared in laboratory from fruits of three different cultivars (Castagnaro, Fantastico, and Femminello) grown in Calabria. HPLC-DAD-MS-MS analyses were carried out on samples diluted in DMF, centrifuged and filtered through 0.45 μm membrane. Bergapten and bergamottin were also isolated by preparative HPLC and characterized by spectroscopic methods. Juice obtained from Femminello fruits presented the highest values of furocoumarins, as well as the highest values of flavonoids.

Similarly to Gattuso et al. (2007), also Gardana et al. (2008) studied the composition of flavonoids and furocoumarins in bergamot juice prepared in laboratory from Calabrian fruits at the end of their maturation period. They used HPLC-DAD-MS-MS instrumentation.

More recently, Dugo P. et al. (2009) determined coumarins and furocoumarins in laboratory-made bergamot juice by HPLC-DAD analysis after solvent extraction using ethyl acetate. The same composition of bergamot essential oil was found, with psoralens being the main components. The

TABLE 8.18
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Bergamot Juice

	[1]			[2]	[3]
Trivial Name	**Castagnaro***	**Fantastico***	**Femminello***		
Citropten					0.8
Bergapten	6.4–7.2	7.2–8.5	9.5–11.8	9.0	30.4
Bergamottin	26.4–29.5	22.5–24.6	38.5–43.7	18.2	61.0
5-Geranyloxy-7-methoxycoumarin					1.1

Notes: [1] Gattuso et al., 2007; [2] Gardana et al., 2008; [3] Dugo P. et al., 2009.
*Cultivars of bergamot.

ratio between bergamottin and bergapten was very different from that of the essential oil, probably due to the lower solubility of bergamottin in the aqueous juice.

Values reported by other authors are significantly different. Dugo P. et al. (2009) reported higher values than other authors. A possible explanation is that the filtration step performed by Gattuso et al. (2007) and Gardana et al. (2008) may cause loss of furocoumarins due to the poor solubility of these components in the juice.

With regards to the analysis of citrus liquors, data on the presence of coumarins and psoralens in lemon (limoncello) and bergamot (bergamino) liquors have been reported, as shown in Table 8.19.

The production of these liquors is traditionally based on the alcoholic maceration of the external part of citrus peel. Water and sugar are the other two main ingredients of the liquor, that presents an alcoholic grade around 30 to 32 degrees.

Versari et al. (2003) analyzed 12 commercial samples of limoncello by HPLC, without any sample pretreatment except filtration on 0.2 μm cellulose acetate membrane. Qualitative composition of the oxygen heterocyclic fraction resembled that of lemon essential oil. Quantitative results varied from sample to sample. Bergamottin was almost always the main constituent, followed by 5-geranyloxy-7-methoxycoumarin, citropten, and imperatorin.

A few years later, Crupi et al. (2007) determined the composition of limoncello liquors, both homemade (2 samples) and commercial (16 samples). They found higher amounts of oxygen heterocyclic fraction in the homemade limoncello, which was prepared following the traditional recipe. Some samples were found to contain herniarin and bergapten, reported in literature in some varieties of lemons. Byakangelicol and oxypeucedanin were absent in some samples, due to their possible hydrolysis to their corresponding diols. However, quantitative differences can be related to the procedures used to prepare limoncello, and, sometimes, to the use of terpeneless oils or oils free from nonvolatile components, which greatly reduce the amount of coumarins and psoralens in the final product.

Dugo P. et al. (2009) have analyzed homemade limoncello, finding a total amount of oxygen heterocyclic components of 17.44 mg/L similar to that reported by Crupi et al. (2007) for the

TABLE 8.19
Qualitative and Quantitative (mg/L) Data Found in Literature for Oxygen Heterocyclic Compounds of Liquors

Trivial Name	Limoncello			Bergamino
	[1]	[2]	[3]	[3]
Herniarin		nd–0.6		
Oxypeucedanin hydrate			3.9	
Byakangelicin			3.3	
Citropten	nd–4.0	0.2–3.0	3.6	0.9–2.6
Bergapten		nd–0.4		1.9–12.9
Byakangelicol		nd–4.9	0.5	
Oxypeucedanin		nd–6.7	0.5	
Imperatorin	nd–4.4			
5-Isopentenyloxy-7-methoxycoumarin	nd–1.0			
8-Geranyloxypsoralen			0.6	
Bergamottin	0.9–21.5	0.1–3.1	1.9	1.5–12.8
5-Geranyloxy-7-methoxycoumarin	0.2–7.0	0.1–2.4	1.2	<0.05–0.9
Citropten derivate	nd–0.3			

Notes: [1] Versari et al., 2003; [2] Crupi et al., 2007; [3] Dugo P. et al., 2009.
nd = non detected.

TABLE 8.20
Oxygen Heterocyclic Compounds Identified in Citrus Essential Oils (1999–2009)

Trivial Name and Systematic Name	Lem	M	SO	BO	B	G	Lim
Coumarins							
Citropten (5,7-dimethoxy-2H-chromen-2-one)	X			X	X	X	X
Meranzin (7-methoxy-8-((3,3-dimethyloxiran-2-yl)methyl)-2H-chromen-2-one)				X		X	
Isomeranzin (7-methoxy-8-(3-methyl-2-oxobutyl)-2H-chromen-2-one)				X		X	
Meranzin hydrate (2H-7-methoxy-8-(2′,3′-dihydroxyisopentyl)-1-benzopyran-2-one)				X		X	
Osthol (7-methoxy-8-(3-methylbut-2-enyl)-2H-chromen-2-one)				X		X	X
Aurapten (7-((*E*)-3,7-dimethylocta-2,6-dienyloxy)-2H-chromen-2-one)						X	X
Epoxyaurapten (7-((*E*)-3-methyl-5-(3,3-dimethyloxiran-2-yl)pent-2-enyloxy)-2H-chromen-2-one)						X	
5-Isopentenyloxy-7-methoxycoumarin (5-(3-methylbut-2-enyloxy)-7-methoxy-2H-chromen-2-one)	X						X
5-Geranyloxy-7-methoxycoumarin (5-[(*E*)-3,7-dimethylocta-2,6-dienyloxy)]-7-methoxy-2H-chromen-2-one)	X				X		X
Herniarin (7-methoxy-2H-chromen-2-one)							X
6,7-Dimethoxy-5-geranyloxycoumarin (5-[(*E*)-3,7-dimethylocta-2,6-dienyloxy)]-6,7-dimethoxy-2H-chromen-2-one)							X
5,7,8-Trimethoxycoumarin (5,7,8-trimethoxy-2H-1-benzopyran-2-one)							X
7-Isopentenyloxycoumarin (7-[(3-methyl-2-buten-1-yl)oxy]- 2H-1-benzopyran-2-one)							X
5-Methoxy-7-idroxycoumarin (5-methoxy-7-idroxy-2H-1-benzopyran-2-one)					X		
Marmin (7-[[(2*E*,6*R*)-6,7-dihydroxy-3,7-dimethyl-2-octen-1-yl]oxy]-2H-1-benzopyran-2-one)						X	
Auraptenol (7-[[(2*E*,6*E*)-8-hydroxy-3,7-dimethyl-2,6-octadien-1-yl]oxy]- 2H-1-benzopyran-2-one)						X	
Methylumbelliferone (7-methoxy-2H-1-benzopyran-2-one)							X
Psoralens							
Bergapten (4-methoxy-7H-furo[3,2-g]chromen-7-one)	X			X	X	X	X
Byakangelicol (9-[(3,3-dimethyloxiran-2-yl)methoxy]-4-methoxy-7H-furo[3,2-g]chromen-7-one)	X						X
Byakangelicin (9-(2,3-dihydroxy-3-methylbutoxy)-4-methoxy-7H-furo[3,2-g]-chromen-7-one)	X						
Oxypeucedanin (4-[(3,3-dimethyloxiran-2-yl)methoxy]-7H-furo[3,2-g]chromen-7-one)	X			X	X	X	X
Oxypeucedanin hydrate (4- (2,3-dihydroxy-3-methylbutoxy)-7H-furo[3,2-g]- chromen-7-one)	X						X
Imperatorin (9-(3-methylbut-2-enyloxy)- 7H-furo[3,2-g]chromen-7-one)	X						X
Isoimperatorin (4-(3-methylbut-2-enyloxy)-7H-furo[3,2-g]chromen-7-one)	X						X
Phellopterin (2H-5-methoxy-8-(3′-methylbut-2′-enyloxy)-furo-(3,2-g)-1-benzopyran-2-one)	X			X			X

Isopimpinellin (4,9-dimethoxy-7H-furo[3,2-g]chromen-7-one)	X						X
Bergamottin (4-[(*E*)-3,7-dimethylocta-2,6-dienyloxy]-7H-furo[3,2-g]chromen-7-one)	X			X	X	X	X
Epoxybergamottin (4-[(*E*)-3-methyl-5-(3,3-dimethyloxiran-2-yl)pent-2-enyloxy]-7H-furo[3,2-g]chromen-7-one)				X	X	X	
Epoxybergamottin hydrate (2H-5-((3′,7′-dimethyl-6′,7′-dihidroxy-2′-ottenyl)oxy)-furo-(3,2-g)-1-benzopyran-2-one)				X		X	
8-Geranyloxypsoralen (9-[(*E*)-3,7-dimethylocta-2,6-dienyloxy]-7H-furo[3,2-g]chromen-7-one)	X			X			X
5-(Isopent-2′-enyloxy)-8-(2′,3′-epoxy)-isopentenyloxypsoralen (8-[(3,3-dimethyloxiran-2-yl)methoxy]-5-(3-methylbut-2-enyloxy)-7H-furo[3,2-g]chromen-7-one)	X						X
Cnidicin (4,9-bis(3-methylbut-2-enyloxy)-7H-furo[3,2-g]chromen-7-one)	X						X
Cnidilin (9-methoxy-4-[(3-methyl-2-buten-1-yl)oxy]- 7H-furo[3,2-g][1]benzopyran-7-one)							X
5-Geranyloxy-8-methoxypsoralen (2H-5-((3′,7′-dimethyl-2′,6′-octadienyl)oxy)-8-methoxy-furo-(3,2-g)-1-benzopyran-2-one)	X						X
Xanthotoxin (9-methoxy-7H-furo[3,2-g]chromen-7-one)	X						X
Heraclenin (9-[[(2R)-3,3-dimethyl-2-oxiranyl]methoxy]- 7H-furo[3,2-g][1]benzopyran-7-one)	X						X
Heraclenol (9-[(2R)-2,3-dihydroxy-3-methylbutoxy]- 7H-furo[3,2-g][1]benzopyran-7-one)							X
5-Methoxy-8-isopenten-2′-enyloxypsoralen (9-methoxy-4-[(3-methyl-2-buten-1-yl)oxy]- 7H-furo[3,2-g][1]benzopyran-7-one)	X						
5-(2′,3′-Epoxy-isopentyloxy)-psoralen	X						
Pabulenol (4-[[(2S)-2-hydroxy-3-methyl-3-butenyl]oxy]- 7H-Furo[3,2-g][1]benzopyran-7-one)							X
Isooxypeucedanin (4-(3-methyl-2-oxobutoxy)-7H-furo[3,2-g][1]benzopyran-7-one)							X
Oxypeucedanin methanolate (4-[(2R)-2-hydroxy-3-methoxy-3-methylbutoxy]-7H-furo[3,2-g][1]benzopyran-7-one)							X
5-(Isopent-2′-enyloxy)-8-(2′,3′-dihydroxy-isopentyloxy)psoralen (5-(3-methylbut-2-enyloxy) -8–7H-furo[3,2-g]chromen-7-one)	X						
5-(2′,3′-epoxy-isopentyloxy)-8-(isopent-2′-enyloxy)psoralen (5–8-(3-methylbut-2-enyloxy)-7H-furo[3,2-g]chromen-7-one)	X						
5-(2′,3′-dihydroxy-isopentyloxy)-8-(isopent-2′-enyloxy)psoralen (5–8-(3-methylbut-2-enyloxy)-7H-furo[3,2-g]chromen-7-one)	X						
Neral oxypeucedaninyl acetal (diastereomer a)							X
Neral oxypeucedaninyl acetal (diastereomer b)							X
Geranial oxypeucedaninyl acetal (diastereomer a)							X
Geranial oxypeucedaninyl acetal (diastereomer b)							X
Polimethoxyflavones							
Sinensetin (4H-2-(3′,4′-dimethoxyphenyl)-5,6,7-trimethoxy-1-benzopyran-4-one)		X	X		X		
3,3′,4′,5,6,7-Hexametoxyflavone (4H-2-(3′,4′-dimethoxyphenyl)-3,5,6,7-tetramethoxy-1-benzopyran-4-one)			X				
Nobiletin (5,6,7,8-tetramethoxy-2-(3,4-dimethoxyphenyl)-4H-chromen-4-one)		X	X	X		X	X
Tetra-*O*-methylscutellarein (4H-2-(4′-methoxyphenyl)-5,6,7-trimethoxy-1-benzopyran-4-one)		X	X	X	X	X	X

continued

TABLE 8.20 (continued)
Oxygen Heterocyclic Compounds Identified in Citrus Essential Oils (1999–2009)

Trivial Name and Systematic Name	Lem	M	SO	BO	B	G	Lim
3,5,6,7,8,3′,4′-Heptamethoxyflavone (3,5,6,7,8-pentamethoxy-2-(3,4-dimethoxyphenyl)-4H-chromen-4-one)		X	X	X		X	X
Tangeretin (5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one)		X	X	X		X	
Isosinensetin (2-(3,4-dimethoxyphenyl)-5,7,8-trimethoxy-4H-1-Benzopyran-4-one)		X					
5-Demethyltangeretin (5-hydroxy-6,7,8-trimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one)		X					
5-Demethylnobiletin (2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-4H-1-benzopyran-4-one)		X					
5,6,7,4′-Tetramethoxyflavone (5,6,7-trimethoxy-2-(4-methoxyphenyl)- 4H-1-benzopyran-4-one)			X				
5-Hydroxy-6,7,4′-trimethoxyflavone (2-(4-methoxyphenyl)-5-hydroxy-6,7-dimethoxy-4H-1-benzopyran-4-one)			X				
5-Hydroxy-6,7,8,4′-tetramethoxyflavone (2-(4-methoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-4H-1-benzopyran-4-one)			X				
3-Hydroxy-5,6,7,4′-tetramethoxyflavone (2-(4-methoxyphenyl)-3-hydroxy-5,6,7-trimethoxy-4H-1-benzopyran-4-one)			X				
3-Hydroxy-5,6,7,8,4′-pentamethoxyflavone (2-(4-methoxyphenyl)-3-hydroxy-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one)			X				
5-Hydroxy-3,7,8,3′,4′-pentametoxyflavone (2-(3,4-dimethoxyphenyl)-3-hydroxy-5,7,8-trimethoxy-4H-1-benzopyran-4-one)			X				
5-Hydroxy-6,7,8,3′,4′-pentametoxyflavone (2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-4H-1-benzopyran-4-one)			X				
5-Hydroxy-3,6,7,8,3′,4′-hexametoxyflavone (2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7,8-tetramethoxy-4H-1-benzopyran-4-one)			X				
5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (2-(3,4-dimethoxyphenyl)-5-hydroxy-3,7-dimethoxy-4H-1-benzopyran-4-one)			X				
Hydroxypentamethoxyflavone 1						X	
Hydroxypentamethoxyflavone 2						X	
Hydroxyhexamethoxyflavone						X	

Notes: Lem = lemon; M = mandarin; SO =sweet orange; BO = bitter orange; B = bergamot; G = Grapefruit; Lim = lime.

homemade sample. The composition was in agreement with that of lemon essential oil, with a significant amount of diols formed from the corresponding epoxides during the preparation of the liquor. The same authors also analyzed two commercial bergamot liquors and found very different values. However, in both cases, the four components present in the oxygen heterocyclic fraction of bergamot essential oils were detected. Liquors were previously extracted with ethyl acetate, and the extract was analyzed by HPLC-DAD.

8.10 CONCLUSIONS

The oxygen heterocyclic compounds that have been reported to occur in citrus essential oils are listed in Table 8.20, together with their systematic name. As already discussed in the paragraphs dedicated to specific oils, some identifications reported only in the less recent literature and not confirmed later can be considered incorrect, and were not reported in this table.

Data reported in the time frame 1999–2009 have been obtained using modern analytical techniques for the separation of components, as well as for their identification: HPLC used in combination with photodiode array detector, mass spectrometry, and NMR spectroscopy.

The qualitative and quantitative composition of this fraction can be used as a criteria of authenticity of the oils, as well as quality, if we consider that their presence can be correlated with the geographical origin, the period, and the technology of extraction.

A renewed interest for these components has been due to their biological properties. For this reason, studies on the presence of oxygen heterocyclic components in citrus essential oils, as well as in other products containing citrus are necessary to estimate benefit risk factors in relation to the exposure of these components in foodstuffs, beverage, as well as cosmetic products.

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9 Carotenoids of Citrus Oils

Paola Dugo, Daniele Giuffrida

CONTENTS

9.1 INTRODUCTION

Carotenoids are an important kind of natural pigment that can be widely found in plant-derived food and products. Although these compounds have been traditionally used in the food industry as colorants, nowadays they attract great attention since they have been discovered to possess several important functional properties, mainly antioxidant activity (Beutner et al., 2001; de Quiros and Costa, 2006; El-Agamey et al., 2004), as well as prevention of cardiovascular diseases (Arab and Steck, 2000; Rao and Rao, 2007), cancer (Nishino et al., 1999; Omoni and Aluko, 2005;), and macular degeneration (Snodderly, 1995). These properties make these compounds ideal for the always increasing functional food industry, as well as promoting the consumption of the natural products in which are contained. Citrus species are well known to possess a rich carotenoid pattern and are regarded as the most complex natural source of this type of compounds (Goodner et al., 2001). Although, in general, these compounds are widely distributed in nature and can be found in higher plants, algae, fungi, and bacteria (Jaime et al., 2007; Mendes-Pinto et al., 2004). The chemical structure of carotenoids is usually based in a C_{40} tetraterpenoid structure with a centrally located and extended conjugated double bond system, which acts as a light-absorbing chromophore and is related to the color shown. Taking into account their chemical structure, these compounds can be divided into two different groups: hydrocarbon carotenoids, generally named carotenes; and oxygenated carotenoids, commonly known as xanthophylls. This second group is the more complicated one in terms of number of compounds and variations in their structure. Xanthophylls can be found in either its free form (like are found the carotenes) or in a more stable fatty acid-esterified form, in the case of mono- and polyhydroxylated xanthophylls. During ripening, in some fruits the esterification degree of carotenoids increases; this has been directly linked to the transformation of the chloroplast into the chromoplast. Thus, in view of the fact that a single carotenoid could be found forming different esters, the already complex natural variability of carotenoids is often increased by the formation of these carotenoid esters. The information concerning the natural esterified form could help in the chemical characterization of citrus oils and, therefore, in the detection of contamination or adulteration occurring in the essential oil production industry.

The oils of various citrus plants such as orange (*Citrus sinensis* [L.] Osbeck), mandarin (*Citrus deliciosa* Ten.), lemon (*Citrus limon* [L.] Burm. f.), grapefruit (*Citrus paradisi* Macf.), and lime (*Citrus aurantifolia* [Christm.] Swing.) are important and frequently used raw materials in the

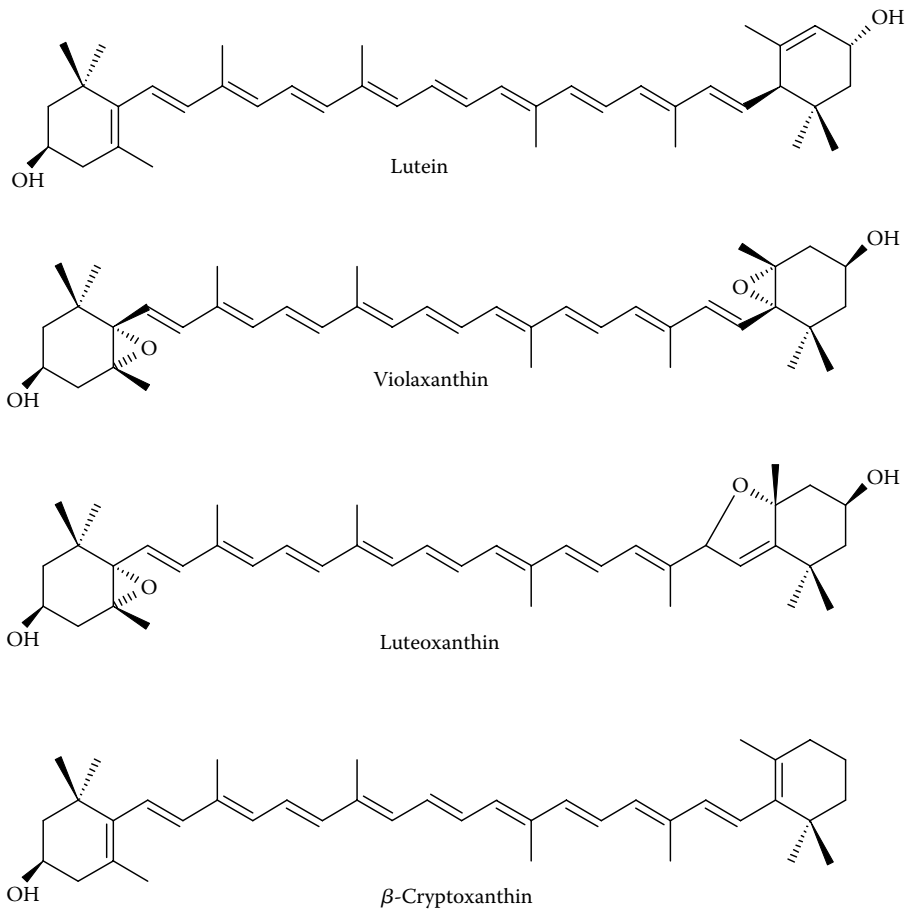

FIGURE 9.1 The chemical structures of some carotenoids reported in *Citrus* species.

perfume and flavor industry. Essential oils are nowadays often produced by pressing the fruit peels. In the literature, a wealth of analytical data is available on the volatile fraction of different citrus peel oils, whereas relatively less information is available on the nonvolatile residues. Moreover, very few papers have addressed the carotenoids composition in citrus essential oils. There are some reports available in the literature on the carotenoid composition in peels of citrus species obtained with solvent extraction of dried peels. Wang et al. (2008) reported the highest total carotenoid content in Ponkan (*C. reticulata*) peel and the lowest in Wendun (*C. grandis* Osbeck) among various citrus species from Taiwan. The level of total carotenoid in citrus peel varies between varieties, but is reported to be abundant in Cara Cara orange (*C. sinensis* [L.] Osbeck) and Star Ruby grapefruit (*C. paradise* Macf.) (Xu et al., 2006). Xanthophylls are also reported to be abundant in Taiwanese orange peel (Yen and Chen, 1995). Moreover, very early reports on the carotenoid composition in citrus peels as well as citrus juices can be found in Di Giacomo (1970). Figure 9.1 shows the chemical structures of some carotenoids reported in citrus species. Among the various citrus essential oils, the carotenoid composition has been so far investigated only in mandarin and orange essential oils.

9.2 CAROTENOIDS IN MANDARIN AND SWEET ORANGE ESSENTIAL OILS

The first study on the carotenoids profiles of sweet orange and mandarin essential oils after alkaline hydrolysis, obtained by reversed-phase liquid chromatography using a C18 column and a photodiode

array detector (PDA), was reported by Bonaccorsi et al. (2003). In this study, violaxanthin and antheraxanthin were reported as the major carotenoids present in orange essential oil and auroxanthin and β-cryptoxanthin were reported as the major carotenoids present in mandarin essential oil.

Most studies on carotenoids composition of various matrices have been carried out after saponification procedures, so the resulting data do not represent the native carotenoid composition of plant tissues. Moreover, artifact formation during the alkaline saponification can be a problem, since the high degree of unsaturation renders carotenoids sensitive to heat, light, and oxygen, with possible isomers formation and degradation.

Giuffrida et al. (2006), reported the first application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry [LC-(APCI)MS] with a C30 reversed-phase column, in the separation and identification of the main carotenoids esters present in native mandarin essential oil. In comparison with classical C18 stationary phase, the use of the much more hydrophobic C30 phase has shown a better resolving power for carotenoids. In fact, the C30 stationary phase provides sufficient phase thickness to enhance interaction with long-chained molecules. Figure 9.2 shows the HPLC chromatogram plotted at 450 nm of native mandarin essential oil, which illustrates the native carotenoid esters profile present in the nonvolatile fraction of this oil. β-cryptoxanthin [(3R)-β, β-caroten-3-ol] was identified as the main carotenoid, which was present both in its free form (1) and esterified as β-cryptoxanthin laurate (2), β-cryptoxanthin myristate (3), and β-cryptoxanthin palmitate (4).

However, even using these C_{30} stationary phases, the separation power of conventional HPLC could be not enough to analyze complex natural matrices. Comprehensive two-dimensional LC (LC × LC) is a novel technique coupling two independent LC separation processes with orthogonal selectivities (Dugo et al., 2008a). In LC × LC, the whole sample is analyzed in the two dimensions independently by using a switching valve as a transfer system between them.

The first application of comprehensive (LC × LC) to elucidate the carotenoid pattern in saponified sweet orange essential oils was reported by Dugo et al. (2006). In this study, a silica micro-HPLC column was used in the first dimension and a monolithic C18 column was used in the second dimension, with photodiode array detection. Under normal-phase conditions, the components were separated according to their increasing polarity. Under reversed-phase conditions, carotenoids were eluted according to their increasing hydrophobicity and decreasing polarity. Figure 9.3 shows the comprehensive LC × LC chromatogram, plotted at 450 nm, of the carotenoid fraction of saponified sweet orange essential oil. Fifty-seven peaks have been detected in the two-dimensional space (2D Plot), which clearly also shows the separation of the carotenoids in different chemical classes, thus identifying a bi-dimensional pattern where the different groups are marked.

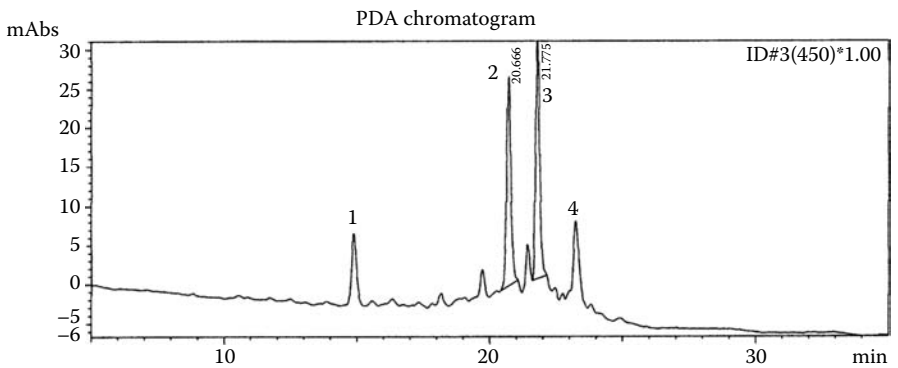

FIGURE 9.2 C30 HPLC chromatogram (PDA, 450 nm) of free carotenoids and carotenoid esters in mandarin essential oil. Peak assignment: free β-cryptoxanthin (1); β-cryptoxanthin-laurate (2); β-cryptoxanthin-myristate (3); β-cryptoxanthin-palmitate (4). (From Giuffrida, D., et al., *Flavour Fragr. J.* 21:319–323, 2006. Printed with permission of John Wiley & Sons, Ltd.)

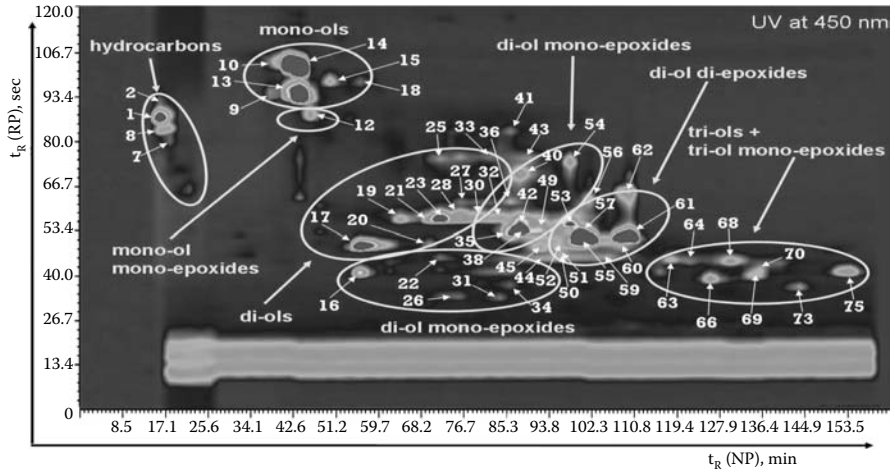

FIGURE 9.3 (**See color insert following page 462.**) Contour plot (PDA, 450 nm) of the comprehensive (LC × LC) analysis of carotenoids present in saponified sweet orange essential oils. (Reprinted with permission from Dugo, P., et al., *Anal. Chem*. 78:7743–7750, 2006. Copyright 2006 American Chemical Society.)

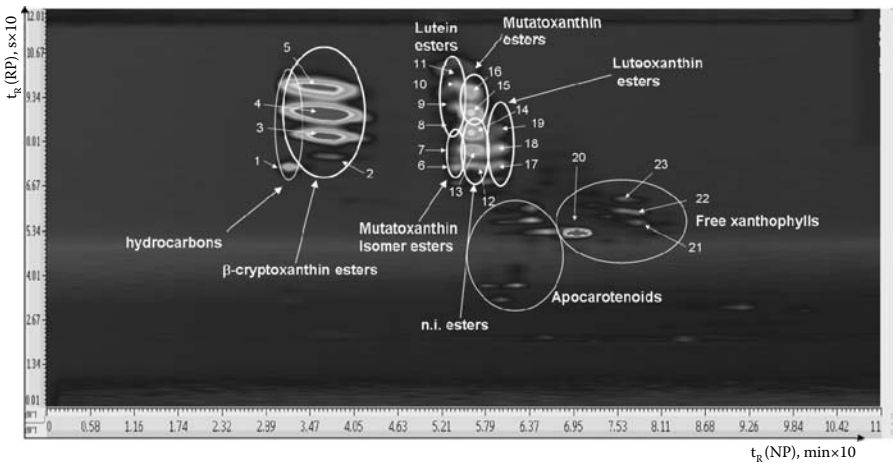

FIGURE 9.4 (**See color insert following page 462.**) Contour plot (PDA, 450 nm) of the comprehensive (LC × LC) analysis of both free carotenoids and carotenoid esters present in native mandarine essential oil. For peak identification see Table 9.1. (Reprinted from Dugo, P., et al., *J. Chromatogr. A* 1189:196–206, 2008b. Copyright 2008 with permission from Elsevier.)

A completely new comprehensive LC × LC method was developed by Dugo et al. (2008b) to determine both the free carotenoids and the carotenoid esters present in intact mandarin essential oil, using NPLC × RPLC coupled to DAD and MS (mass spectrometry) detectors. In this case, the first separation was performed through a microbore cyano column, while the second dimension separation was performed using a C18 monolithic column. In Figure 9.4, the 2D plot corresponding to the carotenoid esters analysis is shown. In this figure, the compounds separated as well as the different groups are marked. The peak identification is shown in Table 9.1. In this table, it is possible to also find the main ions, as well as the UV-Vis spectra information corresponding to each compound. Again, taking into account that most of carotenoids present an absorption maximum at ca. 450 nm, this wavelength was selected to obtain the 2D plot. Free β-cryptoxanthin and carotenoid monoesters were detected in the native composition of mandarin essential oil.

TABLE 9.1
Carotenoid Esters and Free Carotenoids Identified in the Native Mandarin Essential Oil with Their UV-Vis Information Observed and MS Ions Detected

Peak	Identification	UV-Vis Maxima			$[M+H]^+$	$[M+H-FA]^+$
1	ζ-Carotene	379	400	425	541	
	Phytofluene	332	349	367	543	
	Phytoene	275s	286	295s	545	
2	β-Cryptoxanthin-C_{10} ester	428s	454	480	707	535
3	β-Cryptoxanthin-C_{12} ester	428s	454	480	735	535
4	β-Cryptoxanthin-C_{14} ester	428s	454	480	763	535
5	β-Cryptoxanthin-C_{16} ester	428s	454	480	791	535
6	Mutatoxanthin isomer-C_{10} ester	410	430	454		
7	Mutatoxanthin isomer-C_{12} ester	410	430	454		
8	Lutein-C_{14} ester	421s	443	473	779	551
9	Lutein-C_{16} ester	421s	443	473	807	533[b]
10	Lutein-C_{18} ester	421s	443	473	817[a]	533[b]
11	Lutein-$C_{18:1}$ ester	421s	443	473	833	533[b]
12	n.i.-C_{10} ester	397	420	446		
13	n.i.-C_{12} ester	397	420	446		
14	n.i.-C_{14} ester	397	420	446		
15	Mutatoxanthin-C_{16} ester	406	427	449	823	549[b]
16	Mutatoxanthin-C_{18} ester	406	427	449	833[a]	549[b]
17	Luteoxanthin-C_{10} ester	398	419	446		
18	Luteoxanthin-C_{12} ester	398	419	446		
19	Luteoxanthin-C_{14} ester	398	419	446		
20	β-Cryptoxanthin	428s	455	480	553	
21	Free Xanthophyll	397	422	446		
22	Free Xanthophyll	403	423	449		
23	Free Xanthophyll	408	430	455		

[a] $[M+H]-H_2O$; [b] $[M+H-FA]-H_2O$.

Source: Reprinted from Dugo, P., et al., *J. Chromatogr. A* 1189:196–206, 2008b. Copyright 2008 with permission from Elsevier.

A further step in the carotenoid analyses of citrus essential oils was reported by Dugo et al. (2008c). In this study, the native carotenoid composition in a very complex matrix, like red orange essential oil, was reported. Both the free carotenoids and the carotenoid monoesters and diesters were determined. To achieve this goal a comprehensive LC × LC-DAD/APCI-MS method was developed based on a cyano microbore column in the first dimension and a monolithic C18 column in the second dimension. By using this novel analytical technique together with the use of DAD and APCI-MS detectors, it was possible to identify in the sample, without the need of any pretreatment, 40 different carotenoids. Among them, 16 carotenoid monoesters were identified, mainly β-cryptoxanthin palmitate ($C_{16:0}$), myristate ($C_{14:0}$), and laureate ($C_{12:0}$), as well as several lutein, violaxanthin, antheraxanthin, and luteoxanthin monoesters. Moreover, 21 carotenoid diesters, composed by several antheraxanthin, luteoxanthin, violaxanthin, and auroxanthin diesters, were found in the native carotenoid composition of the orange oil. The main carotenoid diesters were the laureate-palmitate ($C_{12:0}$, $C_{16:0}$), myristate-palmitate ($C_{14:0}$, $C_{16:0}$), and di-palmitate ($C_{16:0}$, $C_{16:0}$) diesters, although other diesters were also identified. Besides two different free carotenes, ζ-carotene and phytofluene, and a xanthophyll, lutein, were also determined. The typical 2D plot of the intact red orange essential oil is shown in Figure 9.5. In this figure, the different peaks are numbered and their assignments can be found in Tables 9.2 and 9.3. The elution order observed in the second dimension

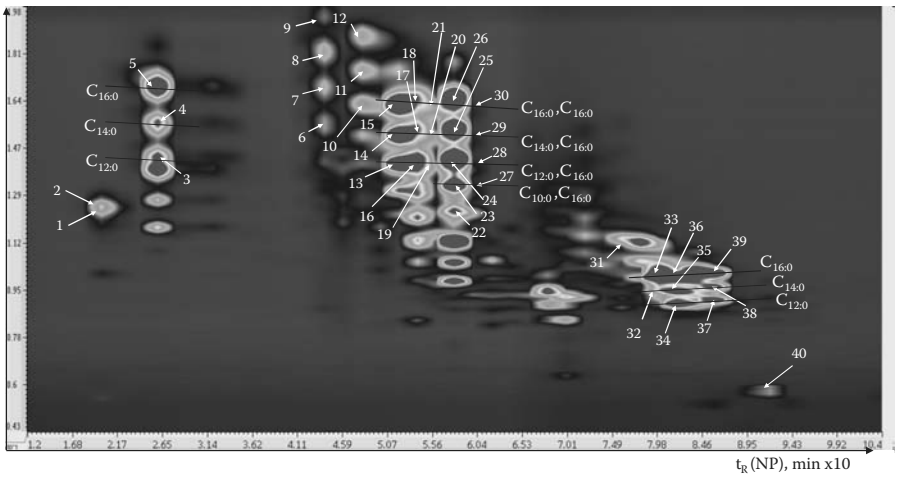

FIGURE 9.5 (**See color insert following page 462.**) Contour plot (450 nm) of the comprehensive HPLC analyses of both free carotenoids and carotenoid esters present in native red orange essential oil. For peak identification see Tables 9.2 and 9.3. (Reprinted with permission from Dugo, P., et al., *J. Agric. Food Chem.* 56:3478–3485, 2008c. Copyright 2008 American Chemical Society.)

TABLE 9.2
UV-Vis and MS Information and Identification of the Free Carotenoids and Carotenoid Monoesters Found in Red Orange Essential Oil

ID	Retention Time (Min)	Identification	UV/Vis Maxima	$[M+H]^+$	$[M+H\text{-}FA]^+$
1	21.2	ζ-Carotene	381, 401, 426	541	
2	21.2	Phytofluene	334, 350, 369	543	
3	27.4	β-Cryptoxanthin-laureate ($C_{12:0}$)	426s, 452, 480	735	535
4	27.6	β-Cryptoxanthin-myristate ($C_{14:0}$)	426s, 452, 480	763	535
5	27.7	β-Cryptoxanthin-palmitate ($C_{16:0}$)	426s, 452, 480	791	535
6	45.6	Lutein-laureate ($C_{12:0}$)	420, 448, 474	733	533
7	45.7	Lutein-myristate ($C_{14:0}$)	420, 448, 474	761	533
8	45.8	Lutein-palmitate ($C_{16:0}$)	420, 448, 474	789	533
9	45.9	Lutein-stearate ($C_{18:0}$)	420, 448, 474	835	533
31	77.1	Antheraxanthin-palmitate ($C_{16:0}$)	422, 443, 472	823	567
32	80.9	Luteoxanthin (b)-myristate ($C_{14:0}$)	401, 420, 446	811	583
33	81.0	Luteoxanthin (b)-palmitate ($C_{16:0}$)	401, 420, 446	839	583
34	82.9	Luteoxanthin (a)-laureate ($C_{12:0}$)	401, 423, 448	783	583
35	82.9	Luteoxanthin (a)-myristate ($C_{14:0}$)	401, 423, 448	811	583
36	83.0	Luteoxanthin (a)-palmitate ($C_{16:0}$)	401, 423, 448	839	583
37	86.9	Violaxanthin-laureate ($C_{12:0}$)	419, 440, 468	783	583
38	87.0	Violaxanthin-myristate ($C_{14:0}$)	419, 440, 468	812	583
39	87.0	Violaxanthin-palmitate ($C_{16:0}$)	419, 440, 468	839	583
40	92.6	Lutein	422, 446, 474	551[a]	

[a] $[M+H\text{-}H_2O]^+$

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separation corresponded to a typical behavior in which the more hydrophobic compounds are more retained and elute later. On the other hand, from the identification carried out and the 2D plot showed in Figure 9.5, it is possible to deduce the elution order in the normal phase separation carried out in the first dimension. It can be appreciated that the first eluted compounds were the

TABLE 9.3
UV-Vis and MS Information and Identification of the Carotenoid Diesters Found in Red Orange Essential Oil

ID	Retention Time (Min)	Identification	UV/Vis Maxima	$[M+H]^+$	$[M+H-FA1-FA2]^+$ and Other Main Ions
10	49.6	Antheraxanthin-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	422, 444, 469	1005	531[b], 805 ($[M+H-C_{12:0}]^+$)
11	49.8	Antheraxanthin-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	422, 444, 469	1033	531[b], 777 ($[M+H-C_{16:0}]^+$)
12	49.9	Antheraxanthin-di-palmitate ($C_{16:0}$, $C_{16:0}$)	422, 444, 469	1061	531[b], 805 ($[M+H-C_{16:0}]^+$)
13	53.4	Luteoxanthin (a)-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	397, 420, 446	1021	565, 821 ($[M+H-C_{12:0}]^+$)
14	53.5	Luteoxanthin (a)-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	397, 420, 446	1049	565, 803 ($[M+H-C_{14:0}]^+$)
15	53.6	Luteoxanthin (a)-di-palmitate ($C_{16:0}$, $C_{16:0}$)	397, 419, 446	1059[a]	565, 803 ($[M+H-C_{16:0}-H_2O]^+$)
16	55.4	Violaxanthin-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	415, 437, 466	1003[a]	565, 765 ($[M+H-C_{16:0}]^+$), 821($[M+H-C_{12:0}]^+$)
17	55.5	Violaxanthin-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	415, 437, 466	1049	565, 775 ($[M+H-C_{16:0}-H_2O]^+$), 803 ($[M+H-C_{14:0}-H_2O]^+$)
18	55.7	Violaxanthin-di-palmitate ($C_{16:0}$, $C_{16:0}$)	415, 437, 466	1059[a]	565, 803 ($[M+H-C_{16:0}-H_2O]^+$)
19	57.4	Auroxanthin-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	381, 401, 425	1021	565, 1003 ($[M+H-H_2O]^+$), 803 ($[M+H-C_{12:0}-H_2O]^+$), 747 ($[M+H-C_{16:0}-H_2O]^+$)
20	57.5	Auroxanthin-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	381, 401, 425	1049	565, 1031 ($[M+H-H_2O]^+$), 803 ($[M+H-C_{14:0}-H_2O]^+$), 775 ($[M+H-C_{16:0}-H_2O]^+$)
21	57.6	Auroxanthin-di-palmitate ($C_{16:0}$, $C_{16:0}$)	381, 402, 426	1078	565, 821 ($[M+H-C_{16:0}]^+$)
22	59.2	Luteoxanthin (b)-di-laureate ($C_{12:0}$, $C_{12:0}$)	401, 420, 447	965	565, 765 ($[M+H-C_{12:0}]^+$)
23	59.3	Luteoxanthin (b)-caproate-palmitate ($C_{10:0}$, $C_{16:0}$)	400, 420, 446	993	565, 975 ($[M+H-H_2O]^+$)
24	59.4	Luteoxanthin (b)-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	398, 420, 446	1003[a]	565, 747 ($[M+H-C_{16:0}-H_2O]^+$), 803 ($[M+H-C_{12:0}-H_2O]^+$)
25	59.5	Luteoxanthin (b)-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	398, 420, 446	1049	565, 1031 ($[M+H-H_2O]^+$), 803 ($[M+H-C_{14:0}-H_2O]^+$), 775 ($[M+H-C_{16:0}-H_2O]^+$)
26	59.6	Luteoxanthin (b)-di-palmitate ($C_{16:0}$, $C_{16:0}$)	400, 420, 446	1077	565, 803 ($[M+H-C_{16:0}-H_2O]^+$)
27	61.3	Auroxanthin isomer-caproate-palmitate ($C_{10:0}$, $C_{16:0}$)	382, 403, 429	993	565, 975 ($[M+H-H_2O]^+$)
28	61.4	Auroxanthin isomer-laureate-palmitate ($C_{12:0}$, $C_{16:0}$)	382, 403, 429	1021	565, 1003 ($[M+H-H_2O]^+$), 747 ($[M+H-C_{16:0}-H_2O]^+$), 803 ($[M+H-C_{12:0}-H_2O]^+$)
29	61.5	Auroxanthin isomer-myristate-palmitate ($C_{14:0}$, $C_{16:0}$)	382, 403, 429	1049	565, 1033 ($[M+H-H_2O]^+$), 775 ($[M+H-C_{16:0}-H_2O]^+$)
30	61.6	Auroxanthin isomer-di-palmitate ($C_{16:0}$, $C_{16:0}$)	382, 403, 429	1077	565, 1059 ($[M+H-H_2O]^+$),821 ($[M+H-C_{16:0}]^+$)

[a] $[M+H-H_2O]^+$; [b] $[M+H-FA_1-FA_2-H_2O]^+$

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hydrocarbons. Regarding the rest of compounds, it could be thought that according to their polarity, diesters would elute faster than monoesters. However, this was only the case when considering a particular carotenoid. Thus, the carotenoid structure was found of great importance, since the presence of free polar groups had a stronger influence than the presence of two fatty acids. Therefore, the elution order of the several mono- and diesters found corresponded to a combination between their esterification degree and the polarity of the carotenoid bound to their structure.

9.3 MANDARIN AND SWEET ORANGE ESSENTIAL OILS: A COMPARISON OF THEIR CAROTENOIDS PROFILES

The carotenoid profiles in the sweet orange and mandarin essential oils were different. The sweet orange essential oil carotenoids' composition was more complex than that of mandarin essential oil. A total of 23 different carotenoids were detected in the native mandarin essential oil composition (Dugo et al., 2008b), where free β-cryptoxanthin and β-cryptoxanthin monoesters were the most abundant and characteristic carotenoids and where diesters were not detected. A total of 40 different carotenoids, including 3 free carotenoids, 16 carotenoid monoesters, and 21 carotenoid diesters, were detected in the native sweet orange essential oil composition (Dugo et al., 2008c), where both violaxanthin mono and diesters and luteoxanthin mono and diesters were the characteristic carotenoids. Considering that citrus oils are largely employed as aromatizers in the food, pharmaceutical, and cosmetic industries, the knowledge of the carotenoids composition in mandarin and sweet orange essential oils, along with other analytical parameters, could be used as indicators of typicality in these citrus oils. Possible adulterations coming, for example, from the addition of sweet orange essential oil into the more valued mandarin oil could therefore be detected by a comparison of their carotenoids profile. The presence of a specific carotenoids profile could in fact be used to guarantee the genuineness of the product, since the quality control of citrus products requires a precise knowledge of the pigments composition of the original products. Further studies should be carried out in order to investigate the carotenoid composition in a wider range of citrus-derived essential oils, thus gaining new analytical data that could also help to a better understanding of the carotenoids metabolism in plants.

9.4 CAROTENOIDS IN ORANGE JUICE

Over the years, more than one hundred carotenoids have been reported to be present in the peel and the pulp of different citrus fruits. The carotenoid profile present in the citrus species depends on several factors, like different genetic factors in the different varieties, geographical origins, climatic factors, degree of ripening and harvesting time, and, for their products like juices, on the industrial processing and storage conditions as well.

As far as citrus juices are concerned, orange juices are probably the most recognized and globally accepted fruit juice, and its carotenoids composition has been investigated far more than juices from other citrus species (Meléndez-Martinez et al., 2007a). Regarding the carotenoids composition of orange juice after the saponification procedure, violaxanthin, luteoxanthin, lutein, β-cryptoxanthin, antheraxanthin, mutatoxanthin, and zeaxanthin have been usually identified as the major carotenoids. This can be observed in Table 9.4, where a list of carotenoids detected by different authors in orange juices is presented. Although more than 60 different compounds are reported in the various literature articles cited, some confusion may arise considering that some compounds reported may effectively be artifacts of the saponification step, or that many compounds are not clearly identified since they are generally indicated as isomers or epimers, or as "type" or "like," in the different literature reports. Moreover, some works even mention coeluting compounds , thus decreasing the identification certainty. In general, paper in which pure standards are used together with the identification power provided by the combination of DAD and MS detectors should be considered as more reliable. Due to the difficulty of the task, very few works have been reported on the separation

TABLE 9.4
Carotenoids in Orange Juice (1996–2009)

Compounds	[1]	[2]*		[3]	[4]	[5]*	[6]	[7]	[8]*		[9]	[10]*	[11]	[12]
		Spain	Belize				μg/100 g		Bonzaga	Cara Cara			mg/L	
Valenciaxanthin	X				X		14.44							
Neochrome	X			X								1.6		X
Trollichrome	X	X			X						X			X
Trollichrome like											X			
Antheraxanthin	X	X		X	X	14.33	236.89				X		6.81[g]	
cis-Antheraxanthin	X	X		X			26.88				X			
9-*cis*-Antheraxanthin														X
(*Z*)-Antheraxanthin isomer													0.26	
(9*Z*)- or (9′*Z*)- Antherxanthin													2.59	
Neoxanthin	X	X			X	5.73	X[c]				X			X
Neoxanthin type														X
Neoxanthin like											X			
Auroxanthin A	X	X							5.04[e]	0.81[e]	X			
Auroxanthin B	X	X							X[e]	X[e]	X			
Auroxanthin isomer											X			
cis-Violaxanthin	X	X	34.6	25.0		7.26			2.91	0.60				X
trans-Violaxanthin														X
Violaxanthin					X						X	13.6[f]		
9-*cis*-Violaxanthin							738.96[c]		30.08	7.48		33.8	X[g]	X
(13*Z*)-Violaxanthin												1.4		
Luteoxanthin	X				X				11.74	1.91	X	2.9		
Luteoxanthin a														X
Luteoxanthin a-type														X
Luteoxanthin b														X
Luteoxanthin b-type														X

continued

TABLE 9.4 (continued)
Carotenoids in Orange Juice (1996–2009)

Compounds	[1]	[2]*		[3]	[4]	[5]*	[6]	[7]	[8]*		[9]	[10]*	[11]	[12]
		Spain	Belize				μg/100 g		Bonzaga	Cara Cara			mg/L	
Luteoxanthin isomer											X			
(*Z*)-Luteoxanthin isomer													0.66	
Mutatoxanthin A	X	X		X	X						X			X
Mutatoxanthin B	X	X									X			
Mutatoxanthin isomer														X
Mutatoxanthin epimer													0.61	
Mutatoxanthin epimer													1.20	
Lutein	X	X	6.4	10.5	X	X	35.65[b]	53.55	X		X	6.6	0.93	X
Lutein 5,6-epoxide							15.69							
cis-Lutein														X
Lutein isomer											X			
Isolutein	X	X[a]	31.6[a]	45.5[a]	X									
Zeaxanthin	X	X[a]	X[a]	X[a]	X		X[b]		2.80	0.99	X		2.00	X
Zeinoxanthin								X					0.50	X
α-Cryptoxanthin	X	X	4.9	2.4	X	X	4.66	35.09			X	0.3		
β-Cryptoxanthin	X	X	12.3	8.9	X	X	11.68	42.29	X	12.66	3.10	X	3.5	1.19	X
(*Z*)-β-Cryptoxanthin												1.2		
9-*cis*-β-Cryptoxanthin														X
Cryptoxanthin 5,6-epoxide											X			
Phytofluene	X	X	1.8	0.5		X		36.54[d]		0.55	19.19	X			X
Phytoene						X		X[d]		2.12	39.89	X			X
Trollixantin						X									
α-Carotene	X	X	1.3	2.3	X	X	1.57	22.41	X			X		0.15	
β-Carotene	X	X	4.9	1.8	X	X	3.40	20.36	X	0.26	3.28	X		0.36	X
ζ-Carotene	X	X	2.2	3.1		X				0.79	0.38	X			X

9-*cis*-α-Carotene		41.45				
9–*cis*–β-Carotene		22.72				
(*Z*)-ζ-Carotene isomer						0.40
ζ–Carotene	X				1.2	
Lycopene			16.56			
Flavoxanthin	X			X		
β-Citraurin					10.8	
(*Z*)-β-Citraurin					1.9	
Monofuranoid like (λ_{max}= 450, 423 nm)					0.9	
Cryptochrome					3.5	
(*E*)-Violaxanthin + (*Z*)-violaxanthin isomers						3.38
Luteoxanthin + (*Z*)-antheraxanthin isomers						1.22

[1] Rouseff et al., 1996; [2] Mouly et al., 1999; [3] Goodner et al., 2001; [4] Verzera et al. 2002; [5] Meléndez-Martinez et al., 2003; [6] Cortés et al., 2004; [7] Schlatterer and Breithaupt, 2005; [8] Xu et al., 2006; [9] Dugo et al., 2006; [10] Agòcs et al., 2007; [11] Meléndez-Martinez et al., 2007b; [12] Dugo et al. 2008d.

X = detected.

* Results expressed as a % of the total peak area.

[a] Isolutein + zeaxanthin; [b] Lutein + zeaxanthin; [c] 9-*cis*-Violaxanthin + neoxanthin; [d] Phytofluene + phytoene; [e] Auroxanthin A + auroxanthin B; [f] Violaxanthin + furanoid; [g] Antherxanthin + (9*Z*)-violaxanthin.

of free carotenoids and carotenoids esters in intact native orange juices. An investigation on the native carotenoid pattern of orange juices of different varieties, studied by LC × LC-DAD/APCI-IT-TOF-MS, was reported by Dugo et al. (2009). As an example of these separations, in Figure 9.6, the contour plot (450 nm) of the comprehensive HPLC analyses of both free carotenoids and carotenoid esters present in native orange juice of the Valencia (A) and Moro (B) varieties is shown. In this work, the ability of the comprehensive LC method to separate intact epoxycarotenoids esters in real matrices like orange juices and its usefulness in estimating some juice quality-related parameters was demonstrated for the first time. Another approach to the separation of free carotenoids and carotenoids esters in native orange juices using C30 columns in tandem was reported by Dugo et al. (2008d). The separation and quantification of free carotenoids and carotenoids esters in orange juices of different varieties using conventional LC with a C30 column and DAD and MS detectors was carried out by Giuffrida et al. (2010). The results shown in Table 9.5, which were obtained after the HPLC analysis of native orange juices of eight different varieties (Moro, Valencia, Brasiliana, Washington, Ovale, Tarocco, Biondo comune, and Sanguinello) using a C30 column with DAD and MS detectors, clearly indicate that in these juices the xanthophylls are mostly esterified (93% of the total carotenoid content). In Table 9.5, a complete characterization of the compounds found in terms of UV/Vis and MS spectra and their content (ppm) in each sample analyzed is shown. Although

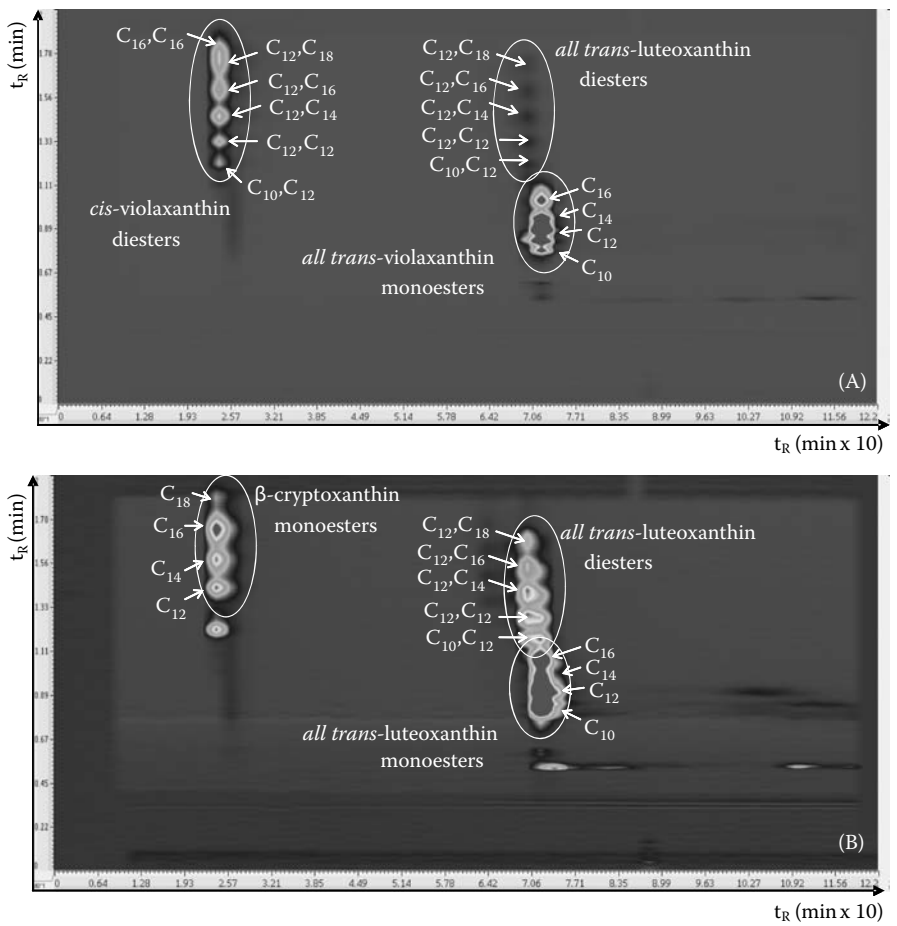

FIGURE 9.6 (**See color insert following page 462.**) Contour plot (450 nm) of the comprehensive HPLC analyses of both free carotenoids and carotenoid esters present in native orange juice of the Valencia (A) and Moro (B) varieties. (From Dugo, P., et al., *J. Sep. Sci.* 32:973–980, 2009. Printed with permission of John Wiley & Sons, Ltd.)

TABLE 9.5
Compounds Identified and Quantified (ppm) in Orange Juices of Different Varieties and Their UV/Vis and MS Spectra Characteristics

Identification	UV/Vis Maxima (nm)			[M+H]$^+$	Fragments Detected (*m/z*)	Carotenoids Content (ppm) in Orange Juices of Different Varieties							
						Valencia	Moro	Biondo Comune	Brasiliana	Ovale	Washington	Tarocco	Sanguinello
cis-Violaxanthin	328, 415	437	466	601	583, 509, 491	0.37	n.d.	n.d.	n.d.	0.12	n.d.	0.1	0.13
Luteoxanthin	398	420	446	601	583, 509, 453, 429	0.14	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	n.d.
Antheraxanthin	419	442	471	585	567, 493, 479	0.38	n.d.	n.d.	n.d.	0.11	n.d.	0.11	n.d.
Mutatoxanthin	405	425	450	585	565, 549, 529, 493	0.12	n.d.	0.12	n.d.	0.15	n.d.	0.12	0.13
Lutein	422	447	471	551		0.18	n.d.	0.21	n.d.	0.16	0.12	0.19	0.15
cis-Violaxanthin-$C_{10:0}$	328, 414	438	467	755	737, 565	0.11	n.d.	n.d.	n.d.	0.14	n.d.	n.d.	0.2
Phytoene	276	285	297	545		0.13	0.11	0.12	n.d.	n.d.	n.d.	n.d.	0.08
cis-Violaxanthin-$C_{12:0}$	328, 415	439	467	783	765, 583, 565	0.80	n.d.	n.d.	n.d.	0.42	n.d.	n.d.	0.13
Luteoxanthin-$C_{12:0}$	398	420	446	783	765, 565	n.d.	0.28	0.23	n.d.	0.3	n.d.	0.28	0.47
β-Cryptoxanthin	421	450	476	553	535, 461	0.15	0.25	n.d.	n.d.	n.d.	0.14	n.d.	0.59
cis-Violaxanthin-$C_{14:0}$	328, 414	438	466	811	793, 565	2.52	0.31	n.d.	n.d.	n.d.	0.1	n.d.	0.56
Luteoxanthin-$C_{14:0}$	398	420	446	811	793, 565	0.59	0.55	1.1	0.26	1.07	0.1	0.61	1.1
Auroxanthin-$C_{16:0}$	380	400	425	839	565	0.50	n.d.	0.19	n.d.	0.19	n.d.	n.d.	n.d.
cis-Violaxanthin-$C_{16:0}$	328, 414	438	466	839	821, 583, 565	2.09	0.22	n.d.	n.d.	0.2	n.d.	n.d.	0.24
Luteoxanthin-$C_{16:0}$	398	419	445	839	821, 565	n.d.	0.69	0.85	n.d.	0.65	n.d.	0.55	0.95
cis-Violaxanthin-$C_{12:0}$-$C_{12:0}$	328, 414	438	467	965	947, 873, 765, 747, 565	0.46	0.32	n.d.	0.38	0.29	0.12	0.32	0.38
cis-Violaxanthin-$C_{12:0}$-$C_{14:0}$	329, 415	437	466	993	975, 793, 775, 765, 747, 565	2.1	0.96	0.49	1.05	0.74	0.14	0.68	1.01
Antheraxanthin-$C_{16:0}$	420	442	471	823	805, 567, 549	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mutatoxanthin-$C_{16:0}$	405	425	450	823	805, 567, 549	n.d.	n.d.	0.21	0.32	n.d.	n.d.	n.d.	n.d.

continued

TABLE 9.5 (continued)
Compounds Identified and Quantified (ppm) in Orange Juices of Different Varieties and Their UV/Vis and MS Spectra Characteristics

Identification	UV/Vis Maxima (nm)			[M+H]$^+$	Fragments Detected (*m/z*)	Carotenoids Content (ppm) in Orange Juices of Different Varieties							
						Valencia	Moro	Biondo Comune	Brasiliana	Ovale	Washington	Tarocco	Sanguinello
cis-Violaxanthin-$C_{14:0}$-$C_{14:0}$	328, 414	438	466	1021	1003, 793, 565	1.13	1.08	0.98	0.85	0.53	0.22	0.59	0.88
β-Cryptoxanthin-$C_{12:0}$	420	450	476	735	535	0.82	n.d.	n.d.	1.59	n.d.	0.46	n.d.	1.85
cis-Violaxanthin-$C_{14:0}$-$C_{16:0}$	328, 414	437	466	1049	1031, 803, 565	0.92	1.05	1.4	0.83	0.66	0.23	1.3	2.57
β-Cryptoxanthin-$C_{14:0}$	420	450	476	763	535	0.73	n.d.	n.d.	1.71	1.41	0.31	n.d.	1.14
cis-Violaxanthin-$C_{16:0}$-$C_{16:0}$	328, 414	437	466	1077	1059, 821. 803, 565	0.72	0.67	0.69	0.71	0.81	0.15	0.6	0.92
β-Cryptoxanthin-$C_{16:0}$	420	450	476	791	535	0.84	0.56	0.72	0.55	0.32	0.33	0.46	1.11
Free carotenoids						1.47	0.36	0.45	0	0.54	0.26	0.64	1.08
Monoesters						9	2.61	3.3	4.43	4.7	1.3	1.9	7.75
Diesters						5.33	4.08	3.56	3.82	3.03	0.86	3.49	5.76
Total carotenoids						15.8	7.05	7.31	8.25	8.27	2.42	6.03	14.59

esterification does not change the visible light absorption properties, esterification increases the solubility of xanthophylls in lipids with which they are associated in nature. This is related to specific objectives for the plants and may also be related to an improvement of the carotenoids bioavailability and of the carotenoid stability against possible thermo-, photo-, and enzymatic oxidation reactions. Moreover, although esterification does not change the chromophore properties of the carotenoid molecule, it does modify the immediate molecular environment, and therefore the chemical activities may be altered depending on the kind of fatty acid bound to the xanthophylls. The different varieties investigated showed variations in their carotenoids profiles and carotenoids concentrations, which could be used as markers of their authenticity or as indicators of the fruits ripening stage or juices ages, as also proposed in the recent study on the characterization of carotenoids from apricots and pumpkins for the evaluation of fruit products authenticity by Kurz et al. (2008) and in the study by Hornero-Mendez et al. (2000), on the use of the xanthophylls esterification in *Capsicum annuum* as a ripeness index. In the eight orange juice varieties studied, the total carotenoid contents ranged from 2.42 ppm in the Washington variety to 15.8 ppm in the Valencia variety. Among the varieties studied, only the Sanguinello variety showed a total carotenoid content similar to that of the Valencia variety (14.59 ppm). The information, provided in Table 9.5, also shows that the main carotenoid in the native samples were β-cryptoxanthin, violaxanthin, and luteoxanthin, which were mainly present in their more stable mono and diester forms. The fatty acids esterifying the xanthophylls were ranging from C_{10} to C_{16}. The formation of luteoxanthin is probably due to the acidity of the juice, which could be enough to promote the 5,6-epoxides to 5,8-epoxides rearrangements during time, thus transforming a portion of violaxanthin into luteoxanthin. In general, the total amounts of monoesters (50.2%) was higher than the total content of diesters (42.9%). Among the monoesters, the β-cryptoxanthin esters were present in the highest amounts. Only diesters of violaxanthin were detected in the samples investigated. Interestingly, the ester contents were different among the varieties studied, showing variability in carotenoid compositions. Among the monoesters, the Valencia variety showed the highest violaxanthin esters amounts (61%), the Brasiliana variety showed the highest β-cryptoxanthin esters content (86.9%), and the Tarocco variety had the highest luteoxanthin esters amount (75.7%). Moreover, in general, in the Valencia, Brasiliana, Ovale, Washington, and Sanguinello varieties, the monoester contents were higher than the diesters, whereas in the Moro, Biondo comune, and Tarocco varieties, the opposite was determined. In fact, the mean value for the ratios between the monoester and diester fractions (monoesters/diesters) among the Valencia, Brasiliana, Ovale, Washington, and Sanguinello varieties was 1.45, whereas the mean value for the ratios between the monoester and diester fractions (monoesters/diesters) among the Moro, Biondo comune, and Tarocco varieties was 0.68. As far as the individual compounds quantified are concerned, the *cis*-violaxanthin-$C_{14:0}$-$C_{16:0}$ was the most abundant diester found, reaching the value of 2.57 ppm in the Sanguinello variety, and the *cis*-violaxanthin-$C_{14:0}$ and *cis*-violaxanthin-$C_{16:0}$ reached the highest amounts among the monoesters in the Valencia variety—2.52 ppm and 2.06 ppm, respectively. These findings seem to indicate that myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids are actively used in the esterification reactions in the samples studied.

9.5 CONCLUSIONS

The present chapter reviews the limited literature available on the carotenoids composition in citrus essential oils and also provides some updated information on the literature reports available on orange juices. Moreover, it also provides information on the new analytical techniques applied to the carotenoids analyses. Considering all the applications of citrus essential oils and the fact that orange juice is probably the most consumed fruit juice worldwide, together with the numerous health benefits attributed to the carotenoids, the broadening of the knowledge on the carotenoid composition in those matrices becomes important, since these phytochemicals can be considered as nutraceuticals or functional.

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10 Minor Components in Extracts of Citrus Fruits

Regula Naef

CONTENTS

10.1 INTRODUCTION

The unique organoleptic properties of citrus fruits are due to the unique composition of their constituents. In general, the flavor and fragrance of citrus peel oils and citrus juice extracts consist of dominant amounts of the nearly odorless limonene accompanied by monoterpenes such as myrcene, γ-terpinene, α- and β-pinene, or sabinene, and sesquiterpenes such as caryophyllene, together with bisabolenes, bergamotenes, or valencene, and fatty aldehydes such as octanal and decanal. Detailed reports on the composition of citrus oils are established by Dugo et al. (2002) and Sawamura (2000). Despite of this common general profile, each single *Citrus* species can be distinguished by an individual characteristic organoleptic signature, which is developed by complex and well-balanced mixtures of trace components. Thanks to the recent evolution of analytical methods and techniques, we now are able to identify potent key compounds of extremely low abundance and extraordinarily low odor thresholds.

In this present chapter, we aim to highlight these minor constituents. Due to the fact that in recent years the number of publications dealing with such compounds has increased exponentially, it would be audacious to claim to give an exhaustive overview. We rather discuss selected compounds identified in various citrus fruits that present either structural and/or organoleptic originality. These compounds are organized according to their functional groups or their biochemical origin. Selected items are sesquiterpenoid hydrocarbons (germacrenes), hydrocarbons with a

homoterpenoid skeleton, linear C_{11} compounds derived from fatty acids, nitrogen-containing compounds, sulfur-containing compounds, saturated branched and unsaturated linear and branched fatty acid aldehydes, phenylpropanoids, methyl jasmonate and related compounds, some lactones, and norisoprenoids. To resume the inexhaustible diversity of mono- and sesquiterpenoid derivatives identified in citrus fruits would have been a hopeless task. Therefore, we decline to discuss them here after reviewing the detailed literature, which did not reveal such trace components having a special impact on the sensory properties (with the exception of wine lactone). The germacrenes as the only example of sesquiterpenoids are selected to discuss an interesting observation in the research on natural products. Systematic botanical names of the *Citrus* species are only given if they are clearly defined in the references in question. Following some recent discussions about reclassification of the genera within the subtribe Citrineae (Mabberley, 1998), some species of the former genera *Microcitrus*, *Fortunella*, and *Poncirus*, and now integrated in the genus *Citrus*, are included in our study.

10.2 SELECTED MINOR COMPOUNDS

10.2.1 Germacrenes (Sesquiterpene Hydrocarbons)

Germacrenes B, D and bicyclogermacrene have been routinely observed in citrus peel oils, whereas germacrenes A and C, thermally very labile compounds, could only be detected as their products of *Cope*-rearrangement, the β- and δ-elemenes, respectively. Feger et al. (2001) investigated 20 typical commercial citrus peel oils. They applied mild GC-conditions, injector and oven temperature not exceeding 100°C. All samples showed the presence of germacrene A and germacrene C, which were most abundant in Key lime oil at 0.46% and 0.59%, respectively. On the other hand, when the temperature of the oven and the injector was held at 200°C, only β- and δ-elemene could be identified. Germacrene B was most abundant in Key lime oil (0.90%) and bicyclogermacrene was found in yuzu at 0.63%; germacrene D was present in all citrus oils with the exception of lemon. The alcohol germacra-1(10),5-dien-4-ol, also called germacrene D-4-ol, has been identified, among others, by Dugo et al. (2005) in Mexican Dancy tangerine oil. Flamini et al. (2007), studying the volatile emission of different plant organs of *Citrus limon*, has discovered bicyclogermacrene in the pollen (4.4%) and germacrene D-4-ol in the adult leaves (0.7%). The same compound is the most abundant volatile constituent (25.9%) in the peel oil of *Microcitrus australasica* var. *sanguinea* (F.M. Bail) Swing (Ruberto et al., 2000) (Figure 10.1).

10.2.2 Undecatriene, Undecatetraenes, and Related Compounds

The linear C_{11} hydrocarbons (3*E*,5*Z*)-1,3,5-undecatriene (galbanolene), (3*E*,5*Z*,8*Z*)-1,3,5,8-undecatetraene, and (3*Z*,5*E*,8*Z*)-1,3,5,8-undecatetraene, metabolites of polyunsaturated fatty acids (Moore, 1977), were originally identified in seaweeds and then later in galbanum and pineapple.

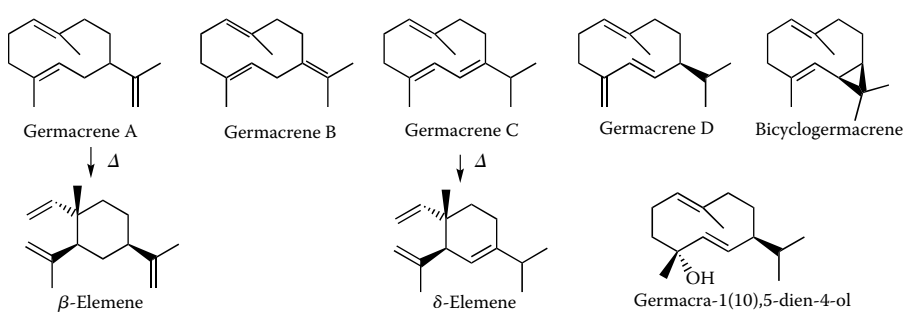


FIGURE 10.1 Germacrenes.

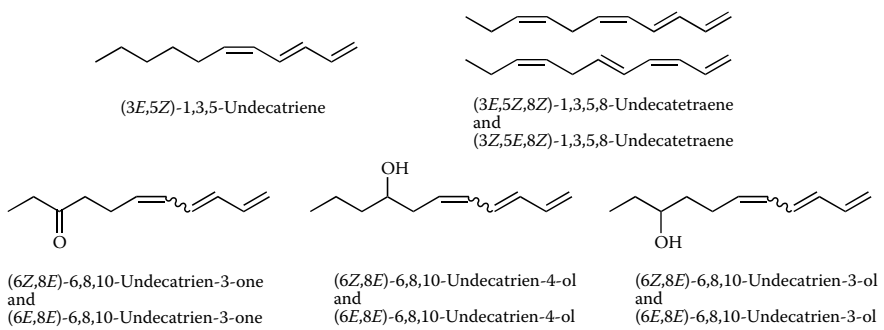


FIGURE 10.2 Linear C_{11} hydrocarbons and related compounds.

Berger et al. (1985) studied various fruits in search of these very potent compounds, exhibiting green, marine, fruity, metallic notes with extremely low odor perception thresholds. They reported on (3*E*,5*Z*)-1,3,5-undecatriene as a constituent of mandarin juice. Later, Escher (1985) discovered the undecatetraenes in yuzu (*Citrus junos*), and Naef and Velluz (2001) identified them in mandarin peel (*Citrus deliciosa*). Very recently, Miyazawa et al. (2009) isolated oxidized metabolites of the undecatetraenes, such as (6*Z*,8*E*)-6,8,10-undecatrien-3-one (yuzunone, already found in galbanum oil by the same authors), (6*E*,8*E*)-6,8,10-undecatrien-3-one, (6*Z*,8*E*)-6,8,10-undecatrien-3-ol, (6*E*,8*E*)-6,8,10-undecatrien-3-ol, (6*Z*,8*E*)-6,8,10-undecatrien-4-ol (yuzuol), and (6*E*,8*E*)-6,8,10-undecatrien-4-ol from a yuzu peel extract. In particular, yuzunone and yuzuol increased the balsamic, sweet, and floral characteristics that distinguished the yuzu aroma from other *Citrus* fruits (Figure 10.2).

10.2.3 4,8-Dimethyl-1,3,7-nonatriene and 4,8,12-trimethyl-trideca-1,3,7,11-tetraene

Maurer et al. (1986) identified the two homoterpenes, (3*E*)- and (3*Z*)-4,8-dimethyl-1,3,7-nonatriene as well as (3*E*,7*E*)-4,8,12-trimethyl-trideca1,3,7,11-tetraene, in cardamom oil, and proved that the C_{11} compounds are metabolites of nerolidol, whereas the C_{16} compound is a degradation product of geranyl linalool. Naef and Velluz (2001), as well as Katayama and Iwabuchi (2002), found the C_{11} compounds in extracts of mandarin peel and in ki-mikan (*Citrus flaviculpus*) peel, respectively. In addition, both groups detected these same compounds in the emission of the volatiles of the intact fruit by solid phase micro extraction (SPME). Naef (2004) extended the study to various citrus fruits and confirmed the presence of the C_{11} homoterpenes in intact fruits of Navel orange, bergamot, bitter orange, sweet lemon (*Citrus limetta*), *C. medica*, and white grapefruit (in ascending amounts). The C_{16} homoterpene, despite its lower volatility, was present as trace amounts in the SPME's of bergamot and bitter orange. These same homoterpenes are constituents of a blend of volatile compounds that are emitted by a plant (e.g., faba beans) after its mechanical damage by herbivores. Carnivorous enemies of these herbivores are then attracted by these volatiles, which indirectly results in reducing further injury of the plant (Donath and Boland, 1995). Citrus plants take advantage of similar defense systems (Farmer, 2001; Yamasaki et al., 2007) (Figure 10.3).

10.2.4 Nitrogen-Containing Compounds

Leaves and flowers of citrus fruits show the highest concentrations of nitrogen-containing compounds. Most abundant are the anthranilates, especially methyl *N*-methyl anthranilate, which in petitgrain mandarin reaches amounts of up to 60%. The only source reported for 2-(*N*-methylamino)-benzaldehyde, reminiscent of citrus, mandarin with pleasant green, floral notes, is the basic extract of mandarin peel (Naef and Velluz, 2001). Compounds such as pyridines and pyrazines or derivatives related to indole have an important flavor impact in spite of their presence as trace components (Figures 10.4 and 10.5).

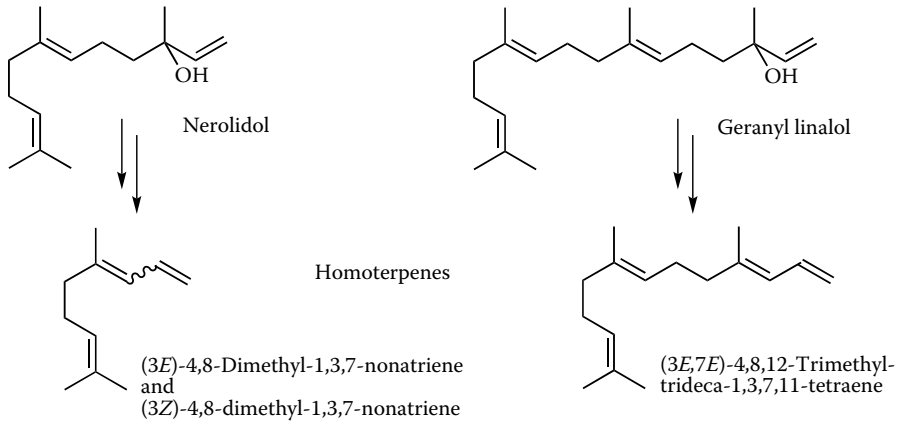


FIGURE 10.3 Homoterpenes.

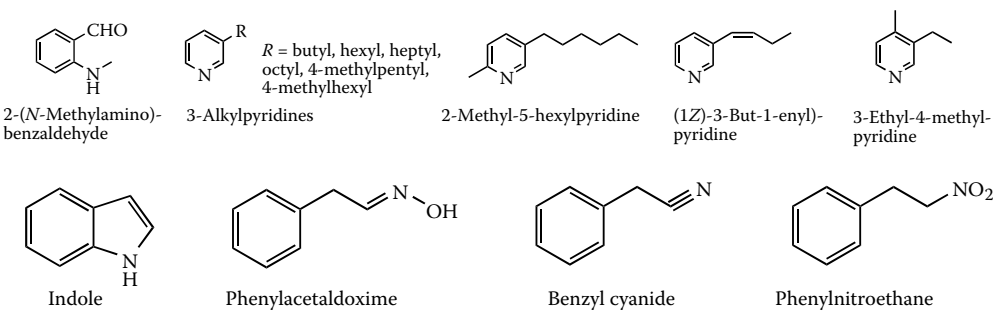


FIGURE 10.4 Nitrogen-containing compounds.

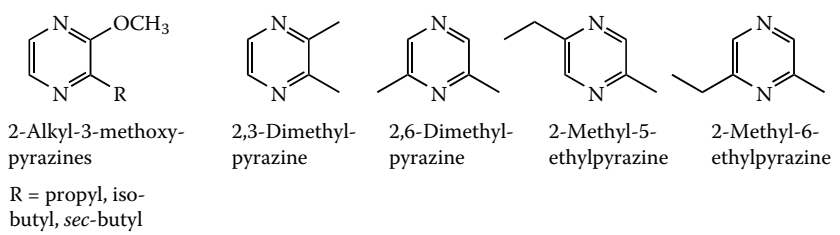


FIGURE 10.5 Pyrazines.

Thomas and Bassols (1992) identified higher alkyl-pyridines, such as 3-butyl-, 3-hexyl-, 3-heptyl-, 3-octyl-, 4-methylpentyl-, 4-methylhexyl-, or 2-methyl-5-hexylpyridines, in cold-pressed sweet orange peel oil from Florida (Valencia) and Brazil (Pera). 3-Hexylpyridine, with a flavor detection threshold of 0.28 ppb in water and exhibiting fatty citrus- and orange-like notes, appears to be the most important organoleptically. Maurer and Hauser (1992) found (1*Z*)-3-(but-1-enyl)pyridine in petitgrain oil (*Citrus aurantium*); when diluted, this compound exhibits green and flowery odors. In the basic fraction of the absolute of *Citrus unshiu,* 3-ethyl-4-methylpyridine was discovered as a new citrus ingredient by Sakurai et al. (1979).

The same authors identified the biogenetically interesting, phenylalanine-derived volatiles phenylacetaldoxime, benzyl cyanide, and 2-phenyl-nitroethane, all related to indole, which is a common constituent in citrus chemistry.

2-Alkyl-3-methoxypyrazines are key compounds in the flavors of green peas and bell peppers. Their odor descriptors are green, earthy, and pea-like, and their odor detection value is very low. Boelens and Sindreu (1988) describe trace amounts of 2-methoxy-3-isobutylpyrazine in the leaf oil of bitter oranges (*Citrus aurantium*). Schieberle et al. (2003) found them in homogenized clementine (*Citrus reticulate blanco cv. Clementine*) segments; 3-*sec*-butyl-2-methoxypyrazine, with the highest odor activity value (OAV) of 2250, was among the most intense odorants of clementine, exhibiting floral, woody notes. OAV is defined as the concentration of a compound divided by its odor threshold in water. Compounds with values exceeding 50 actively contribute to the quality of a flavor. A mandarin peel extract was treated with 20% aq. HCl to isolate the disubstituted dimethyl- and ethylmethylpyrazines (Naef and Velluz, 2001), which have flavor descriptors from green, earthy, and sweet to nutty, chocolate, and roasted.

10.2.5 Sulfur-Containing Compounds

Sulfur-containing compounds, in particular free thiols known for their extremely low detection thresholds, are signature molecules that give characteristic imprints to a flavor. Progress in the sensitivity of analytical equipment (time-of-flight MS, high resolution MS, multidimensional GC-MS, HRGC/olfactometry, and "aroma extract dilution analysis") together with sophisticated methods of isolation (HPLC, affinity chromatography) allows to measure spectral data of unknown compounds present in minute concentrations and to establish new structures with outstanding organoleptic properties. Nevertheless, one of the most potent molecules ever detected, *p*-menth-1-ene-8-thiol with an odor threshold of 10^{-4} μg/l in water, was already identified as a constituent of grapefruit juice in 1982 by Demole et al., using traditional fractionation techniques and a good portion of intuition. Schieberle and Buettner (2000) confirmed its importance for grapefruit juice flavors and determined an OAV of 100, whereas its role in orange juice was negligible (OAV > 1). The same authors used aroma extract dilution analysis, or the "stable isotope dilution assay"-method (Buettner and Schieberle, 2000), to measure 0.8 μg/l in the white, and 0.4 μg/l in the pink grapefruit juice. Yukawa et al. (1994) identified it as key odorant of the pleasant, fresh, sulfurous, green odor of yuzu (*Citrus junos*). 4-Mercapto-4-methyl-2-pentanone is an even more potent ingredient of grapefruit juice and is described as sulfury, catty, tropical fruit-, buchu-, grapefruit-, and green tea-like. First identified in carob beans by McLeod et al. (1992), its importance for citrus flavors was recognized by Buettner and Schieberle (2000) in grapefruit juice with an OAV of 1333. Its positive contribution to the yuzu aroma with a flavor dilution factor (FD) of 128 was proved by Miyazawa et al. (2009), confirming the findings of Escher et al. (2006). An unusual compound is responsible for the sulfury odor of the pontianak orange (*Citrus nobilis* var. Lour. *microcarpa* Hassk.): 1-phenylethanethiol ((*R*):(*S*) = 76:24), which is described as sulfurous and resinous with an odor threshold of 0.005 ng/l in air (Fischer et al., 2008). Cold on-column injection is primordial to avoid its decomposition into styrene and H_2S. (*S,S′*)-Ethylidene dithioacetate is a unique constituent of blood orange juice (Näf and Velluz, 1996). It is probably formed by acetalization of acetaldehyde with thioacetic acid. The odor exhibits sulfury, tropical, fruity, green, and blackcurrant-like tonalities (Figure 10.6).

3-Mercapto-3-methyl-1-butanol and 3-mercapto-1-hexanol, as well as their esters, have been recently identified in *Poncirus trifoliata* or *Citrus trifoliata*, a spiny shrub native to China and resistant to low temperatures, used mainly as hardy, disease-resistant rootstocks for cultivated citrus plants by Starkenmann et al. (2007). For the esters of both alcohols, the carboxylate moieties are derived from the even, linear saturated fatty acids C_4 to C_{18}. These compounds have interesting organoleptic properties and may find use in various flavor applications. Both free alcohols are reminiscent of tropical fruits, especially mango. The esters exhibit fruity notes and turn into "fatty, meaty, smoky" when the alkyl-chain is longer. Astonishingly, even the less-volatile compounds are still well-detected at 10 ppm.

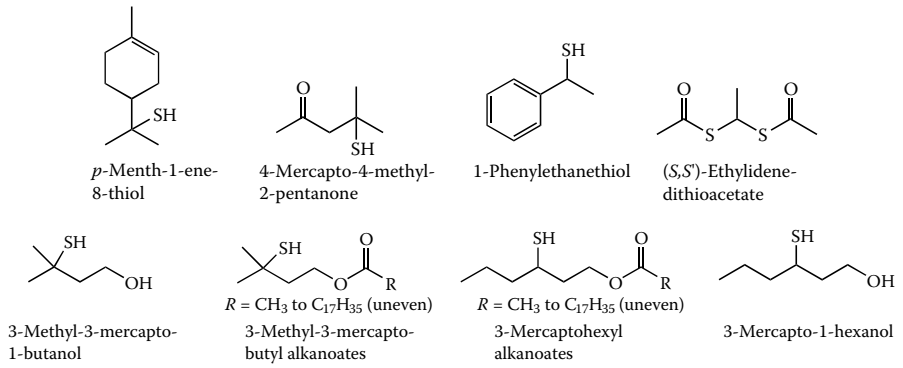


FIGURE 10.6 Sulfur-containing compounds.

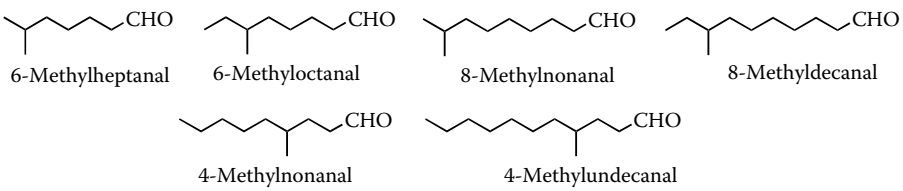


FIGURE 10.7 Branched, saturated fatty acid aldehydes.

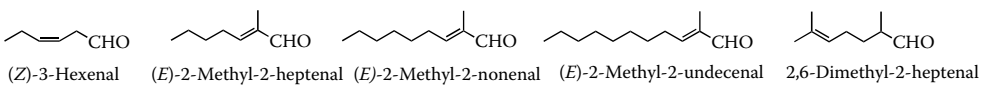


FIGURE 10.8 Unsaturated fatty acid aldehydes.

10.2.6 Fatty Acid Aldehydes

The linear fatty acid aldehydes, especially octanal, decanal, and dodecanal, together with some terpenes (limonene, citral, citronellal) form the basis of all citrus flavors. (2*E*)-2-Alkenals with chain length from C_4 to C_{12} contribute with their green, fresh notes to blond oranges (Naef and Velluz, 1996), to the flavor of bitter oranges (Boelens and Sindreu, 1988), to clementine (Chisholm et al., 2003), or to yuzu (Tajima et al., 1990). The concentration of the saturated linear aldehydes is in the order of magnitude of 50–100 μg/kg, for the 2-alkenals it falls to 0.5–1.5 μg/kg (Buettner and Schieberle, 2001a,b). It is still possible to perceive this range of concentrations without preseparation of the fruit extracts. Isolation and identification of the branched saturated, the branched unsaturated and the linear unsaturated aldehydes require an enrichment, either by column chromatography or treatment with the Girard T reagent. This is mostly followed by preparative gas chromatography, in particular by two dimensional capillary gas chromatography (Figures 10.7 through 10.10).

Branched saturated aldehydes, mainly 6-methylheptanal, 6-methyloctanal, 8-methylnonanal, and 8-methyldecanal, have been found in yuzu (Tajima et al., 1990), in orange peel oil (Widder et al., 2003), in a bitter orange extract (Naef, 2005), and in Australian fingerlime (*Citrus australasica*) by Delort and Jaquier (2009). Their odor qualities vary from fruity, fresh green, and juicy for the smaller molecules to fatty, soapy, and peel-like for the longer-chain molecules with odor thresholds of 1.2 ppb, 5.6 ppb, 42 ppb, and 28 ppb, respectively, in water. 4-Methylnonanal and 4-methylundecanal are constituents of a lemon peel (*Citrus limon*) extract (Naef and Jaquier, 2006); the odor of

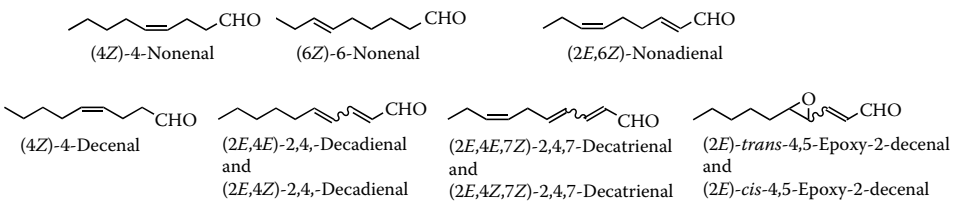


FIGURE 10.9 Unsaturated fatty acid aldehydes C_9 and C_{10}.

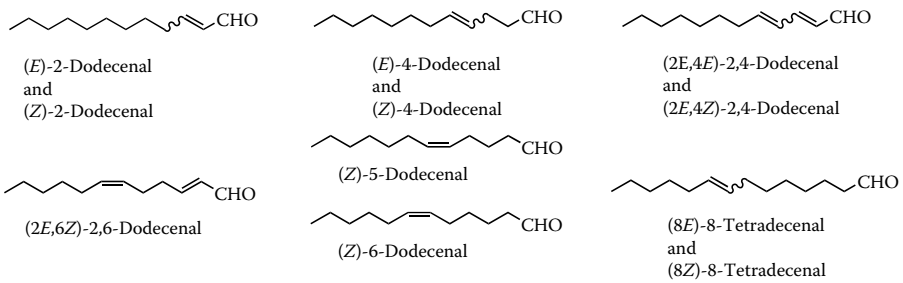


FIGURE 10.10 Unsaturated fatty acid aldehydes C_{12} and C_{14}.

the first is complex and reminiscent of iris, citrus, coriander, parsley, melon, and cucumber, whereas the descriptors of the second are oily and fatty.

(3*Z*)-3-Hexenal, formed by enzymatic degradation of linolenic acid, has the typical green-banana smell of cut leaves and is the unique ingredient of freshly squeezed orange juice (Buettner and Schieberle, 2001b). The three branched unsaturated aldehydes (2*E*)-2-methyl-2-heptenal, (2*E*)-2-methyl-2-nonenal, and (2*E*)-2-methyl-2-undecenal have been isolated from lemon peel (Naef and Jaquier, 2006). They exhibit strong notes of lemon grass for the first, of fish, chicken, citrus, melon and green tea for the second, and oily, fatty for the last compound. (2*E*)-2,6-Dimethyl-2-heptenal is a nor-monoterpene and has well earned its trivial name, "Melonal," due to its powerful floral, green, melon-like odor with a detection threshold in water of 1 ppb. It was a highly appreciated yet artificial flavor ingredient long before its identification in eucalyptus by Chen et al. (1983). Sato et al. (1990) mention it in the leaf oil of *Citrus hystrix*, and Yang et al. (1992, 2000, 2001) identified it in various citrus products such as the peel oils of lemon, lime, sudachi (*Citrus sudachi*), yuzu (*C. junos*), and kabosu (*C. sphareocarpa*).

(4*Z*)-4-Nonenal and (6*E*)-6-nonenal are minor components of yuzu only (Miyazawa et al., 2009), and have never been detected in oils of other *Citrus* species. (6*E*)-6-nonenal, with its powerful peely, citrusy, and albedo-like odor, is a key ingredient of the yuzu aroma. (2*E*,6*Z*)-2,6-nonadienal, reminiscent of cucumbers, was described in clementines by Chisholm et al. (2003).

(4*Z*)-4-Decenal, with its flowery, green, citrus-like flavor is an important constituent of several citrus fruits and was found by Naef and Velluz (2001) in mandarin, by Chisholm et al. (2003) and Buettner et al. (2003) in clementine, by Naef (2005) in bitter orange, by Fischer et al. (2008) in the Pontianak orange, and by Miyazawa et al. (2009) in yuzu. Decadienals, both (2*E*,4*E*)- and (2*E*,4*Z*)-isomers, are ubiquitous citrus ingredients, and are reminiscent of hot oil, meat, and cereal. However, the corresponding trienals, (2*E*,4*E*,7*Z*)- and (2*E*,4*Z*,7*Z*)-2,4,7-decatrienal, are reported only in mandarin and tangerine extracts by Naef and Velluz (2001) and Naef et al. (2001). They have a fishy, oily odor and a fatty, round mouthfeel. Applied in appropriate concentrations to an orange flavor, the mixture containing 75% of the (2*E*,4*Z*,7*Z*)-isomer increases the typical tangerine character. (2*E*)-*trans*-4,5-Epoxy-2-decenal, possessing a very intense metallic odor, has been widely detected by the group of Schieberle (e.g., in navel oranges with OAV = 387) (Buettner and Schieberle, 2001b).

Widder et al. (2003) confirmed its presence in orange peel and juice. Kumazawa et al. (2007) and Miyazawa et al. (2009) characterized it together with its (*cis*)-isomer in orange and grapefruit juice extracts and in yuzu, respectively. Chisholm et al. (2003) identified its C_9- and C_{12}-homologues by deduction from spectral data.

The unsaturated C_{12}-aldehydes (2*E*)- and (2*Z*)-2-dodecenal, (4*Z*)- and (4*E*)-4-dodecenal, (2*E*,4*E*)- and (2*E*,4*Z*)-2,4-dodecadienal, (2*E*,6*Z*)-2,6-dodecenal, (5*Z*)-5-dodecenal, and (6*Z*)-6-dodecenal, seem to be markers for mandarin, tangerine, and clementine oils (Chisholm et al., 2003; Naef and Velluz, 2001). From these, (*Z*)-4-dodecenal especially emphasizes the juicy, green, and natural character of the mandarin flavor. In addition, (*Z*)-5-dodecenal was recently identified as a key odorant in pontianak oranges (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) (Dharmawan et al., 2009). Widder et al. (2003, 2008) detected new interesting C_{14}-aldehydes, (8*Z*)-, and (8*E*)-8-tetradecenal, which intensify the citrus character of fragrances and flavors.

10.2.7 Phenylpropanoids

Degradation of lignin leads to the class of plant metabolites called phenylpropanoids. The volatile derivatives cinnamaldehyde, cinnamic alcohol, ethyl cinnamate, and ethyl 3-phenylpropanoate have sweet, balsamic, fruity and woody tonalities and contribute essentially to the characteristic notes of blood orange juice (Näf and Velluz, 1996). Cinnamaldehyde was identified in redblush grapefruit (Njoroge et al., 2005), cinnamic alcohol in Tahiti lime (Njoroge et al., 1996), and ethyl cinnamate in mandarin oranges (Schieberle et al., 2003). Safrole is a sweet, spicy, warm, and floral flavor ingredient of nutmeg and was identified at a level of 0.24% in the leaf oil of *Citrus hystrix* (Sato et al., 1990). The less-volatile hydroxycinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid) may act as antioxidants and free radical scavengers. They serve as markers for blood orange juice (Rapisarda et al., 1998) to detect adulterations with the cheaper juice of blond oranges (Figure 10.11).

10.2.8 (*Z*)-Jasmone, Methyl Jasmonates, and Methyl Dihydrojasmonates

(*Z*)-Jasmone is a typical constituent of neroli (bitter orange flower) oils with a floral, jasmine-, tea-, peach-, and hay-like fragrance and was found, together with methyl jasmonate, in bitter orange flower oil by Boelens and Sindreu (1988). Recently, it was also discovered in calamondin juice (*Citrus madurensis*) (Takeuchi et al., 2005) and in the emission of blossoms of yuzu measured by SPME (Ono et al., 2008). The structure of the odoriferous lipid metabolite methyl jasmonate, a constituent of jasmine oil, was elucidated by Demole et al. (1962). Having two asymmetric centers, this compound can occur as two diastereoisomers, methyl jasmonate, and methyl epijasmonate. The latter, exhibiting a powerful lemon- and jasmine-like odor, was identified for the first time in lemon peel by Nishida and Acree (1984) in a classical analytical procedure using column chromatography and microreactions such as ozonolysis and hydrogenation. In one lemon fruit, they

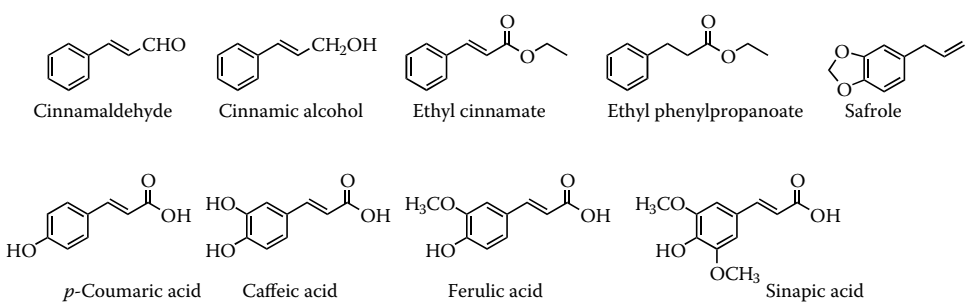


FIGURE 10.11 Phenylpropanoids.

determined the presence of 70 μg of methyl epijasmonate and 5 μg of methyl jasmonate. Del Mar Caja et al. (2008) pretended, in a questionable publication, that the methyl epijasmonate exists as the (+)-enantiomer in lemon peel, referring to already published data without checking the enantiomeric distributions on chiral GC columns. Methyl epijasmonate contributes to the typical flavor of the ki-mikan fruit (*Citrus flaviculpus*) (Katayama and Iwabuchi, 2002). In a remarkable work, Werkhoff et al. (2002) undertook the difficult task of determining the enantiomeric distribution of methyl dihydrojasmonate (MDHJ) in natural raw materials. As this famous fragrance ingredient is ubiquitous in perfumes, detergents, and homecare products, careful blank experiments have to prove the absence of MDHJ in the environment of the analytical investigation. Werkhoff et al. (2002) identified it in 21 food products, among which included the peel oil of Lima orange, a nonacidic, intensely sweet orange popular in Brazil. The amounts were 2.1 mg/kg of the *trans*- and 0.2 mg/kg of the *cis*-epimer. The chiral analysis revealed an enantiomeric distribution of (–)-(1*R*,2*R*):(+)-(1*S*,2*S*) = 35.3 : 64.7 for the *trans*-isomer and (+)-(1*R*,2*S*):(–)-(1*S*,2*R*) = 36.1:63.9 for the *cis*-isomer. According to Werkhoff et al., the (+)-(1*R*,2*S*)-enantiomer exhibits the most powerful odor, with an odor threshold of 15 ppb possessing floral, jasmine, woody, slightly fatty, and β-ionone-like notes, followed by the (–)-(1*R*,2*R*)-enantiomer with an odor threshold of 240 ppb and floral, sweet jasmine-notes. The remaining enantiomers are much weaker (thresholds of 12000 ppb and 15000 ppb), with floral, fatty, tea-, and hay-like descriptors. Hence, the enantiomer identified in Lima orange at the lowest concentration gives the most important organoleptic contribution. Methyl jasmonate is known to be an important plant growth regulator (Del Mar Caja et al., 2008 and references cited therein). (*Z*)-Jasmone and methyl epijasmonate play an important role in plant defense (Farmer, 2000) (Figure 10.12).

10.2.9 Lactones

It cannot be imagined that γ- and δ-lactones of linear fatty acids are absent in fruit flavors such as peach, apricot, or mangoes. However, to our knowledge, they have never been reported in any extract of citrus fruits. Nevertheless, a branched lactone, *trans*-2-methyl-4-decanolide, with a strong lactonic, coconut-like smell, was identified by Naef and Velluz (2005, and references cited therein) in mandarin peel and blood orange juice. Unsaturated macrolactones are a curiosity in natural product chemistry, a famous example is ambrettolide ((*Z*)-7-hexadec-16-enolide) from *Hibiscus abelmoschus* (Kerschbaum, 1927). (3*Z*)-3-Dodecen-12-olide is a pheromone from the flat grain beetle (Millar et al., 1985). Interestingly, (9*Z*)-9-dodecen-12-olide is a constituent isolated by Matsuura et al. (1980) from yuzu, and therefore called yuzu lactone. It exhibits minty, spicy, and camphor-like notes. Recently, it has also been identified as a constituent of the Australian *Microcitrus australasica* var. *sanguinea* (Ruberto et al., 2000) and of Australian finger lime (*Citrus autralasica*) (Delort and

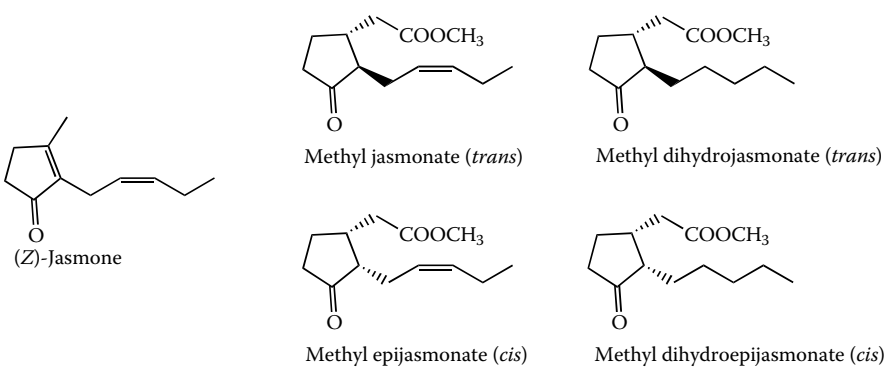
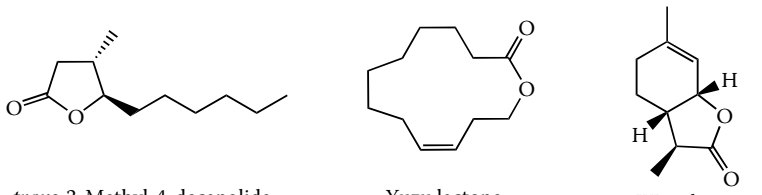
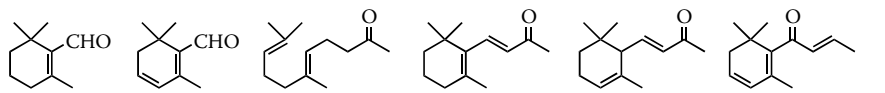


FIGURE 10.12 Methyl jasmonate and related compounds.



trans-3-Methyl-4-decanolide Yuzu lactone Wine lactone

FIGURE 10.13 Lactones.



β-Cyclocitral Safranal Geranylacetone α-Ionone β-Ionone (*E*)-β-Damascenone

FIGURE 10.14 Norisoprenoids C_{10} and C_{13}.

Jaquier, 2009). Wine lactone, (3*S*,3a*S*,7a*R*)-3,6-dimethyl-3a,4,5,7a-tetrahydro-2(*3H*)-benzofuranone, with a *p*-menthane skeleton and first described in white wine by Guth (1997), is one of the most powerful, spicy, woody, and intensely sweet odorants with an odor threshold in air of 0.00001 ng/l and an OAV of 269 in Navel oranges (Buettner and Schieberle, 2001b). This compound gives an important contribution to the clementine flavor (Buettner et al., 2003). Katayama and Iwabuchi (2002) identified the last two lactones in a peel extract of ki-mikan (*Citrus flaviculpus*) (Figure 10.13).

10.2.10 Norisoprenoids C_{10} and C_{13}

Despite the fact that carotenoids like lycopene and α-and β-carotene, cryptoxanthin or lutein are the main coloring pigments in the flavedo and the juice sacs of citrus fruits with the highest concentrations in oranges, mandarins, and pink grapefruits (Fanciullino et al., 2006), their degradation compounds, the norisoprenoids C_{10}, C_{11}, and predominantly C_{13}, otherwise often primordial in essential oils, play only a minor role in citrus oils. β-Cyclocitral, possessing powerful green, floral, and minty tonalities, was found in blond orange juice whereas safranal, with spicy, saffron-like notes, increases the characteristic flavor of blood oranges (Naef and Velluz, 1996). (*E*)-Geranylacetone was identified in the pollen (9.0%), the stamens (1.9%), and the adult leaves (1.9%) of lemon by Flamini et al. (2007). It is mentioned in the flowers (Sakurai et al., 1979) and the juice sacs (Takahashi et al., 2000) of *Citrus unshiu*. (*E*)-β-Damascenone contributes to the fruity and sweet odor quality of mandarin oranges (OAV = 50, Schieberle et al., 2003). Buettner and Schieberle (2001b) listed β-ionone as a constituent of orange juice, but without any positive influence on its flavor. Mahattanatawee et al. (2005) attribute some importance of β-cyclocitral, α- and β-ionone, and β-damascenone to the flavor of orange juice (Figure 10.14).

10.3 CONCLUSION

The structural identification of new compounds present in trace amounts is laborious and time-consuming. Nevertheless, experience demonstrates that compounds that have been isolated from one natural product will be easily recognized in many other extracts, on the condition that the spectral and physical data of the compound have been introduced into the available databanks. A typical example is (6*Z*,8*E*)-6,8,10-undecatrien-3-one. Its identification in galbanum oil was established by Miyazama et al. (2009), who shortly afterward also identified it in a yuzu peel extract. *p*-Menth-1-ene-8-thiol is, at least up to now, an exclusive constituent of citrus extracts, whereas for example, 2,4,7-decatrienals or 4,5-epoxy-2-decenals can be potentially found in any product rich

in unsaturated fatty acids. However, in the environment of citrus constituents such as limonene, citral, terpineols, and fatty acid aldehydes, these compounds effectively contribute to characteristic citrus notes. A thorough study of the literature on the chemistry of the volatile constituents of oils and extracts of various citrus fruits brings to light an astonishing diversity of organic compounds, which, when combined in appropriate concentrations, create the perception of unique, distinctive aromas. Although, as shown by recent work, the exploration of the composition of authentic flavors is unlimited, nature is unwilling to reveal all its secrets. Flavors and fragrances produced by natural sources will always remain unbeatable.

ACKNOWLEDGMENTS

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11 Advanced Analytical Techniques for the Analysis of Citrus Oils

Peter Quinto Tranchida, Paola Dugo, Luigi Mondello, Giovanni Dugo

CONTENTS

11.1 INTRODUCTION

Citrus essential oils are obtained from the fruit peel and are widely used both in the food and perfume industries. From a commercial viewpoint, citrus oils are characterized by considerable economic importance. Unfortunately, the production and introduction of low-quality and/or adulterated oils in the market is a common occurrence. Consequently, the development and availability of high-resolution, rapid, sensitive, and selective analytical methodologies is considered a prime requirement by many citrus essential oil analysts.

From a compositional standpoint, citrus essential oils contain 200-plus compounds (Shaw, 1979), which can be grouped essentially into:

- Volatile fraction, which constitutes 85%–99% of the whole oil and contains the monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters
- Nonvolatile residue, which ranges from 1% to 15% of the whole oil and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, psoralens, and flavonoids

From an analytical instrumental perspective, citrus essential oils are generally considered as matrices of medium complexity. Consequently, traditional single-column chromatography

techniques can often, but not always, provide acceptable results; it is obvious that the satisfactory outcome of any analysis is strictly dependent on the initial analytical objectives. For example, if a routine characterization of the volatile fraction is required, then conventional gas chromatography (GC) with a variety of stationary phases is certainly a prime choice (Dugo G. et al., 1994a). However, if the entire elucidation of the volatile or nonvolatile profile is desired, then a comprehensive 2D GC (Mondello et al., 2005) or 2D liquid chromatography (LC) (Dugo, P. et al., 2004) approach would be the best choice. If a very fast separation of the volatile fraction is needed, then a conventional GC column would be of little help, whereas a microbore capillary would be much more suitable (Mondello et al., 2004a,b).

The present chapter describes the use of advanced mono- and bi-dimensional GC and LC methods, as well as mass spectrometry technologies, in various citrus essential oil applicational fields. Emphasis will be placed on three very powerful multidimensional chromatography methods, namely comprehensive two-dimensional gas chromatography (GC × GC), liquid chromatography (LC × LC), and liquid–gas chromatography (LC × GC). Comprehensive chromatography techniques have had a revolutionary effect in the field of separation science and will probably conquer the chromatography scene in the next decades.

11.2 MULTIDIMENSIONAL GAS CHROMATOGRAPHY: HEART-CUTTING AND COMPREHENSIVE TECHNIQUES

Single-column GC is the most commonly applied method for the analysis of citrus essential oil volatile constituents (Dugo G. et al., 1994a). If an open-tubular capillary column is operated under ideal conditions for a given analysis, then the two aspects that govern a GC separation are (a) stationary-phase selectivity and (b) peak capacity (n_c). The first feature is related to the stationary-phase chemical composition, while the second is dependent on the column characteristics, *viz*., length, internal diameter, stationary-phase thickness, and intensity of volatile-stationary phase interactions. Ideally, a chromatographic analysis for a given citrus sample will be achieved in the minimum time if a column is characterized by the minimum required n_c and by the most appropriate stationary phase.

The number of peaks that can be positioned side by side (with a specific R_S value), along the retention time axis generated by a capillary column method, is usually in the 200–600 range. Unfortunately, it has been demonstrated from a theoretical viewpoint that "no more than 37% of the peak capacity can be used to generate peak resolution," and that "many of the peaks observed under these circumstances represent the grouping of two or more close-lying components.", to conclude that "s (the number of single component peaks) can never exceed 18% of n_c" (Giddings, 1990). For example, if a GC method is characterized by an n_c value of 400, then ca. 70 compounds can be fully isolated, which is far less than the average number of volatiles contained in a citrus essential oil. Although the latter calculation is an approximate one, and does not consider stationary-phase selectivity, it provides a good indication of the separation power of a GC capillary column.

As mentioned above, the complete separation of the compounds of interest with the least time cost must always be the foremost objective in any citrus essential oil application. However, unsatisfactory GC resolution is a common occurrence, and, on the basis of the previous considerations, can be related essentially to the following issues: (1) the lack of column selectivity, (2) the lack of column peak capacity, (3) the combination of factors 1 and 2, (4) nonideal GC conditions, and (5) excessive sample complexity. Such events can lead to difficulties in the qualitative and quantitative analysis of target essential oil analytes. It must be added that, in several instances, co-elution is altogether acceptable if selective detection or a mass spectrometric separative dimension are available.

In the field of separation science, analysts tend to fall in one of two well-defined groups:

1. Mass spectrometry (MS) experts who do not pay excessive attention to a poor chromatographic separation because the mass analyzer can achieve ion separation on an *m/z* basis. Mass spectrometry has the potential to separate and reliably identify compounds that overlap at the GC outlet. It is clear that, in the case of a excessive peak crowding, identification can become both difficult and unreliable.
2. Chromatography specialists who tend to have great faith in their capacity to generate an ideal separation, and hence in the deliverance of pure analytes to the ion source. However, chromatography experts can encounter problems whenever peak overlapping arises, and a deeper knowledge of the MS potential is necessary.

In truth, both separation processes are complementary, equally important, and should be pushed to their full capacity.

11.2.1 Heart-Cutting Two-Dimensional Gas Chromatography

Whenever unsatisfactory resolution occurs in a given citrus essential oil analysis, the most effective way of enhancing the selectivity and resolving power of a GC system (considering equivalent detection conditions) is by using multidimensional gas chromatography (MDGC). MDGC approaches can be divided into heart-cutting and comprehensive methodologies. The former technique (also called classical MDGC) enables the transfer of selected chromatographic bands, from a primary to a secondary capillary, connected to one another by using either a switching valve or a Deans switch. Preliminary monodimensional experiments are necessary to select the primary (defined as precolumn) chromatography bands, which require analysis on the second dimension (defined as analytical column) (Bertsch, 1999). The main advantage of classical MDGC is that the secondary separation is usually carried out on a full-length conventional column (i.e., 30 m × 0.25 mm ID × 0.25 μm d_f). It must be emphasized that the number of heart cuts subjected to a second separation is restricted because excessive transfers could lead to the overlapping of target analytes with other interfering compounds. The peak capacity of an MDGC method equals the sum of that of the first and second column, the latter multiplied by the number (x) of transfers [$n_{c1} + (n_{c2} \times x)$]: for example, if both capillaries are characterized by an n_c value of 400, and three heart cuts are carried out, then a final n_c value of 1600 is attained.

Classical MDGC has been employed since the mid-1950s (Simmons and Snyder, 1958; Deans, 1968; Schomburg et al., 1973; Deans, 1981; Schomburg et al., 1982), using either two packed columns, a packed and a capillary one, or two capillaries. In the primordial years, MDGC systems were invariably characterized by a rotary switching valve, the installation and operation of which were rather easy. However, dead volumes and adsorption effects, as well as low maximum operational temperatures, gas leaks, and flow path plugging, were the substantial disadvantages of these systems.

In 1968, Deans reported a valveless transfer system with no moving parts in either the gas flow path or the high-temperature zones. The Deans switch technique functioned on the basis of a pressure balance mechanism between the two columns (Deans, 1968, 1981; Deans and Scott, 1973), and was made possible by in-line restrictors and the use of additional make-up gas. Further improvement on the initial Deans system was achieved by Schomburg et al. (1982), who introduced the "live" switching system: two columns were inserted over a thin platinum capillary, which was the central component of the coupling piece, while supplementary gas was provided through two control lines and adjusted with needle valves.

A decade after the introduction of the Deans switching technique, a commercial instrument enabling solvent flushing, heart cutting, and backflushing was developed by Siemens. Many papers, reporting the use of this instrument for the analysis of citrus essential oils, can be found in the liter-

ature (Casabianca et al., 1995; Mosandl, 1995; Juchelka et al., 1996; Juchelka and Mosandl, 1996; Mosandl and Juchelka, 1997).

Substantial technological advances in valve design were accomplished in the 1980s with the introduction of microvolume connections, use of thermally stable elasteromeric material, and elimination of unswept volumes. Satisfactory results were attained on real-world samples, with no apparent problems related to valve activity observed (Jennings, 1984).

Classical MDGC applications in the citrus essential oil field mainly refer to the determination of the enantiomeric distribution of a series of specific constituents. Enantioselective GC, using derivatized cyclodextrin stationary phases, is frequently employed for the determination of the enantiomeric ratio of volatile chiral compounds. However, the complexity of citrus oils often exceeds the peak capacity of a single chiral column and a preseparation step is necessary. In particular, enantioselective MDGC, with the combination of a nonchiral precolumn and a chiral analytical column, has been demonstrated to be an effective method for the analysis of chiral volatiles without any further clean-up or derivatization procedures. In 1998, a twin-oven MDGC system, equipped with a high-temperature valve and a system to maintain constant flow conditions during heart cutting, was used for the analysis of lemon oil enantiomers (Mondello et al., 1998a). The MDGC system and subsequent versions (Mondello et al., 1998b,c,d,e, 1999) enabled both multiple heart-cut operations and the independent employment of the two GCs when the MDGC option was not exploited (Figure 11.1). The pneumatic and electronic circuits allowed the maintenance of constant first-dimension retention times, even for those components that eluted after several transfers. The system has been used to determine the enantiomeric distribution of β-pinene, sabinene, limonene, linalol, terpinen-4-ol, and α-terpineol in various citrus essential oils. The analysis of linalyl acetate, in bergamot essential oils, has also been carried out (Mondello et al., 1997; 1998a,b,c,d,e, 1999; Dugo G. et al., 2001).

The analytical potential of the previously illustrated MDGC system has been boosted by using a mass spectrometric detector (MDGC-MS) (Mondello et al., 1998b). The MDGC analysis of a bergamot oil, using an apolar precolumn (SE-52) and a polar analytical column (Carbowax 20M), is illustrated in Figure 11.2. The chromatographic band, which was injected onto the Carbowax column, is shown (~23 min) in the same figure, as well as the correspondent TIC MDGC-MS chromatogram. As can be seen, the chromatographic band contained seven compounds, four of which were identified. The MDGC-MS method described was characterized by three separation dimensions: the primary and secondary capillaries achieved separation on a boiling point and polarity basis, respectively; the third MS dimension discriminated between different *m/z* values.

Since the introduction of relatively cheap, benchtop GC-MS instruments and the later comprehensive two-dimensional gas chromatography, heart-cutting MDGC has suffered a gradual decline over the past 8 to 10 years. At present, however, this powerful technique has been recently gaining popularity in both academia and industrial areas, due to the development of highly effective MDGC systems equipped with accurate electronic pressure control units and automatically controlled by user-friendly software. Schemes of an MDGC transfer system (in the "stand-by" and "cut" modes), based on the Deans switching principle and used in an ultimate generation, twin-oven (defined as GC1 and GC2) MDGC-MS instrument, are shown in Figure 11.3. In both configurations of use, an electronic pressure control unit (Aux) supplies gas at constant pressure (defined as P_{Aux}) to an external (outside GC1) fused-silica restrictor (defined as R_3) and to a three-way electrovalve (EV). EV is linked to two metal branches, one with another fused-silica restrictor (defined as R_2) and the other without: the dimensions of R_2 are such that a specific pressure drop is generated (ΔP_2) that is slightly higher than that produced by R_3 ($\Delta P_2 > \Delta P_3$). The primary and secondary capillaries are connected by using a low dead volume, inert, and thermally stable stainless steel transfer device; the latter is located in GC1, is also connected to the 1D (R_3 line) and 2D (EV line) metallic branches, and to Det1 (in this case a flame ionization detector). The second column is located in GC2, which is connected to GC1 through a heated transfer line. In the stand-by

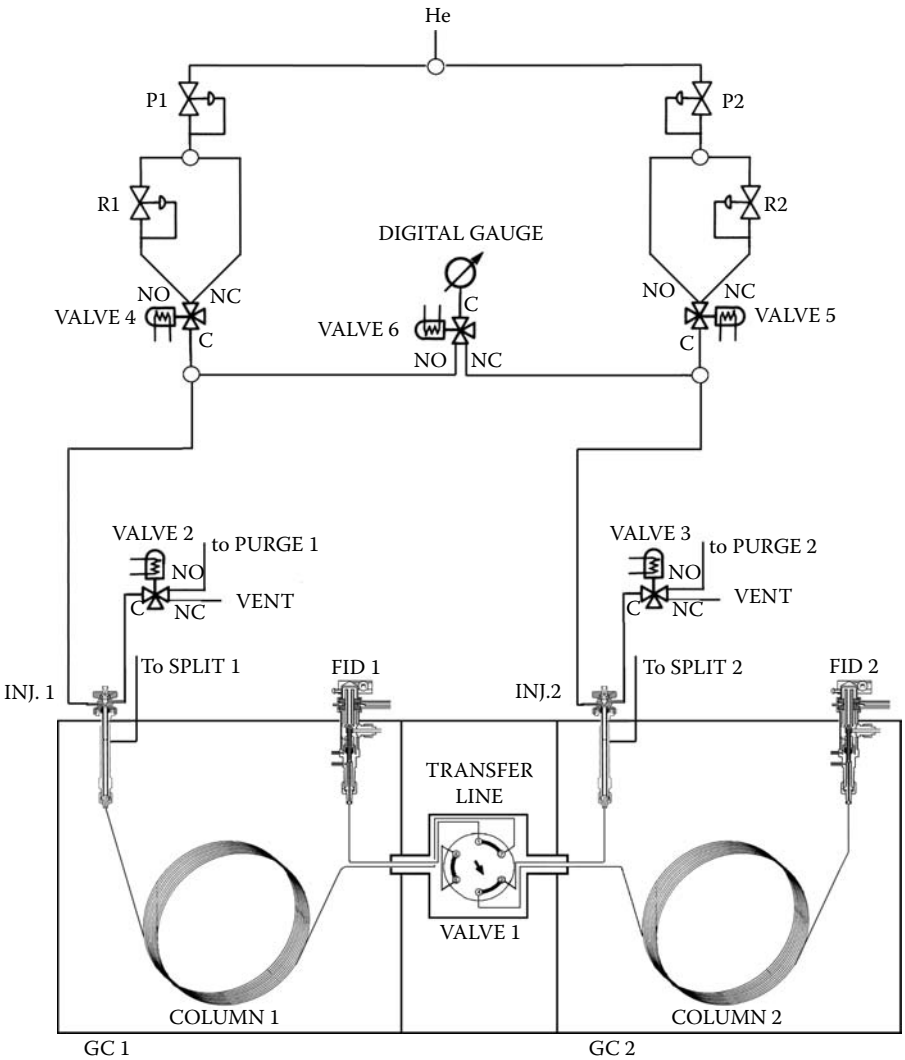

FIGURE 11.1 Pneumatic and electronic scheme of the MDGC system, in the stand-by configuration. (From Mondello, L., et al., *J. High Resolut. Chromatogr.* 22, 350–356, 1999. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

mode (Figure 11.3A), the Aux pressure reaches the second dimension unaltered and is reduced on the first dimension side ($P - \Delta P_3$) after passing through R_3. It is obvious that, under such pressure conditions, the solutes leaving the primary column are directed to the FID. In the heart-cut mode (Figure 11.3B), the pressure at the first-dimension branch does not alter while it becomes $P - \Delta P_2$ at the second dimension branch; under such pressure conditions ($P - \Delta P_3 > P - \Delta P_2$), the primary column flow is directed onto the secondary column. The instrument is automatically controlled using a dedicated software, which also enables the calculation of fundamental operational parameters such as linear velocities, gas flows, and analyte recovery. Recently, the system was used by Sciarrone et al. (2010) in a comparative system with enantio-GC in the analysis of mandarin essential oil (see Chapter 7).

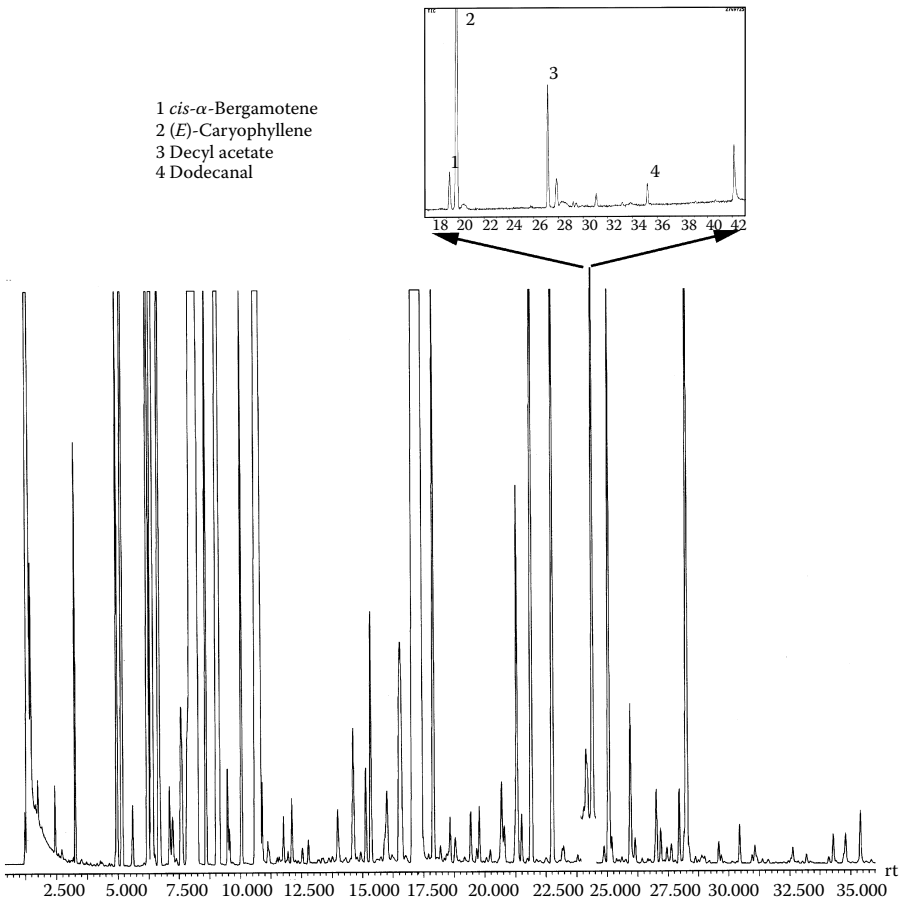

FIGURE 11.2 Stand-by MDGC–FID chromatogram relative to a bergamot essential oil sample, and carried out on an SE-52 column; the single heart cut has been moved from its original position to make it more visible. Upper chromatogram expansion: TIC GC-GC-MS analysis of the single heart cut, carried out on a polar column.

11.2.2 Comprehensive Two-Dimensional Gas Chromatography

The invention of comprehensive 2D GC in 1991 can be certainly considered as a milestone in the GC field (Liu and Phillips, 1991). The resolution power enhancement, observed when passing from GC to GC × GC, is probable greater than that between the packed and capillary column. A typical GC × GC system consists of two columns connected in series and with a different selectivity. A transfer device, defined as modulator, is the most important part of the set up, and is (usually) situated at the head of the secondary column. The modulator functions in a continuous manner throughout the GC × GC application, enabling the isolation, reconcentration, and injection (a process defined as modulation) of chromatographic bands from a primary conventional column (e.g., 30 m × 0.25 mm ID × 0.25 μm d_f) onto a short microbore capillary segment (e.g., 1–2 m × 0.10 mm ID × 0.10 μm d_f). The time necessary to achieve the modulation process is defined as the modulation period, and corresponds to the time window of each analysis in the second dimension (typically 4–8 seconds). Normally, an apolar column is used as primary column, enabling the modulation of heart cuts containing isovolatile constituents. The latter are subjected to a high-speed 2D analysis, generally on a medium or highly polar capillary: isovolatile solutes are isolated in relation to the degree of functional group polarity-based interactions. Such a column combination,

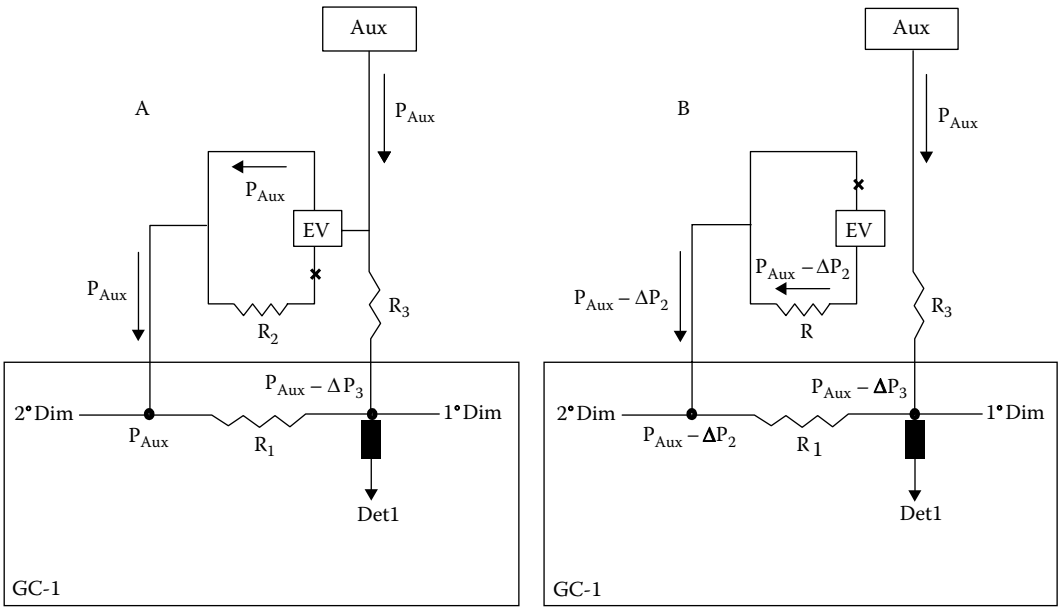

FIGURE 11.3 Schemes of a Deans switching MDGC interface in the stand-by (A) and cutting (B) modes (see text for abbreviations).

defined as orthogonal, guarantees slow and fast peak production in the first and second dimension, respectively. It is obvious that a GC × GC separation will be all the more effective in relation to the degree of difference between the two separation mechanisms. The possible overlapping of two differently structured molecules is statistically improbable, as this would require equal elution times on two columns characterized by distinct stationary phases. In an ideal GC × GC experiment, the total peak capacity is equivalent to the product of the peak capacities ($n_{c1} \times n_{c2}$) relative to each dimension. Although such a value certainly exceeds the "realistic" peak capacity, comprehensive 2D GC is certainly the most powerful tool today available for the analysis of complex volatile samples.

Although GC × GC experiments are currently achieved through a variety of modulation systems, the principles of the process have remained essentially unaltered. In an ideal GC × GC analysis, each peak eluting from the first dimension is subjected to several modulations (at least three) in order to avoid loss of resolution achieved on the primary column. An example of how the (dual-stage) thermal modulation process was initially carried out is shown in Figure 11.4. In phase 1, a narrow chromatography band—in this case containing two hypothetical overlapping compounds—is formed at the head of the modulator; the latter is maintained at a sufficiently low temperature to generate this primary reconcentration effect. In phase 2, a ms duration heating pulse (ΔI) is directed to the first segment of the modulator, causing band remobilization. In phase 3, the released band hits a second cold modulator spot and is again compressed; at the same time, analytes begin to accumulate at the modulator head, which has rapidly cooled down. In phase 4, a ms duration heating pulse (ΔII) is directed to the second segment of the modulator, and the narrow band is injected into the second dimension. Thermal modulation has a beneficial effect on sensitivity: band compression produces a signal-to-noise increase in the 10–50 factor range, depending on the operational conditions and the modulator type. Band compression also generates very narrow peaks, both in space and time, hence fast detectors (a sampling frequency of minimum 50 Hz is necessary) are mandatory. In phase 5, the two compounds are subjected to a rapid GC separation and (ideally) reach the detector at different times. During the fast 2D analysis, modulation is carried out on the subsequent fraction. The two analytes resolved on the second column are characterized by the same first-dimension t_R (expressed

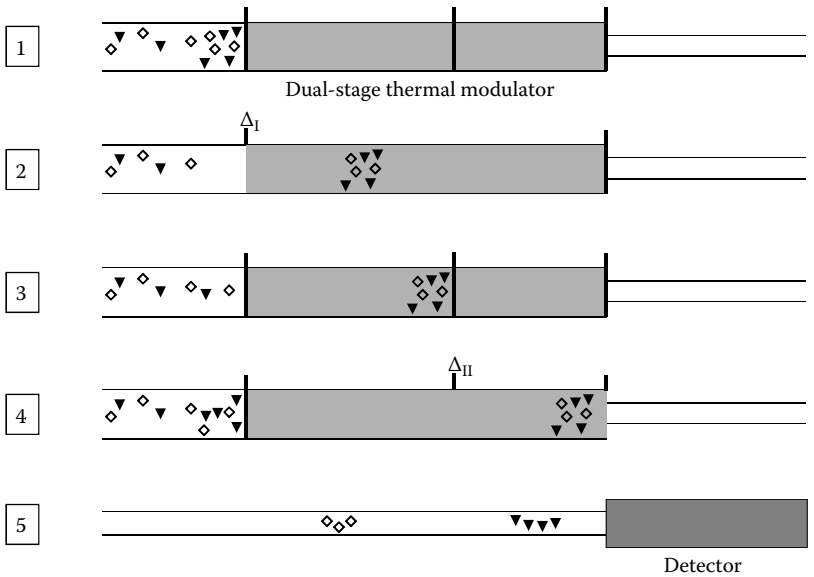

FIGURE 11.4 Schematic illustration of the GC × GC modulation process. The symbols represent two different analytes.

in minutes) and different second dimension t_R (expressed in seconds). An important requisite is that all compounds reach the detector before the next 2D injection and, thus, within the modulation time window. If second-dimension retention times exceed the modulation period, then a phenomenon called "wrap around" occurs.

If a 3000 second GC × GC experiment is considered, with a 4-second modulation period, then 750 sequential 4-second 2D chromatograms will form a "raw" (monodimensional) GC × GC chromatogram. Dedicated software is mandatory for visualizing the raw chromatogram in a bi-dimensional format (contour plot): the single fast GC chromatograms, positioned at a 90° angle to an *x*-axis, are characterized by first-dimension t_R that are usually expressed in minutes. The compounds separated in the second dimension, aligned along a *y*-axis, are characterized by an oval form and with t_R values that are expressed in seconds. The color and dimension of each blob is directly related to the third unvisualized axis (*z*), namely detector response. Dedicated software is also mandatory for GC × GC quantitation: the peak areas relative to the same compound in each fast 2D chromatogram are summed. No differences should be observed between quantitative results for a single component in modulated and nonmodulated experiments.

The advantages of comprehensive 2D GC over conventional GC are selectivity (two separation dimensions, related to volatility and polarity), sensitivity (band compression), separation power, structure (formation of group-type patterns on the 2D plane), and speed (comparable to ultrafast GC experiments, if the number of peaks resolved per unit of time is considered). If a third MS dimension is added to a GC × GC instrument, then the most powerful tool presently available for volatile analysis is generated (GC × GC-MS).

An interesting cryogenic modulation GC × GC-FID experiment, carried out on a lemon essential oil, was described in 2005 (Mondello et al.). Initially, a GC-MS application was performed on the lemon oil sample using a conventional polar column. Compound identification was achieved by combining MS spectral and retention data (linear retention indices). It was observed that considerable interferences occurred between the sesquiterpene hydrocarbons and the oxygenated monoterpenes. It must be emphasized that while the monoterpene hydrocarbon fraction is similar in all citrus oils, the sesquiterpene hydrocarbon and the oxygenated monoterpene fractions are characteristic. The GC × GC separation was carried out on a 1D polar and 2D apolar column set and achieved the thorough

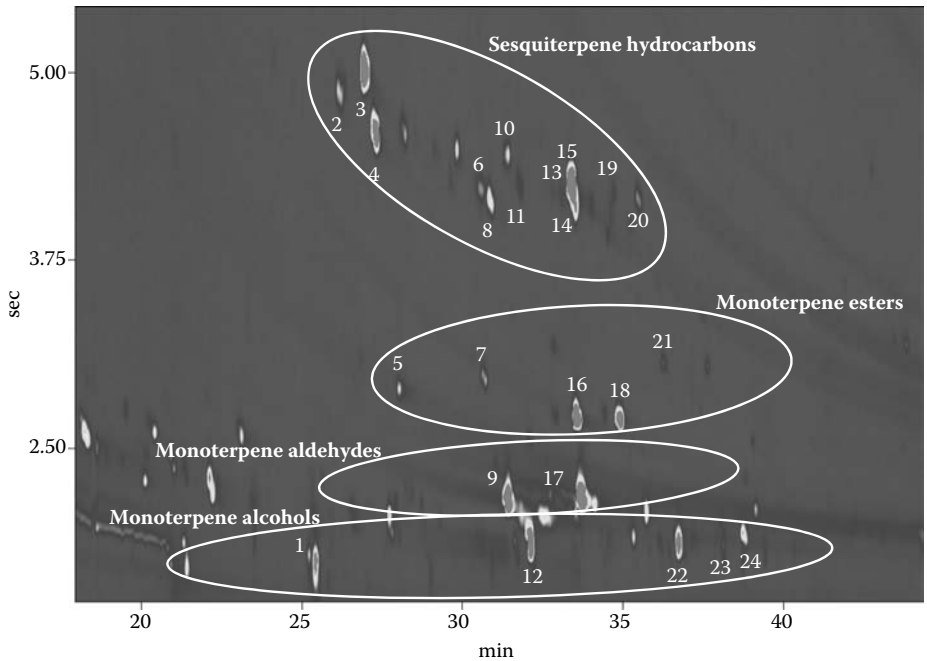

FIGURE 11.5 (**See color insert following page 462.**) 2D expansion relative to the GC × GC-FID analysis on lemon essential oil. Peak identification: 1. linalol; 2. *cis*-α-bergamotene; 3. (*E*)-caryophyllene; 4. *trans*-α-bergamotene; 5. linalyl acetate; 6. (*Z*)-β-santalene; 7. citronellyl acetate; 8. α-humulene; 9. neral; 10. (*Z*)-β-farnesene; 11. germacrene D; 12. α-terpineol; 13. valercene; 14. bicyclogermacrene; 15. β-bisabolene; 16. neryl acetate; 17. geranial; 18. geranyl acetate; 19. *cis*-α-bisabolene; 20. *trans*-α-bisabolene; 21. citronellyl formiate; 22. nerol; 23. *p*-cymen-8-ol; 24. geraniol. (From Mondello, L., et al., *Flav. Frag. J.* 20, 136–140, 2005. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

resolution of the sample constituents, which were identified by using pure standard compounds. The reversed column combination proved to be the most suited, since the use of a polar second dimension was the cause of excessive retention toward the more polar components. A chromatogram expansion, relative to the sesquiterpene hydrocarbon and the oxygenated monoterpene zone, is illustrated in Figure 11.5. Observing the 2D chromatogram, it is clear that a series of first dimension overlapping compounds are separated on the second fast apolar capillary (1 m × 0.10 mm ID × 0.10 μm d_f): peaks 15, 16, and 17 (β-bisabolene, neryl acetate, geranial), peaks 9 and 10 [neral, (*Z*)-β-farnesene], and peaks 6, 7, and 8 (β-santalene, citronellyl acetate, α-humulene). The 2D space plane is also characterized by a certain degree of structure, as can be seen in the circled areas in Figure 11.5—in fact, monoterpene oxygenated and sesquiterpene hydrocarbon compounds all fall within specific 2D zones. While the distribution of sesquiterpenes inside their 2D zone appears to be random, the positions of the monoterpene oxygenated analytes with the same functional group are certainly more related. The alcohols 1, 12, 22, 23, 24; the aldehydes 9 and 17; and the esters 5, 7, 16, 18, 21 tend to align themselves in distinct bands, even if these components have different hydrocarbon skeletons.

A GC × enantio-GC-MS experiment, developed for the analysis of bergamot oil, has been described by Shellie and Marriott (2002). Of high interest was GC × GC method optimization, namely the proper combination of column dimensions in order to exploit the vacuum outlet conditions. A microbore primary apolar column (10 m × 0.1 mm ID × 0.1 μm d_f) and a secondary 0.25 mm ID chiral column (1 m × 0.25 μm d_f) were employed. The primary microbore column was exploited for solute separation and as a restrictor, while the secondary wider-bore column was suited

for reduced-pressure rapid chiral separations. By using a short, 0.25 mm ID column, subatmospheric conditions extended across the entire capillary length, and an increase in the optimum linear velocity was attained. A GC × enantio-GC-MS experiment was carried out on a bergamot essential oil and, although wrap around was rather evident, some very nice second-dimension enantiomer separations were shown.

11.3 COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY

In the GC field, it is well known that retention is more or less dependent on analyte boiling points, hence there is always a certain degree of correlation between the two dimensions in a GC × GC system. On the contrary, HPLC methodologies are characterized by a series of separation mechanisms with entirely independent selectivities. Consequently, there is a high number of theoretical MDLC combinations with no or little correlation. Unfortunately, the hyphenation of a series of HPLC techniques can be a rather complicated issue, if not an impossible one. In fact, problems related to the immiscibility of solvents, 1D liquid phase-2D stationary-phase incompatibility, or the precipitation of buffer salts can arise (Cortes and Rothman, 1990). Off-line MDLC approaches are often used for the preseparation of complex samples, and are characterized by a series of disadvantages that derive from sample manipulation, such as the loss of sample through evaporation or even spilling, as well as contamination.

Classical online MDLC is achieved by using a high pressure switching valve, which connects the first and second dimensions. The valve is usually equipped with a loop that accumulates a specific volume of primary column effluent, and re-injects it into a secondary column. Using heart-cutting online techniques, the aforementioned off-line disadvantages are avoided, since manipulation of the liquid sample is no longer required. However, such methods do not achieve a bi-dimensional separation on the entire initial sample.

Comprehensive 2D LC was introduced more than a decade before GC × GC, by Erni and Frei (1978), followed by Bushey and Jorgenson (1990). The LC × LC systems, up until now developed, can be basically divided in the following two groups: (1) a switching valve is connected to a primary conventional column and to two parallel rapid secondary columns; (2) a switching valve, equipped with two sample loops, is linked to a primary microbore LC column and to a secondary fast column.

Considering the second option, the employment of a microbore column is a good analytical solution for various reasons. First, the small column ID avoids excessive dilution, generating flow rates that are compatible with second-dimension sample volumes. Furthermore, a preconcentration step at the head of the secondary column is not necessary, while incompatibility between solvents can be circumvented by using different separation modes. On the other hand, the limited sample capacity is to be considered as a slight drawback.

The first combination of normal-phase (NP) and reversed-phase (RP) modes, in an LC × LC system, was applied to the analysis of the oxygen heterocyclic components (coumarins and psoralens) of a lemon essential oil (Dugo, P. et al., 2004). A microbore silica column was used in the first dimension (isocratic elution: 75% *n*-hexane/25% acetonitrile), while a 2.5 cm monolithic-type column was employed in the second dimension (gradient elution). The columns were connected to a ten-port valve equipped with two 20 μL sample loops that were alternately filled with first-dimension 60-second chromatography bands. While loop 1 was in the filling position, an LC pump flushed the contents of loop 2 onto the second dimension. At the end of the modulation period (in this case 60 seconds), the switching interface inverted the position of the two loops (loop 1: re-injection stage; loop 2: filling stage). In this way, all the effluent from the first column was subjected to a bi-dimensional separation. It is noteworthy that no problems were encountered in the transfer of small volumes of immiscible 1D effluent onto the monolithic column (an aqueous mobile phase was used). Furthermore, the 1D mobile phase composition was stronger than that at the head of the monolithic column (50% acetonitrile), generating a band compression effect during each second-dimension

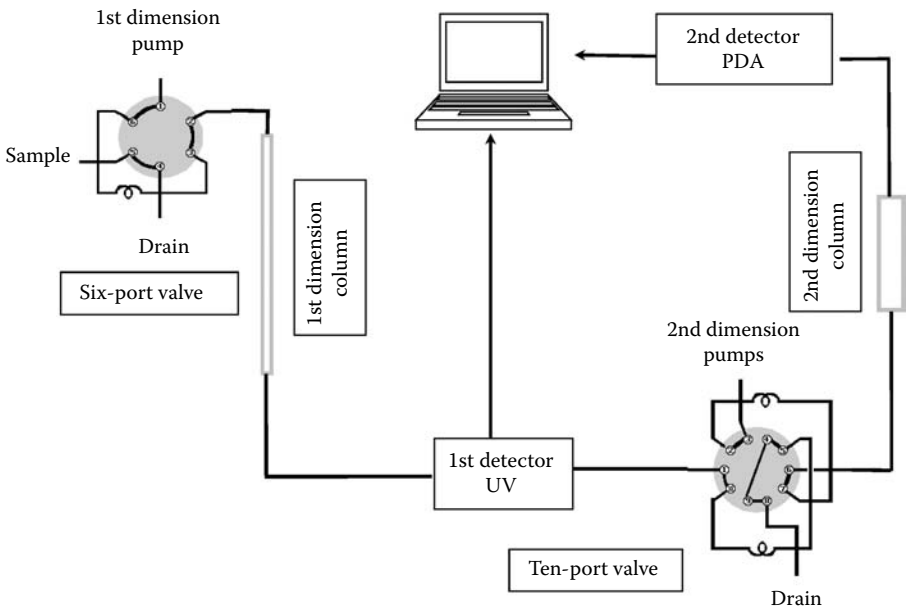

FIGURE 11.6 Schematic of the LC × LC instrument used for the analysis of lemon essential oil. (Reprinted with permission from Dugo, P., et al., *Anal. Chem*. 76, 9, 2525–2530, 2004. Copyright 2004 American Chemical Society.)

injection. A gradient, applied every 60 seconds, was then necessary to elute the 1D heart cut within 48 seconds (plus 12 seconds equilibration time). The use of a monolithic column enabled both high flow rates (with no loss in resolving power) and fast equilibration times. The LC × LC instrumentation employed is shown in Figure 11.6, while the bi-dimensional chromatogram relative to the oxygen heterocyclic fraction of the lemon oil sample is illustrated in Figure 11.7. Peak identification (two coumarins and eight psoralens) was achieved through the comparison of retention times in each dimension and on the 2D space plane with those of standard compounds (when available), and UV spectra generated by using a photo diode array detector.

On the basis of the elution order in the two dimensions, a series of considerations can be made: under NP conditions, psoralens elute before the corresponding coumarins. This can be seen by observing the elution order of bergamottin (peak 2) and 5-geranyloxy-7-methoxycoumarin (peak 5). Furthermore, psoralens substituted in position 5 elute before those substituted in position 8 (bergamottin [peak 2] elutes before 8-geranyloxypsoralen [peak 8]). In accordance with the NP separation mechanism, isomers are well resolved, as can be observed for bergamottin and 8-geranyloxypsoralen. On the contrary, homologues are not very well separated because hydrocarbon substituents contribute little to analyte retention. In fact, bergamottin (peak 2) and isoimperatorin (peak 3), which differ in the alkyl chain length, elute very closely in the first dimension. As expected, compounds of different polarity are easily resolved under NP conditions. For example, isoimperatorin (peak 3) and oxypeucedanin (peak 10), which differ for the presence of an epoxy substituent in the side chain, are well resolved. The NP separation mechanism was useful in the full resolution of a series of analytes that would have co-eluted under RP conditions (e.g., peaks 6, 10, and 11).

Under RP conditions, a combination of polarity and hydrophobicity dominates retention. Components with a long geranyloxy side chain are highly retained in the second dimension (peaks 2, 4, 5, and 8), while those with a shorter alkyl chain are less retained (peaks 3, 7, and 9), with the two groups located in distinct 2D zones. The position of the geranyloxy side chain discriminates less than in NP LC, and components such as bergamottin and 8-geranyloxypsoralen elute very

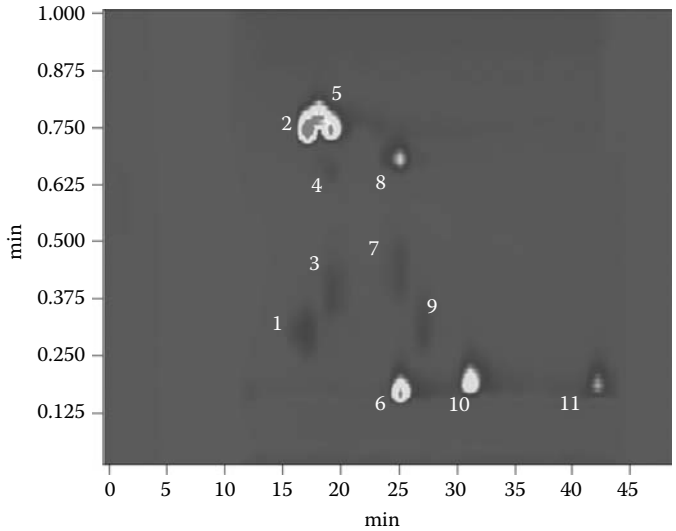

FIGURE 11.7 Comprehensive 2D normal-phase–reversed-phase LC separation of the oxygen heterocyclic fraction of a lemon oil sample. Peak identification: 1. unknown; 2. 5-geranyloxypsoralen (bergamottin); 3. 5-isopentenyloxypsoralen (isoimperatorin); 4. 5-geranyloxy-8-methoxypsoralen; 5. 5-geranyloxy-7-methoxycoumarin; 6. 5,7-dimethoxycoumarin (citropten); 7. 5-methoxy-8-isopentenyloxypsoralen (phellopterin); 8. 8-geranyloxypsoralen; 9. 5-isopentenyloxy-8-epoxyisopentyloxypsoralen; 10. 5-epoxyisopentyloxypsoralen (oxypeucedanin); 11. 5-methoxy-8-(2,3-epoxyisopentyloxy)psoralen. (Reprinted with permission from Dugo, P., et al., *Anal. Chem.* 76, 9, 2525–2530, 2004. Copyright 2004 American Chemical Society.)

closely. The RP separation mechanism was useful in the full resolution of a series of analytes that would have co-eluted under NP conditions (peaks 3, 4, and 5).

Recently, dual-gradient NP-LC × RP-LC has been applied to the analysis of carotenoids in orange essential oil, a particularly challenging application (Dugo, P. et al., 2006). In fact, the carotenoid profile in citrus essential oils is a rather complex one, due to the structural diversity and extreme instability of these constituents. In the LC × LC experiment, the carotenoids formed group-type patterns on the 2D space plane: the hydrocarbons (carotenes) were well separated from the oxygenated counterparts, namely xanthophylls. The latter were subdivided into mono-, di- and triols, and their corresponding epoxides. Most of the carotenoids were identified through retention behavior and UV–Vis spectra because very few carotenoid standards are commercially available.

In 2008, an interesting and rather complex LC × LC system, characterized by two two-position ten-port switching valves and second-dimension parallel columns, was developed and applied to the analysis of a lemon oil extract (François et al., 2008). A scheme of the system (defined as LC × 2LC) is shown in Figure 11.8: valve 1 (V1), characterized by two loops (30 μL volume each), is altogether similar to the valve system described by Dugo, P. et al. (2004), and is exploited to accumulate 1-minute first dimension (NP) fractions (flow rate: 30 μL/min). When V1 is in position 1 (Figure 11.8A), loop 1 begins to collect a 30-μL fraction, while the content of loop 2 is flushed onto the second column (RP column 2) by using RP pump 2 (the gradient starts), which is connected to valve 2 (V2). After a specific time period (defined as t_x), namely the time necessary to inject the loop 2 content onto the secondary column, V2 is switched and the analysis is continued on RP column 2 through a valve shortcut (Figure 11.8B). After 1 minute, V1 is switched and the content of loop 1 is injected onto the second column (RP column 1) using RP pump 1, which is connected to V2 (Figure 11.8C). After t_x, V2 is switched and the analysis is continued on RP column 1 through a valve shortcut (Figure 11.8D). During the second minute, loop 2 is filled with first-dimension effluent, and both RP columns are

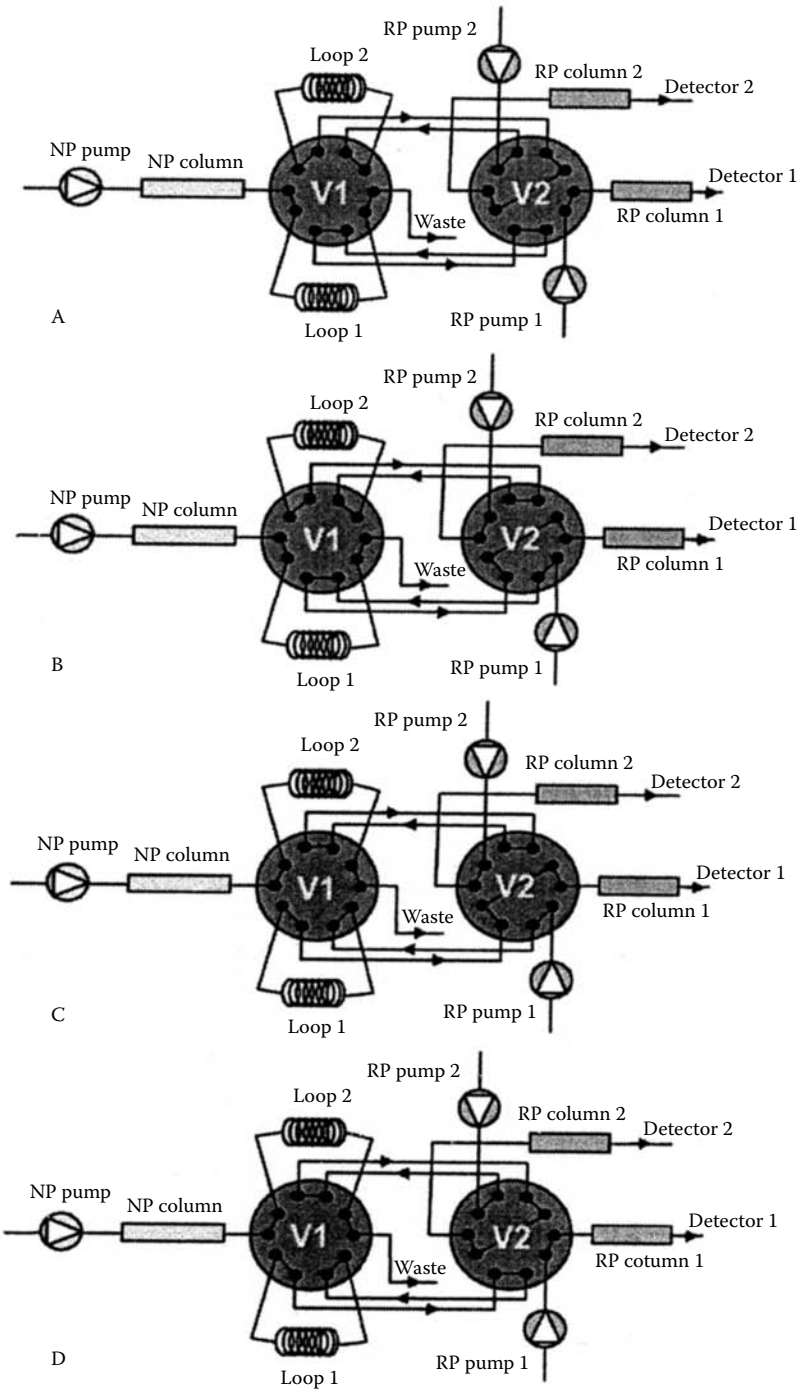

FIGURE 11.8 A scheme of the LC × 2LC system. (A) V1: position 1, V2: position 1. (B) V1: position 1, V2: position 2. (C) V1: position 2, V2: position 2. (D) V1: position 2, V2: position 1. (Reprinted from Francois, I., et al., *J. Chromatogr. A*. 1178, 33–42, 2008. Copyright 2008 with permission from Elsevier.)

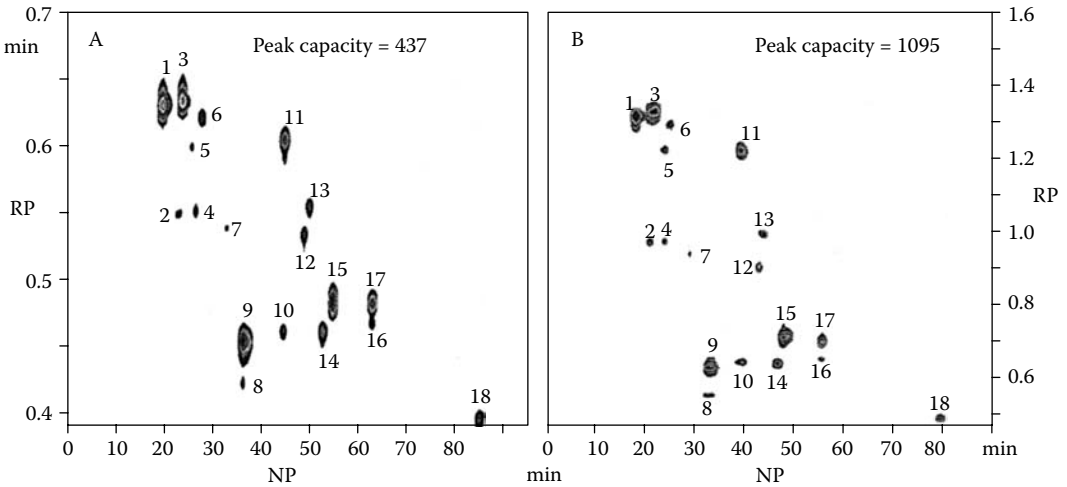

FIGURE 11.9 (A) Single-valve LC × LC and (B) LC × 2LC analyses of lemon oil extract. For peak identification refer to François et al. (2008). (Reprinted from Francois, I., et al., *J. Chromatogr. A*. 1178, 33–42, 2008. Copyright 2008 with permission from Elsevier.)

engaged in the analysis of previous fractions. After 2 minutes, the RP analysis and equilibration of RP column 2 is ultimated, and the cycle starts again: V1 is switched to position 1 (Figure 11.8A), loop 1 begins to collect its fraction, while the content of loop 2 is injected into RP column 2. The main advantage of the LC × 2LC system proposed is that the second-dimension analysis time, and thus the gradient time, can be doubled, enabling a significant peak capacity gain.

The performance of the LC × 2LC instrument was compared to that of a single-valve LC × LC system in the analysis of a lemon oil extract. The bi-dimensional chromatograms, illustrated in Figure 11.9A and B, demonstrate the improved resolving power of the LC × 2LC approach: second-dimension peak capacities of 15 and 40 and overall peak capacities of 437 and 1095 were calculated for the single-valve (Figure 11.9A) and twin-valve (Figure 11.9B) systems, respectively.

11.4 MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY: HEART-CUTTING AND COMPREHENSIVE TECHNIQUES

The complete separation of complex mixtures, through single-column GC, is often hindered by the fact that sample components belong to numerous chemical families and are present in a wide range of amounts. In many situations, it is much more convenient to isolate more simple and homogenous mixtures prior to a GC separation.

The exploitation of an off-line LC preseparation step prior to a GC analysis is very useful because LC enables the isolation of chemical classes or subclasses of compounds. Hence, simplified mixtures of chemically similar compounds are subjected to a conventional GC analysis. The off-line combination of LC and GC generates a bi-dimensional method that exploits the selectivity of the first dimension and the high resolving power of the second one. Notwithstanding the usefulness of off-line LC–GC, as previously seen a high degree of manual intervention, is certainly a disadvantage.

High-performance liquid chromatography, coupled to high-resolution GC, in an online mode, is a very powerful analytical technique because of its enhanced selectivity and sensitivity. Online LC–GC multidimensional methods are particularly suited to the separation of compounds with

similar physicochemical properties, in samples characterized by a great number of chemical classes, such as citrus essential oils. Multidimensional LC–GC approaches can be divided in heart-cutting (LC–GC) and comprehensive (LC × GC) methodologies.

11.4.1 Heart-Cutting Two-Dimensional Liquid Chromatography–Gas Chromatography

Several interesting LC–GC studies, in the citrus oil field, have been described, mainly in the 1990s (Munari et al., 1990; Dugo, G. et al., 1994b,c; Mondello et al., 1994a,b,c, 1995a, 1996a,b, 1998f,g). In LC–GC, the sample is first separated by HPLC using a single column or a combination of columns, to isolate the components of interest; the latter are then directly transferred onto a GC capillary, where a further separation is carried out. The hyphenation of the GC system to a mass spectrometer introduces a third separation dimension, generating a very powerful analytical method (LC–GC–MS). The mass spectra attained after an LC–GC separation are normally highly pure, enabling a much easier interpretation if compared to those generated from a straightforward GC–MS analysis.

The main requisites for any LC–GC experiment are that

1. The target analytes are amenable to GC analysis.
2. A large amount of liquid solvent needs to be removed, prior to the GC step.

Considering the first obvious requisite, it must be noted that practically all LC–GC methods, derive from a previous GC one. Considering solvent elimination, this was the most problematic technological obstacle, inhibiting the development of LC–GC. In the field of online LC–GC, various interfaces have been developed to enable the introduction of first-dimension heart cuts onto the secondary column (Grob, 1987a,b). This process is achieved through the selective elimination of the solvent, thus allowing the re-injection of a sharp analyte band into the GC column. Retention gap methods are based on the employment of an uncoated capillary column (precolumn), located before the analytical GC column. The eluent is directed onto the precolumn and is retained as a liquid film on the column wall. The solvent starts to evaporate at the rear of the liquid film, while the high-boiling compounds are left on the column inner surface. The liquid film beyond the evaporation site entraps the highly volatile constituents, thus avoiding their co-evaporation with the solvent (solvent trapping). The retention gap method represents the best approach in the case of qualitative and quantitative analysis of samples containing highly volatile compounds. However, the method is restricted to fractions of only modest volumes.

Partially concurrent solvent evaporation, which is an alternative retention gap method, enables a more efficient exploitation of the uncoated capillary: during GC introduction, part of the eluent is evaporated concurrently. Figure 11.10 shows an HPLC–GC on-column type interface that allows "partially concurrent eluent evaporation." If the elimination of the entire volume of LC solvent is

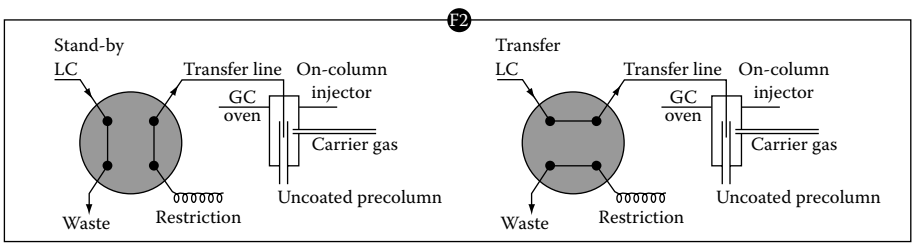

FIGURE 11.10 On-column interface. The HPLC effluent enters the valve and flows to waste when the LC–GC system is in the stand-by configuration. When the valve is switched to the cutting position, the effluent is pumped through the transfer line into the inlet of the on-column injector. At the end of the transfer the valve is switched to the stand-by configuration, and the effluent again flows to waste.

TABLE 11.1
LC-GC Analyses: HPLC Conditions

	Time Interval (min)	Eluents	Flow (μL/min)	Fraction	Transfer Time (min)	Solvent Vapor Exit Closed At (min)
Sweet orange oil aldehydes	0–2	Pentane				
	3–12	Pentane:diethyl ether, 97:3	180	F_1 [a]	9.5–10.5	10.9
	13–17	Pentane:diethyl ether, 90:10		F_2 [a]	13.0–14.3	15.0
	18–23	Pentane:diethyl ether, 85:15		F_3 [a]	16.5–17.8	18.5
	Back-flush with diethyl ether (1 mL)					
Monoterpene and sesquiterpene	0–5	Pentane	180		1.6–3.6	
Citrus oil hydrocarbons	Back-flush with diethyl ether (1 mL)					
Bergamot oil	0–3	Pentane	180	F_1 [b]	1.0–3.0	3.8
	4–13	Pentane:diethyl ether, 97:3		F_2 [b]	12.0–15.0	16.5
	14–19	Pentane:diethyl ether, 90:10		F_3 [b]	17.0–19.0	19.9
	20–30	Pentane:diethyl ether, 80:20		F_4 [b]	21.0–23.5	24.9
	Back-flush with diethyl ether (1 mL)					
Neroli oil	0–5	Pentane	180	F_1 [a]	1.5–3.5	4.0
	Back-flush with diethyl ether (1 mL)			F_2 [c]	7.5–9.5	10.1
Enantiomeric distribution of linalol and terpinen-4-ol*	0–3	Pentane	220	F_1 [d]	17.0–19.0	
	4–15	Pentane:tert-butylmethyl ether, 95:5				
	16–30					
	31–50	Pentane:tert-butylmethyl ether, 90:10				
		Pentane:tert-butylmethyl ether, 70:30				
Petitgrain oils	0–5	Pentane	180	F_1 [c]	1.5–3.0	
	Back-flush with diethyl ether (1 mL)			F_2 [c]	5.5–7.0	

Notes: F_1 [a]: aliphatic aldehydes; F_2 [a]: monoterpene aldehydes; F_3 [a]: sesquiterpene aldehydes; F_1 [b]: mono- and sesquiterpene hydrocarbons; F_2 [b]: aliphatic aldehydes and esters; F_3 [b] and F_4 [b]: alcohols; F_1 [c]: mono- and sesquiterpene hydrocarbons; F_2 [c]: oxygenated compounds; F_1 [d]: linalol and terpinen-4-ol.

Source: Mondello, L., et al., *Perfum. Flav.* 21, 25–49, 1996a.

required, this can be attained by using another approach, namely fully concurrent solvent evaporation. Using the latter method, there is only limited liquid spreading inside the precolumn, enabling the transfer of large volume fractions.

A variety of successful LC–GC and LC–GC–MS methods have been developed and applied to the analysis of citrus essential oils (Dugo, G. et al., 1994b,c; Mondello et al., 1994a,b,c, 1995a, 1996a,b, 1998f,g). HPLC and GC operational conditions relative to a series of experiments and using a fully automated instrument (DualChrom 3000 series, Fisons) are reported in Tables 11.1 and 11.2, respectively. As can be observed, LC–GC can be used to analyze only selected components, such as sweet orange oil aldehydes (Mondello et al., 1994a); the identification and quantitation of such constituents is difficult because of the possible overlapping of peaks. In sweet orange oil, the aliphatic aldehyde content can provide useful information concerning the origin of the oil and the ripeness of the fruit at harvest time.

TABLE 11.2
LC-GC Analyses: GC Conditions

	Capillary Column	Carrier Gas	Temperature Program	Eluent Evaporation Rate (μL/min)
Sweet orange oil aldehydes	SE-52: 25 m × 0.32 mm ID × 0.4–0.45 μm d_f	He, 120 kPa	45°C (6 min) to 220°C at 3°C/min	151
Monoterpene and sesquiterpene citrus oil hydrocarbon	DB-5: 21 m × 0.25 mm ID × 0.25 μm d_f	He, 140 kPa 32 cm/s	45°C (6 min) to 240°C at 3°C/min	138
Bergamot oil	SE-52: 25 m × 0.32 mm ID × 0.4–0.45 μm d_f	He, 120 kPa	45°C (6 min) to 220°C at 3°C/min	145
Neroli oil	SE-52: 26 m × 0.32 mm ID × 0.4–0.45 μm d_f	He, 120 kPa 33 cm/s	45°C (6 min) to 250°C at 3°C/min	150 (pentane) 139 (diethyl ether)
Enantiomeric distribution of linalol and terpinen-4-ol	2,3-Dimethyl-6-pentyl-β-cyclodextrin 30% and OV-1701 70%: 25 m × 0.32 mm ID × 0.25 μm d_f	He,120 kPa	45°C (3 min) to 150°C at 1°C/min	155

Source: Mondello, L., et al., *Perfum. Flav.* 21, 25–49, 1996a.

An interesting citrus oil LC–GC experiment was carried by isolating and subjecting to GC analysis two fractions named as F_1 and F_2. The first contained monoterpene and sesquiterpene hydrocarbons, while the second contained oxygenated constituents (Table 11.1). Figure 11.11A illustrates the TIC GC–MS chromatogram of a bitter orange petitgrain oil and is characterized by considerable peak overlapping: 6-methyl-5-hepten-2-one and myrcene; 1,8 cineole and limonene; *trans*-linalol oxide and terpinolene; δ-elemene and an unknown oxygenated compound, α-copaene, and citronellyl acetate. As can be observed in Figure 11.11B and C, an HPLC preseparation enabled the satisfactory resolution of all the detectable components present in the oil.

Online LC–GC has also been applied to the analysis of the enantiomeric distribution of trace components present in citrus oils. Monoterpene alcohols represent about 0.5% of the volatile fraction of lemon, mandarin, sweet, and bitter orange oils. The determination of the enantiomeric distribution of these constituents can provide useful information on authenticity, quality and origin. As can be observed from the data reported in Table 11.3, the enantiomeric distribution of terpinen-4-ol enables a differentiation between cold-pressed and distilled mandarin and lemon oils. Furthermore, the different (–) and (+) linalol ratios between sweet and bitter orange oils allow the detection of low contamination levels (as little as 5%) of sweet orange oil, in the more expensive bitter orange oil. The enantiomeric ratio of (–) and (+)-linalol can also vary with geographical origin (see values reported for genuine Italian and Spanish bitter orange oils compared to those for oils from Brazil and the Ivory Coast) and with fruit cultivar (see values reported for Italian sweet orange oils and for Uruguayan mandarin oils, of different cultivars).

11.4.2 Comprehensive Two-Dimensional Liquid Chromatography–Gas Chromatography

The step from LC–GC to LC × GC is a rather large one from a technological point of view. The first LC × GC system was used for the headspace analysis of volatile organic compounds contained

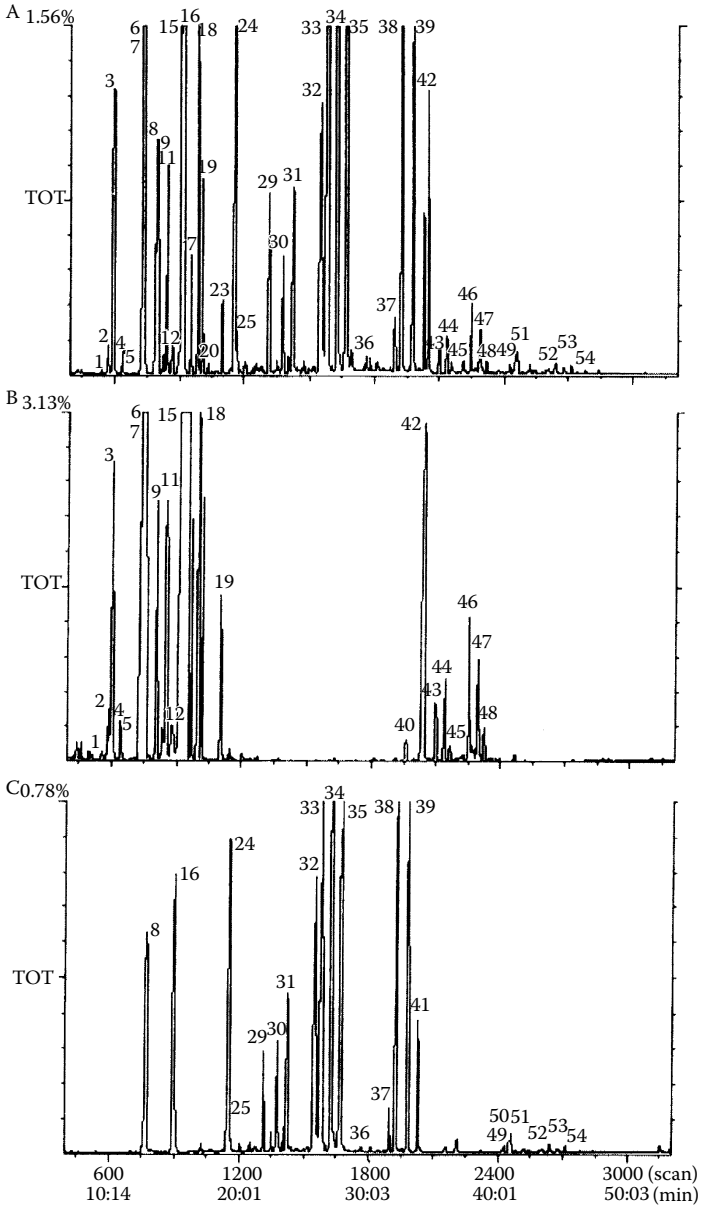

FIGURE 11.11 TIC GC–MS chromatogram of a bitter orange petitgrain oil (A) and of the F_1 (hydrocarbons) (B) and F_2 (oxygenated compounds) (C) LC-separated fractions. See Plate 8. Peak identification: (1) tricyclene; (2) α-thujene; (3) α-pinene; (4) camphene; (5) sabinene; (6) β-pinene; (7) 6-methyl-5-hepten-2-one; (8) myrcene; (9) α-phellandrene; (10) δ-3-carene; (11) α-terpinene; (12) *p*-cymene; (13) limonene; (14) 1,8 cineole; (15) (*Z*)-β-ocimene; (16) (*E*)-β-ocimene; (17) γ-terpinene; (18) *cis*-linalol oxide; (19) terpinolene; (20) *trans*-linalol oxide; (21) linalol; (22) citronellal; (23) terpinen-4-ol; (24) α-terpineol; (25) nerol; (26) neral; (27) linalyl acetate; (28) geranial; (29) δ-elemene; (30) α-cubebene; (31) α-terpinyl acetate; (32) citronellyl acetate; (33) α-copaene; (34) neryl acetate; (35) geranyl acetate; (36) β-elemene; (37) methyl *N*-methyl anthranilate; (38) (*E*)-caryophyllene; (39) *trans*-α-bergamotene; (40) α-humulene; (41) (*Z*)-β-farnesene; (42) bicyclogermacrene; (43) α-farnesene; (44) δ-cadinene; (45) (*E*)-nerolidol; (46) spathulenol; (47) caryophyllene oxide. (From Mondello, L., et al., *American Lab.* December, 41–49, 1996b. Copyright International Scientific Communications Inc. Reproduced with permission.)

TABLE 11.3
Enantiomeric Distribution of Linalol and Terpinen-4-ol in Various Citrus Oils

	(–) Linalol	(+) Linalol	(–) Terpinen-4-ol	(+) Terpinen-4-ol
Lemon oil (cold-pressed)	54	46	80	20
Lemon oil (distilled)	53	47	78	22
Mandarin oil (cold-pressed)	17	83	87	13
Mandarin oil (distilled)	17	83	73	27
Bitter orange oils (Italy)	83	17		
Sweet orange oils (Italy)	7	93		
Bitter orange oils (Spain)	82	18		
Bitter orange oils (Brazil)	68	32		
Bitter orange oils (Ivory Coast)	67	33		
	Italian Sweet Orange Oils			
Blond Oranges				
Biondo commune	8	92		
Navelina	9	91		
Washington navel	8	92		
Valencia late	9	91		
Ovale	5	95		
Red Oranges				
Moro	6	94		
Tarocco	5	95		
Sanguinello	5	95		
	Mandarin Oils			
Mandarin oils (Italy)	16	84		
Mandarin Oils (Uruguay)				
Malvasio	12	88		
Ellendale	14	86		
Ortanique	16	84		
Comun	21	79		
Malaquina	30	70		

Source: Mondello, L., et al., *Perfum. Flav.* 21, 25–49, 1996a.

in water (Quigley et al., 2000). In 2004, an entirely automated LC × GC system was exploited in lipid analysis (de Koning et al., 2004a): the instrument was operated using stop-flow conditions and combined with a time-of-flight (ToF) MS. A six-port switching valve and a dual side-port syringe were tested as interfaces, with both providing good results. In a further LC × GC-ToF MS application, using the dual side-port syringe, a fuel sample was subjected to a primary NP LC separation (de Koning et al., 2004b). Eighty μL fractions, corresponding to a 6 second band width, were transferred onto a nonpolar 30 m × 0.25 ID GC column. The research demonstrated that even after an LC preseparation step, a single GC column was not successful in the full separation of each LC fraction. At present, there is great room for development and applications in the LC × GC research field.

In 2008, an LC × GC method was applied to the analysis of bergamot essential oil (Mondello et al., 2008): the first-dimension analysis was carried out under gradient NP conditions (a 30 cm × 1 mm ID silica microbore column was used) and enabled a chemical class separation. The first

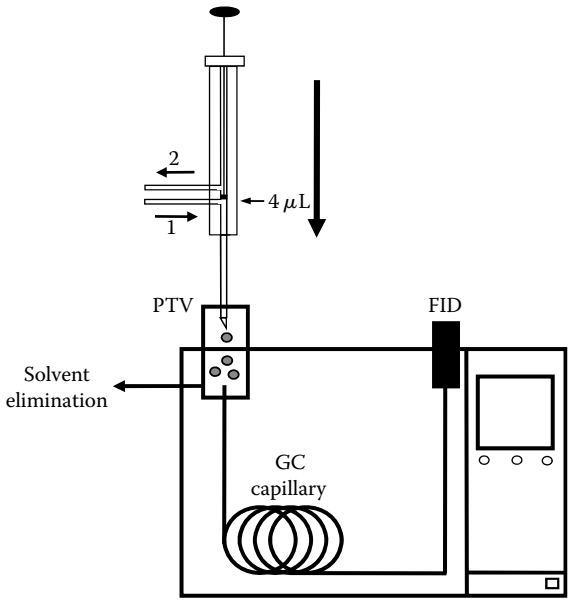

FIGURE 11.12 Schematic of the LC × GC syringe interface used for the analysis of bergamot essential oil.

fraction was composed of monoterpines and sesquiterpenes hydrocarbons that were obviously weakly retained on the silica column; esters, aliphatic, and monoterpene aldehydes, and mono-/sesquiterpene alcohols followed. LC × GC band transfer (60 second fractions, corresponding to 160 μL) was achieved under LC stop-flow conditions through a dual-port 25-μL syringe (Figure 11.12). The latter was connected to the LC detector outlet (line 1) and to waste (line 2), by using two transfer lines. A rubber plug was situated at the terminal part of the syringe plunger, which was characterized by a lower outer diameter with respect to the internal diameter of the syringe barrel; in this way, the LC mobile phase can flow freely inside the syringe. In the waste mode, the plug is located below line 1 and the mobile phase is directed to waste, while in the transfer position, the plug is situated between line 1 and 2 and the effluent is directed to the GC. The internal syringe volume between the plug and the needle exit is very low, namely 4 μL, enabling the direct flow of the LC mobile phase to the GC, during the transfer period, followed by immediate PTV [60°C (1 min) to 280°C at 250°C/min] solvent evaporation. Each LC fraction was analyzed on an apolar 25 m × 0. 25 mm ID × 0.25 μm d_f GC capillary using the temperature program 45°C (4 min) to 220°C at 3°C/min. An LC × GC dedicated software enabled the control of each instrument through the respective native LC and GC softwares. LC × GC chromatogram expansions, relative to the hydrocarbon and ester fractions of bergamot essential oil, are illustrated in Figure 11.13A and B, respectively.

11.5 FAST GAS CHROMATOGRAPHY

The separation of citrus essential oil volatiles is generally carried out by using conventional GC. Although the employment of a 25–30 m × 0.25–0.32 mm ID capillary column does usually ensure satisfactory separations on such sample types, the cost in analytical time (for most citrus oil applications this is typically 60 min) can be a substantial disadvantage. In fact, high time costs become a drawback for laboratories with a high daily sample throughout and/or where there is a need for quick and reliable results. Taking for granted that the most selective conventional GC column is being exploited, the immediate route toward reducing retention times is by increasing the gas linear velocity and/or the temperature program ramp, or by reducing the column length. It is obvious

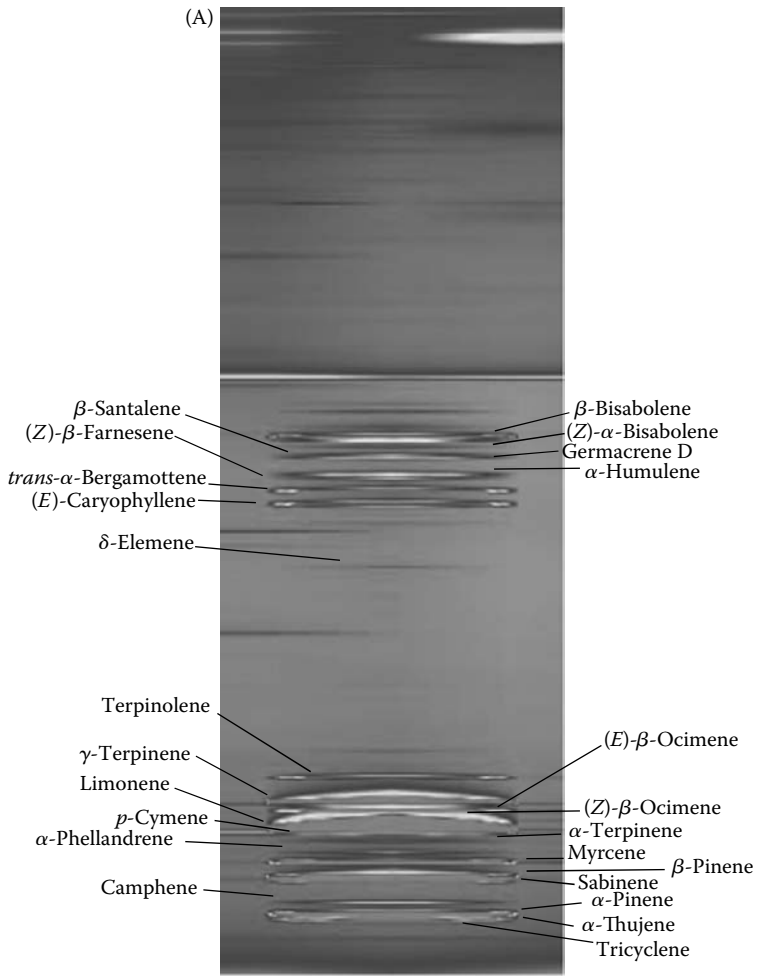

FIGURE 11.13 **(See color insert following page 462.)** LC × GC chromatogram expansion of the bergamot oil (A) hydrocarbon and (B) ester fractions.

that such modifications can be applied until the lowest degree of acceptable analyte separation is attained.

If modifications of the operational conditions or column length are not sufficient, then a specific fast GC method must be considered. Over the past decades, several methods have been theorized and developed with various outcomes, such as multicapillary columns (Sandra et al., 1996), microparticle packed capillary columns (Shen et al., 1997), low pressure outlet conditions (LP-GC) (van Deursen et al., 2000), turbulent flow (van Es et al., 1989), helically coiled columns (Tijssen et al., 1987), resistive heating (Dallüge et al., 2002), reduced column ID, and stationary-phase thickness (microbore capillaries) (van Es, 1990).

The main objective of any rapid GC technique is to maintain (compared with conventional GC) sufficient resolving power for the separation of the compounds of interest. Considering this requisite, the microbore column approach is probably the most effective way of reducing analysis times: conventional GC profiles can be reproduced with a great increase in analysis speed.

As can be derived from the classical Golay-Giddings equation (Golay, 1958; Stewart et al., 1959; Giddings et al., 1960; Cramers et al., 1983), a decrease in column ID reduces resistance to mass transfer in the mobile phase, hence, band broadening leading to an increase in efficiency. Furthermore,

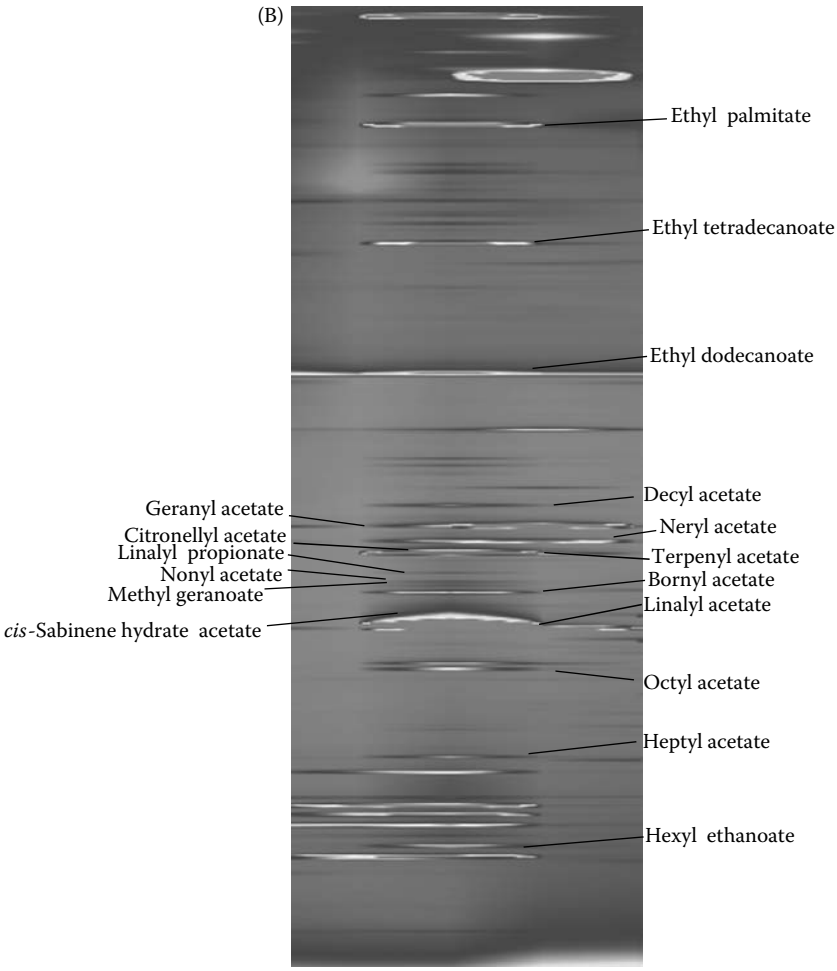

FIGURE 11.13 (Continued)

microbore capillaries are characterized by thin stationary-phase coatings and, thus, the percent influence of resistance to mass transfer in the stationary phase (C_S term) on band enlargement can be neglected. Consequently, the Golay-Giddings equation can be simplified, and an expression derived where the minimum plate height coincides approximately with the internal diameter. In GC, it is a rule of thumb that a 10 m × 0.10 mm ID × 0.10 μm microbore column can generate the same N value (~100,000) as a 25 m × 0.25 mm ID × 0.25 μm capillary if both are operated under optimum conditions.

Hydrogen is the most convenient carrier gas for fast GC applications (safety precautions must be considered) because higher optimum gas linear velocities (u_{opt}) are attained: u_{opt} equals 55–60 cm/s when a 10 m × 0.10 mm ID capillary is employed. Moreover, in the case of reduced-ID columns, if higher-than-ideal gas velocities are applied, resolution losses are rather slight, as the ascending part of microbore column Golay curves rises gradually.

Considering the temperature program, it has been demonstrated that a rate of 10°C/dead time should be employed (Blumberg and Klee, 1998). If a more accelerated ramp is applied, then stationary-phase residence times are reduced below a critical level for satisfactory component resolution, while if lower program rates are used, then resolution gains will be limited (while elution times will

be extended). When using a 10 m × 0.10 mm ID column, under ideal gas velocity conditions, then a rate of 10–20°C/min can be considered as optimum. Excessively high-temperature program ramps (>30°C/min) have a negative effect on resolution, as substantial parts of the column are not exploited for separation. For most fast GC applications, an instrumental capability of maximum 80–100°C/min is usually sufficient.

Microbore columns are characterized by lower sample capacity (C_{max}), a feature which is proportional to ID^3. The amount of a solute that can be tolerated in a 100 μm ID column before efficiency is significantly reduced is about 1–1.2 ng; in the case of 50 μm ID columns, quantities over 0.15 ng will have a considerably negative effect on resolving power. The values aforementioned provide a good indication, but may vary in relation to each specific analyte. Sensitivity is substantially maintained with respect to conventional GC methods (a 10%–20% decrease in peak height is usually observed), because the obliged use of lower sample amounts is counterbalanced, in part, by the generation of tall and narrow peaks.

A further important issue is related to detection capabilities because high-speed elution necessitates fast sampling rates. In fact, insufficient acquisition rates lead to incorrect peak reconstruction (jagged peak shapes are observed) and, therefore, reliable peak quantitation is obstacled. On the contrary, excessively high sampling rates cause an increase in baseline noise width and, thus, a reduction in method signal-to-noise ratios. There are many differing opinions on "how many data points/peak are necessary." As a general rule, 10 data points per peak can be considered enough for proper peak reconstruction. A 50 Hz sampling frequency is sufficient in nearly all fast GC applications (3–15 min analysis time), while in very fast GC (1–3 min analysis time) and ultra fast GC (<1 min analysis time) experiments, higher sampling rates are required (100–200 Hz).

Microbore column fast GC instrumental requirements have been fully satisfied in the last 10 years. If a 10 m × 0.1 mm ID capillary is used, instead of a 25 m × 0.25 mm ID one, with both operated under ideal conditions, then a speed enhancement factor in the 3–6 range can be expected. Such benefits were attained by Tranchida et al. (2006), in a fast headspace solid phase microextraction–gas chromatographic (HS–SPME–GC) application on bergamot essential oil. An entirely automated fast HS–SPME–GC method was developed, using a low-volume (7 μm) polydimethyl siloxane SPME fiber, which enabled a rapid equilibration time (15 min), and a 10 m × 0.1 mm ID apolar capillary, which enabled a fast separation (12.5 min). A CO_2 cryo-trap, located just below the injection port, was used to eliminate desorption-generated band broadening, which effected the more volatile bergamot oil components. The authors reported that the employment of a reduced-ID column, in a SPME-GC experiment, lead to two substantial advantages: the reduction of both the SPME equilibration and compound separation times. The former benefit was related to the necessary use of a low-volume SPME fiber (due to the reduced column sample capacity), if compared to conventional SPME-GC. A conventional HS-SPME-GC (a 30 μm SPME fiber and 0.25 mm ID column were used) analysis was carried out on the bergamot sample, with analyte extraction and separation both requiring 50 minutes. In the fast and conventional applications, peak resolution was altogether comparable, as can be observed in Figure 11.14. Although a slight loss in terms of sensitivity was observed in the rapid approach, this did not hinder the detection of all peaks of interest.

If a very fast microbore column GC method is used in citrus oil analysis, then a price must be payed in resolution terms (considering conventional GC); however, such an event is entirely acceptable if target analytes remain separated. Lime essential oil has been analyzed in a very fast GC mode, using a 5 m × 50 μm ID capillary with a 0.05 μm stationary-phase thickness (Mondello et al., 2004a). The calculated column efficiency, under optimum conditions, was almost 100,000 plates (an experimental H_{min} value of 0.052 mm was calculated); a very fast high-resolution GC analysis of lime essential oil, with a total analysis time of less than 1.5 minutes, was reported. The application was about 33 times faster than the conventional GC technique and proved to be adequate for essential oil quality assurance analysis, and the quantitation of key components. Although resolution was reduced if compared to conventional GC, the end results for target compounds were in good agreement.

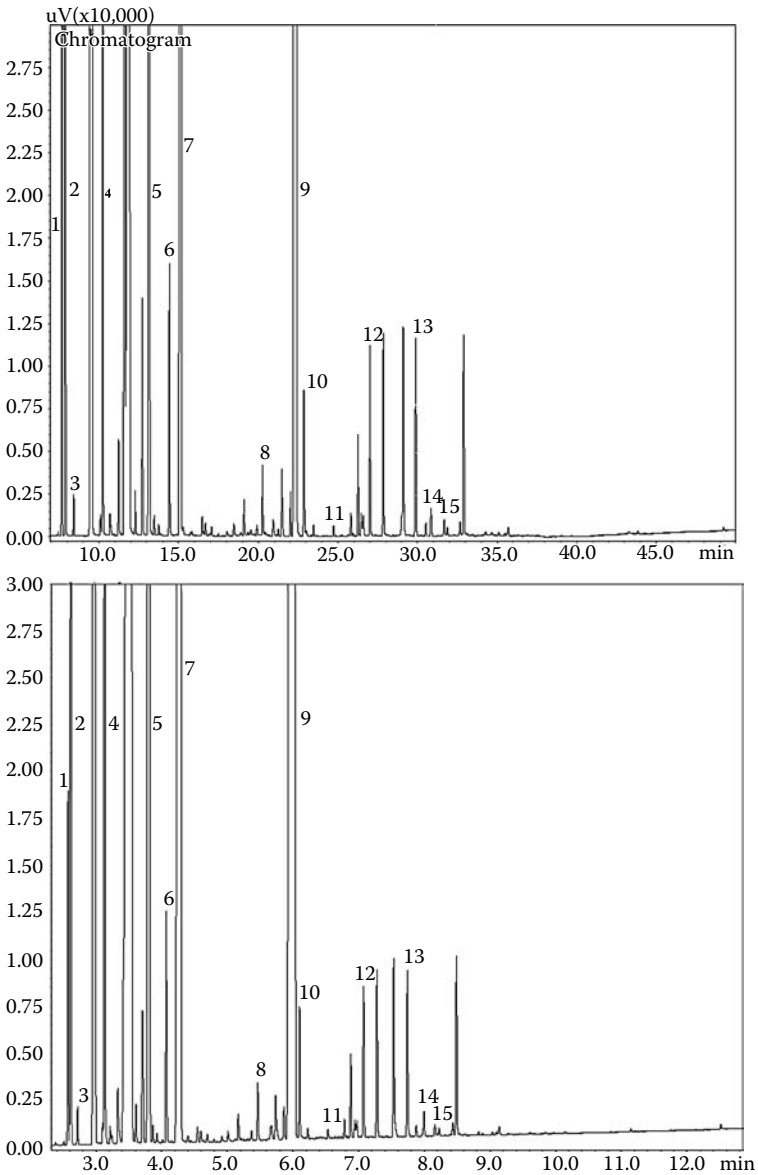

FIGURE 11.14 Upper chromatogram: a 30 μm fiber conventional HS-SPME-GC application on bergamot essential oil. Peaks: (1) α-thujene; (2) α-pinene; (3) camphene; (4) myrcene; (5) γ-terpinene; (6) terpinolene; (7) linalol; (8) octyl acetate; (9) linalyl acetate; (10) geranial; (11) nonyl acetate; (12) neryl acetate; (13) *trans*-α-bergamoptene; (14) *cis*-β-farnesene; (15) germacrene D. Lower chromatogram: a cryo-trap fast HS-SPME-GC application on bergamot essential oil (peak identification as indicated before). (Reprinted from Tranchida, P.Q., et al., *J. Chromatogr. A*. 1103, 162–165, 2006. Copyright 2006 with permission from Elsevier.)

A good example of the use of very fast GC for the discovery of fraud, in citrus products, has been provided by Mondello et al. (2000). It is well known that one of the most common adulterations, in the citrus oil field, is the addition of cheap orange oils to cold-pressed lemon oil. Sweet orange oils are characterized by the presence of about 0.1% of δ-3-carene, a monoterpene hydrocarbon that is either missing or present in traces in other citrus oils. The contrary is true for camphene,

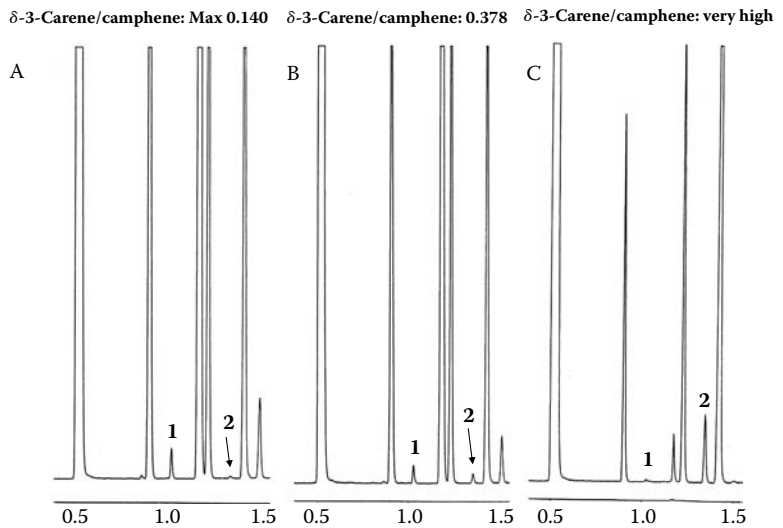

FIGURE 11.15 Very fast GC chromatograms obtained for (A) genuine lemon oil, (B) mixture of lemon oil and 20% of sweet orange oil, and (C) genuine sweet orange oil. Peak identification: (1) camphene; (2) δ-3-carene. (From Mondello, L., et al., *LC GC Europe*. 13, 495–502, 2000. Copyright Advanstar Communications. Reproduced with permission from LCGC Europe.)

another monoterpene hydrocarbon, practically absent in sweet orange oils, but present in higher relative amounts in lemon oil (ca. 0.06%). In a pure lemon oil, the δ-3-carene/camphene ratio is 0.14% maximum. Very fast GC applications were carried out on a lemon oil, an adulterated lemon oil and a sweet orange oil (Figure 11.15). As can been seen from the expansions, the rapid GC method was optimized for the separation of the target components, thus again reaching the analytical objective.

In 2004, Mondello et al. (2004b) described a very fast GC–FID method used for citrus oil analysis (bergamot, lemon, sweet and bitter orange, mandarin oils). A rapid bergamot oil separation was shown as an example: volatiles were separated in just over 180 seconds on a 10 m × 0.1 mm ID apolar capillary, at a hydrogen constant linear velocity of 81.5 cm/s and using a temperature program ramp of 50°C/min. Compared to a conventional GC-FID application (30 m × 0.25 mm ID column; 30 cm/s velocity; temperature program ramp: 3°C/min), on the same sample, a speed gain of almost 14 times was attained, with only slight resolution losses (Figure 11.16). Peak base widths as low as 360 msec were measured, while intra-day retention time and peak area precision were altogether comparable to the values attained in the conventional analysis.

The use of a mass selective detector in microbore column fast GC experiments is obviously desirable, often necessary, and has been several times described (Mastovská and Lehotay, 2003). Nonscanning MS systems, such as time of flight instruments, provide a wide mass range as well as very fast acquisition rates (up to 500 spectra/s). Furthermore, spectrum profiles do not change across the peak (no skewing). Such an instrument is highly suited for very fast and ultrafast GC experiments, but is characterized by high economical costs. In the case of fast GC applications, less expensive scanning systems (i.e., quadrupole) can be used even for quantitative purposes (Mastovská and Lehotay, 2003). In fact, ultimate generation quadrupole mass spectrometers (qMSs), operated in the full scan mode, can generate up to 20–25 spectra/s. Furthermore, qMSs have very good commercial MS library compatibilities. The effectiveness of a rapid scanning qMS, in a microbore column fast GC–MS analysis on lime essential oil, was shown by Mondello et al. (2000). The qMS instrument was operated at a scan speed of 6750 amu/s, and generated ca. 20 spectra/s. A TIC fast GC-qMS

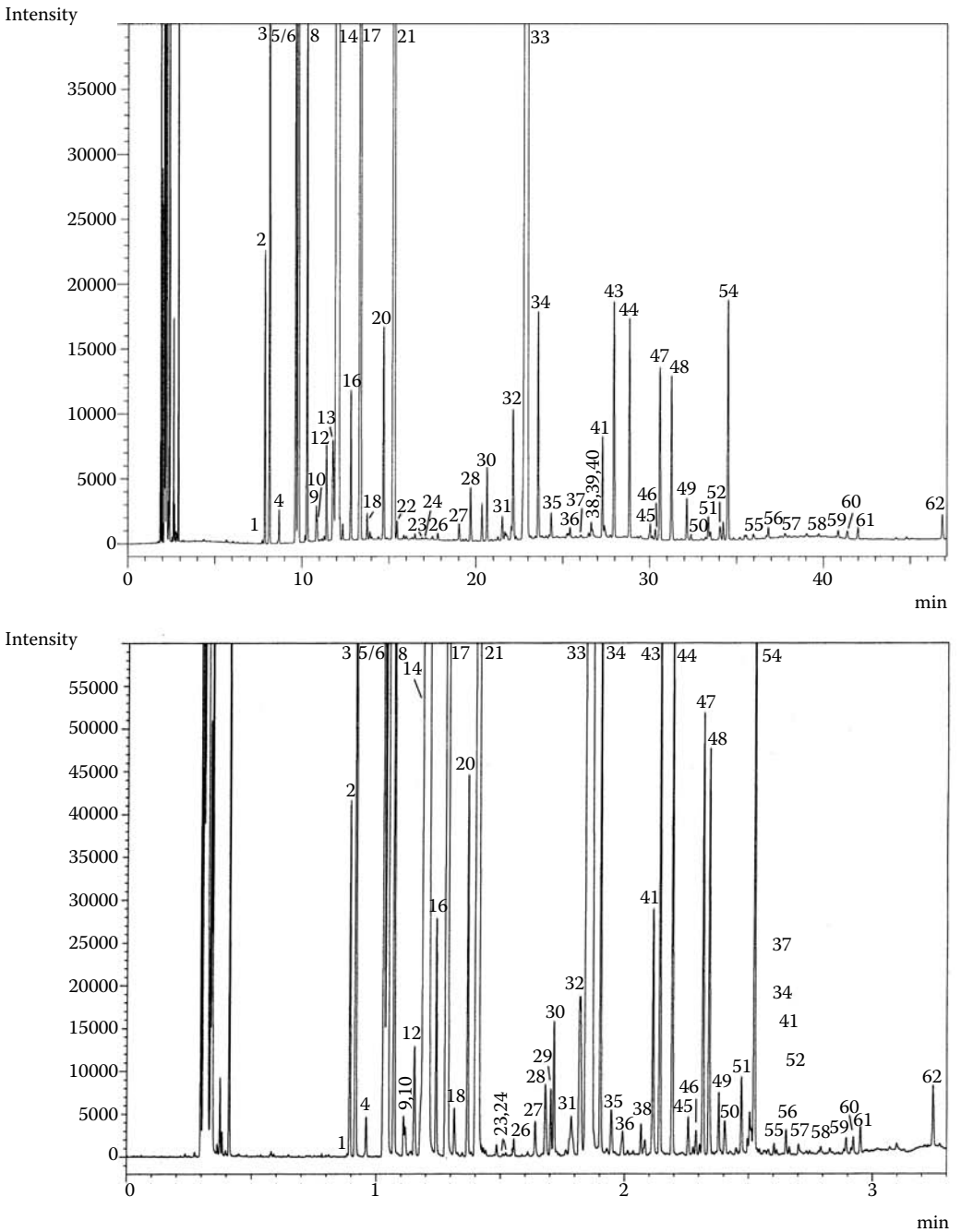

FIGURE 11.16 Conventional and very fast GC–FID chromatograms of bergamot essential oil. For peak identification refer to Mondello et al. (2004b). (From Mondello, L., et al., *J. Chromatogr. Sci.* 42, 410–416, 2004b. Copyright Preston Publications. Reproduced with permission.)

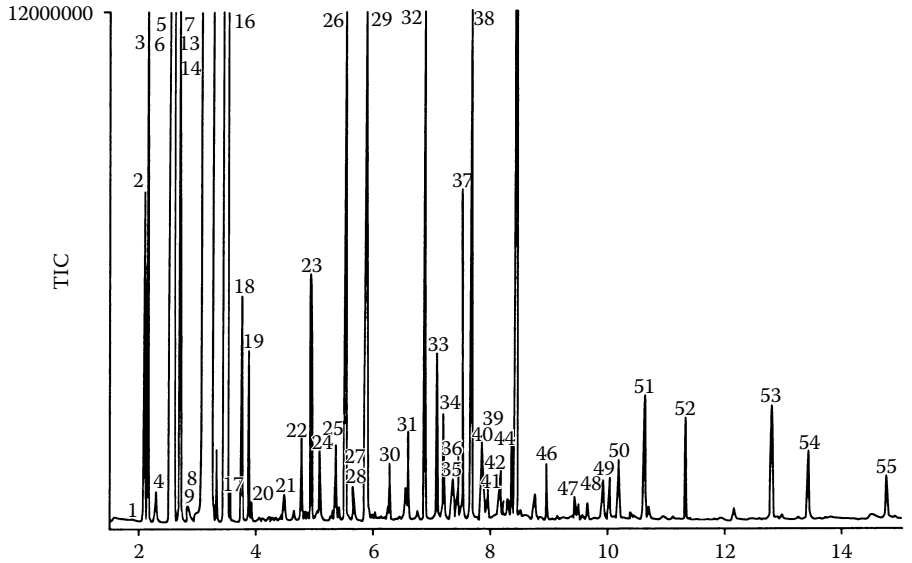

FIGURE 11.17 TIC fast GC-qMS chromatogram of lime essential oil. For peak identification refer to Mondello et al. (2000). (From Mondello, L., et al., *LC GC Europe*. 13, 495–502, 2000. Copyright Advanstar Communications. Reproduced with permission from LCGC Europe.)

chromatogram of lime essential oil is shown in Figure 11.17. The authors reported that a total number of 55 compounds were identified, and that a good agreement was observed with conventional GC-qMS qualitative and quantitative results.

A high-speed GC method with ToF MS detection was developed and exploited for the analysis of citrus essential oils (Veriotti and Sacks, 2002). Rapid GC separation (less than 140 seconds) was achieved through fast temperature programming (50°C/min) and by using a tandem-column stop-flow technique: a 7 m × 0.18 mm ID trifluoropropylmethyl polysiloxane column was connected to a 5% phenyl dimethylpolysiloxane one, of equivalent dimensions. A carrier-gas pressure pulse, applied to the column connection point, was exploited to stop the primary column flow for specific time periods, and to improve the overall GC separation. ToF MS detection provided a high spectral frequency (25 Hz) and spectral deconvolution capabilities, decreasing the need of high-resolution separations. To detect peak co-elution, at least two complete mass spectra between the peak apexes of the co-eluting compounds must be attained and, thus, a minimum peak apex separation of 120 msec was required, considering the 25 Hz spectral frequency. A tandem-column GC analysis of bergamot essential oil is shown in Figure 11.18: the primary column FID chromatogram is illustrated in Figure 11.18A and it is important to note that compounds 24, 25, and 26 are fully resolved from each other. Furthermore, primary column peak coelutions 17, 20, 24 and 18, 25, are entirely separated on the secondary column, while peaks 24, 25, and 26 undergo co-elution, as shown in Figure 11.18B. Through the application of a 6 second stop-flow pulse at 77 seconds after sample injection (first arrow indication in Figure 11.18A), component 24 is completely separated from peaks 25 and 26 (Figure 11.18C). An additional two-second stop-flow pulse, applied at 87.2 seconds (second arrow indication in Figure 11.18A), enabled the resolution of peaks 25 and 26, but caused the co-elution of compounds 22 and 25 (Figure 11.18D). If the stop-flow pulse is increased to five seconds, then peaks 24, 25, and 26 are fully isolated (Figure 11.18E).

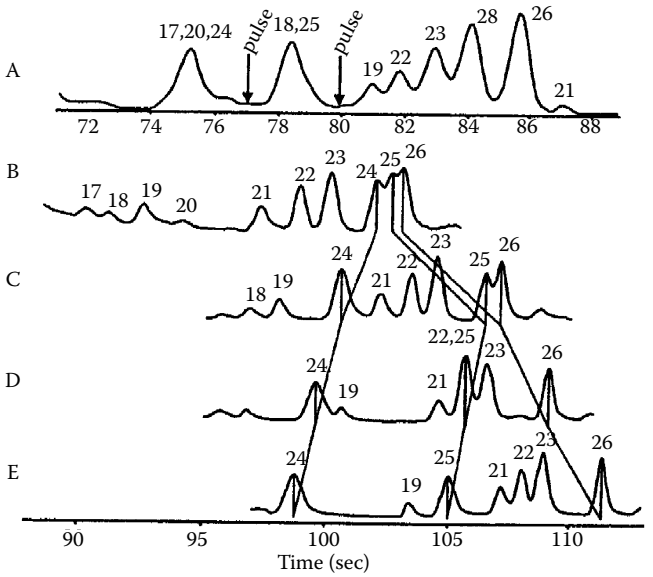

FIGURE 11.18 Bergamot oil GC-ToF MS chromatograms illustrating enhanced resolution of peaks 24, 25, and 26 through stop-flow operation. (A) Primary column GC–FID chromatogram; (B) tandem-column GC-ToF MS chromatogram, without stop-flow operation; (C) tandem-column GC-ToF MS chromatogram, with 6 second stop-flow pulse starting 77 seconds after injection; (D) tandem-column GC–ToF MS chromatogram, with 6 second stop-flow pulse starting 77 seconds after injection and 2 seconds stop-flow pulse starting 87.2 seconds after injection; (E) tandem-column GC-ToF MS chromatogram, with 6 seconds stop-flow pulse starting 77 seconds after injection and 5 seconds stop-flow pulse starting 87.2 seconds after injection. For peak identification refer to Veriotti and Sacks (2002). (Reprinted with permission from Veriotti, T., and Sacks, R., *Anal. Chem.* 74, 5635–5640, 2002. Copyright 2002 American Chemical Society.)

11.6 MASS SPECTROMETRY

Gas chromatography, combined with mass spectrometry, occupies a central position in the identification of citrus essential oil constituents. The most common identification procedure consists in the comparison of experimental mass spectra with those contained in a reference MS library. At the end of an MS library search, a list of possible matches is generated, with the most probable one situated in the first position. Unfortunately, in many cases peak identification is not reliable because many terpenes are characterized by very similar mass spectra.

The reliability of GC–MS data can be greatly increased by exploiting retention data, as proposed by Mondello et al. (1995b). A dedicated dual-filter MS library, defined as flavor and fragrance synthetic and natural compounds (FFSNC), has been recently constructed combining mass spectra and linear retention index (LRI) data (derived from polar and apolar columns). The search procedure functions as follows: prior to a citrus essential oil analysis, a C_7–C_{30} alkane mixture is subjected to GC–MS analysis. At the end of the analysis, the LRI values relative to each unknown peak are automatically calculated by the GC–MS software. During MS library matching, the GC–MS software automatically deletes library hits with a spectral similarity lower than a specific value defined by the analyst (filter 1); normally, a minimum spectral similarity value of 90% is chosen. The second filter enables the automatic elimination of possible matches with an LRI value outside an acceptable

retention index window, and in relation to the LRI of the unknown compound. A typical LRI range is ± 5 units for an apolar column, while less restrictive windows are applied when using a polar column.

As was reported in Chapter 7, useful information on the authenticity, quality, extraction technique, geographic origin, and biogenesis of citrus essential oils can be achieved through enantioselective GC using different chiral phases for the determination of enantiomer ratios. However, considering that modern adulterations of citrus essential oils, as well as many food products, are becoming harder to unveil, the determination of elemental isotopic ratios of target components by means of isotope ratio mass spectrometry (IRMS) has become a rapid and convenient tool for authenticity assessment (Brenna et al., 1997). In fact, the analysis of the isotopic composition can be very useful for the determination of: geographic origin, an adulteration of natural essential oils with synthetic or natural compounds, prohibited farming procedures or production methods.

High-precision IRMS can be defined as the method that deals with the measurement of deviations of isotope abundance ratios from an agreed standard by only a few parts per thousand for C, H, N, O, and S. Each element must be converted from its current chemical state, into the required gaseous species, prior to its introduction into the ion source. The measurement of the $^{13}C/^{12}C$ ratio, in particular, is already well established and is commonly achieved by using a GC separation step (GC–IRMS). Carbon isotope ratio measurements are attained by converting the C atoms into CO_2 (via a combustion furnace), and then by comparing the carbon isotope ratio of specific constituents to that of a known reference. A dimensionless quantity (δ) is used to express the isotope ratio value of a specific compound, in relation to the stated reference and is expressed in parts-per-thousand.

Faulhaber et al. (1997a,b) reported $\delta^{13}C$ values of characteristic mandarin essential oil constituents, by using GC-IRMS. $\delta^{13}C$ values for monoterpene hydrocarbons were attained through direct GC-IRMS, while $\delta^{13}C$ measurements for the oxygenated constituents (α-sinensal, methyl *N*-methylanthranilate, linalol, and octanal) were achieved after a preseparation. Table 11.4 reports $\delta^{13}C$ values for: genuine Italian cold-pressed mandarin oils, commercial mandarin oils of various geographical origins, distilled mandarin and sweet orange oils. As can be concluded from the data reported in Table 11.4, $\delta^{13}C$ values can vary greatly in relation to geographical origin.

It must be emphasized that to overcome the effects of the plant growth conditions, on isotopic ratios, the use of a suitable isotopic standard (myrcene) was recommended by Faulhaber et al. (1997b).

In recent research, Schipilliti et al. (2009) analyzed fifty-three genuine Sicilian mandarin essential oils, by using GC-IRMS. Minimum and maximum $\delta^{13}C$ values (authenticity range) average $\delta^{13}C$ values ($n = 3$) and standard deviations were calculated for: α-thujene, α-pinene, β-pinene, myrcene, limonene, γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, decanal, thymol, methyl *N*-methylanthranilate, (*E*,*E*)-α-farnesene and α-sinensal. Myrcene was selected as internal isotopic standard to eliminate growing condition effects, such as geographic differences, climate, or harvest time; myrcene was used because it is present in sufficient amounts, biogenetically related to most of the investigated components and a characteristic component of lower sensorial importance. A series of commercial mandarin essential oils were subjected to GC-IRMS analysis, and the results relative to three samples (defined as Co1, Co2, and Co3) are illustrated in a graph reported in Figure 11.19. Co1 was characterized by $\delta^{13}C_{myrcene}$ values generally outside the authenticity range, in particular for γ-terpinene and terpinolene. Co2 and Co3 provided similar results: a slight depletion was observed for methyl *N*-methyl anthranilate (MNMA), (*E*,*E*)-α-farnesene and α-sinensal, with respect to the authenticity range.

TABLE 11.4
$\delta^{13}C$ values (n = 3) of Characteristic Mandarin Essential Oil Constituents

Sample	α-Sinensal	Limonene	γ-Terpinene	α-Thujene	β-pin./sab.	Myrcene	Terpinolene	MNM	Linalol	Octanal
Authentic mandarin oils from Italy Au1–10*										
Min	–28.53	–31.01	–30.16	–28.60	–30.08	–27.49	–30.37	–31.07	–28.60	–30.90
Max	–26.41	–29.40	–28.83	–27.18	–28.76	–25.83	–28.63	–29.54	–26.39	–28.30
Mean	–27.97	–30.25	–29.60	–27.81	–29.32	–26.56	–29.17	–30.24	–27.79	–29.68
[σ]	[0.77]	[0.52]	[0.46]	[0.43]	[0.44]	[0.47]	[0.56]	[0.50]	[0.71]	[1.03]
Commercial mandarin oils from Italy										
Co1	–27.30	–30.27	–29.86	–28.55	–29.95	–27.51	–29.52	–32.42	–27.62	–28.48
Co2	–27.99	–30.92	–30.49	–29.29	–30.56	–27.94	–30.56	–31.42	–28.39	–29.81
Co3	–26.90	–30.10	–29.66	27.37	–28.87	–26.32	–29.06	–29.97	–27.00	–29.67
Commercial mandarin oils from Greece										
Co4	–26.05	–29.41	–28.34	–27.06	–28.20	–25.35	–28.54	–29.11	–26.42	–28.25
Co5	–26.81	–29.36	–29.10	–27.35	–29.03	–26.48	–29.08	–30.37	–27.10	–29.31
Commercial mandarin oils from Brazil										
Co6	–30.35	–32.36	–32.00	–30.53	–31.30	–28.55	–31.29	–32.66	–29.68	–31.01
Commercial mandarin oils from Argentina										
Co7	–30.22	–31.91	–30.95	–28.94	–30.62	–27.95	–31.47	–32.66	–29.40	–30.22
Commercial mandarin oils unknown origin										
Co8	–27.02	–30.14	–29.50	–28.16	–29.84	–27.05	–29.21	–30.22	–27.30	nd
Co9	–27.46	–29.94	–28.97	–27.41	–29.11	–25.30	–28.38	–30.42	–27.41	–28.59
Co10	–29.92	–30.50	–31.52	–30.01	–31.16	–28.82	–29.19	–32.05	–28.81	–31.31

Distilled mandarin peel oils from Italy										
Co11	nd	–30.32	–30.50	–29.37	–30.04	–26.93	–29.75	–32.48	–28.03	–30.47
Co12	nd	–31.57	–29.66	–29.18	–30.05	–27.36	–29.97	–33.42	–28.29	–30.89
Au13*	–28.97	–30.45	–30.82	–28.58	–29.73	–26.92	–29.95	–30.30	–28.60	–31.76
Orange peel oils from Italy										
Au15	nd	–29.48	Nd	nd	nd	–25.78	nd	nd	–27.07	–27.01
Au16	nd	–28.86	Nd	nd	nd	–25.45	nd	nd	–26.02	–25.60
Au17	nd	–28.68	Nd	nd	nd	–26.29	nd	nd	–26.30	–26.54
Au18*	nd	–29.61	Nd	nd	nd	–26.25	nd	nd	–27.50	–28.00

Notes: Au = authentic; *Au7–10 and Au18 were laboratory-prepared by solvent extraction, while Au13 was laboratory-prepared by distillation; Co = commercial; nd = not determined; β-pin./sab. = β-pinene/sabinene; MNM = methyl *N*-methyl anthranilate.

Source: Faulhaber, S., et al., *J. Agric. Food Chem*. 45, 4719–4725, 1997b.

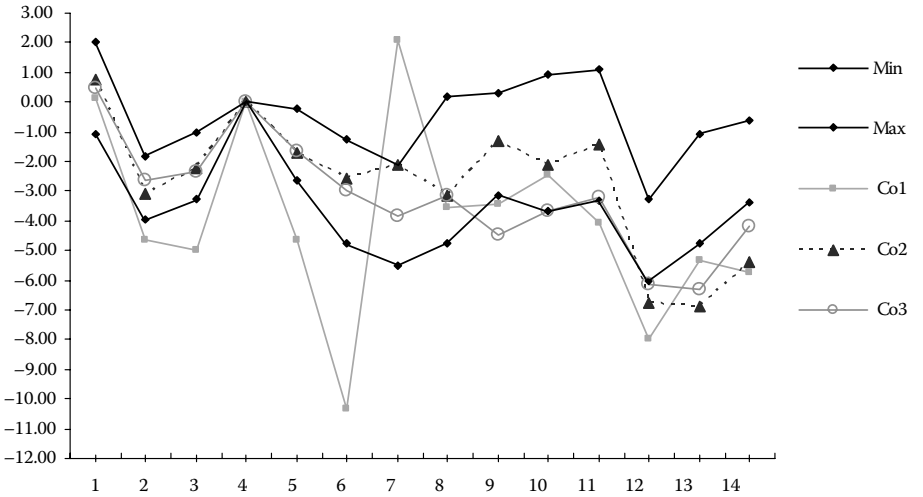

FIGURE 11.19 Graph reporting the mandarin essential oil authenticity range and the $\delta^{13}C_{myrcene}$ results for three commercial mandarin oils (denominated Co1, Co2, and Co3). Compound numbers: (1) α-thujene; (2) α-pinene; (3) β-pinene; (4) myrcene; (5) limonene; (6) γ-terpinene; (7) terpinolene; (8) terpinen-4-ol; (9) α-terpineol; (10) decanal; (11) thymol; (12) methyl *N*-methyl anthranilate; (13) (*E*,*E*)-α-farnesene; (14) α-sinensal. (From Schipilliti, L., et al., 2010. Genuineness assessment of mandarin essential oils employing gas chromatographycombustion-isotope ratio mass spectrometry (GC-C-IRMS). *J. Sep. Sci.* 33, 617–625. Copyright Wiley-VCH Verlag GmbH & Co. Reproduced with permission.)

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12 Contaminants in Citrus Essential Oils: State of The Art (2000–2009)

Giacomo Dugo, Giuseppa Di Bella, Marcello Saitta

CONTENTS

12.1 INTRODUCTION

The contamination of citrus essential oils (CEOs) has been a well known problem since 1960s. The CEOs production mainly consists of oils from lemons, mandarins, and oranges. Bergamots, which grow almost only in a small area of the Reggio Calabria province (Italy), permit the production of high valuable oils, even if in small quantities.

The competition among world producers of citrus derivatives on the international market is very hard. Therefore a serious and constant quality control examination is needed.

The determination of pesticide residues and plasticizers in CEOs is well documented. However, up until 2000, the presence of other kind of contaminants were not investigated. In the last 10 years, new studies were conducted to evaluate the presence of organic and inorganic contaminants in CEOs, by using innovative techniques, with the aim of checking the status of the contamination due to those very toxic and ubiquitous chemicals. A brief review was recently published by Lawrence (2009).

These studies still regarded the analysis of organophosphorus and organochlorine pesticides and plasticizers, but also the presence of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), polychlorinated biphenyls (PCBs), heavy metals, chlorohydrins, and spinosad.

In this review, the data regarding citrus oils produced in the last crop years were considered.

12.2 TRACE ELEMENTS

The presence of heavy metals in CEOs depends on many factors, such as the nature of the soil, climatic conditions, genotype of the plant, agronomic techniques, and storage and extraction procedures (Bruno et al., 1978; Chiricosta et al., 1978; Di Giacomo, 1988). Elements such as Cu(II), Zn(II), Mn(II), and Se(IV) naturally occur in citrus fruit; they are important micronutrients both for animals and plants. These metals are essential at low concentrations but may become toxic at high levels (McLaughlin et al., 1999; Nielsen, 1999). Some other elements, including Cd(II), Ni(II), and Pb(II), are potentially toxic and can be found in essential oils as a result of a contamination process (Bruno et al., 1978). CEOs are not consumed as such, but small amounts are used as flavors for food, beverages, and cosmetics. Consequently, it would be quite difficult to estimate both heavy metals concentrations that are ingested by humans through alimentary products, which contain essential oils and heavy metals amounts that come in contact with the skin through the use of cosmetic products. Even so, considering the wide diffusion of CEOs, it is of great concern to assess metal levels of nutritional or toxicological interest.

The use of essential oils as food additives is regulated in Italy by D. L. n. 107, 25/01/1992, which conforms with European Council Directives 88/388/CEE and 91/71/CEE regarding the use of aromas in foods and materials for food preparation.

Even if the present legislation sets maximum levels accepted for some heavy metals (mercury, arsenic, and lead) in aromas used in food and pharmaceutical preparations, the most widely used extracting techniques for CEOs cannot avoid contact between the oils and metal surfaces, washing waters, and metal containers. Therefore, contamination with other metals, such as Cr, Fe, and Ni, is quite possible (Kumar et al., 1994).

This kind of contamination was not treated in a previous review (Dugo and Di Bella, 2002).

12.2.1 Analytical Methods

Methods used to evaluate trace elements in CEOs were derivative potentiometric stripping analysis (dPSA) (La Pera et al., 2003), derivative stripping chronopotentiometry (dSCP) (La Pera et al., 2005), graphite furnace atomic absorption spectrometry (GFAAS) (Cautela et al., 2006, 2007), and inductively coupled plasma optical emission spectrometry (ICP-OES) (Cautela et al., 2005, 2006). The first two methods are less expensive with respect to the others, and also maintain detection limits < 1 ng g^{-1} for all the examined elements (La Pera et al., 2003).

12.2.2 Content in Citrus Oils

Table 12.1 summarizes the results reported for the analyses of trace elements in CEOs.

La Pera et al. (2003) analyzed three Sicilian citrus essential oil samples, respectively extracted from lemon, sweet orange, and mandarin, one Calabrian bergamot essential oil sample. All the oils were produced in the crop years 1999–2000. The paper showed the results obtained comparing dPSA and GFAAS and two different extraction procedures involving concentrated hydrochloric acid treatment or acid-alcoholic dissolution. The analyzed elements were Cd(II), Cu(II), Pb(II), and Zn(II). Cd was the element present in the lowest amount and Zn the highest in all types of essential oils. The highest Cd concentration was 23 ng g^{-1} in the bergamot oil. Cu ranged from 17 ng g^{-1} in the lemon essential oil to 380 ng g^{-1} in the bergamot oil. Pb ranged from 76 ng g^{-1} in the sweet orange essential oil to 180 ng g^{-1} in the mandarin oil. Zn ranged from 804 ng g^{-1} in the lemon essential oil to 1647 ng g^{-1} in the sweet orange oil.

La Pera et al. (2005) used dSCP for the determination of Cd, Cu, Mn, Ni, Pb, Zn, and Se in 55 samples of Calabrian bergamot oil (26 produced in 1999 and 29 produced in 2000) and 66 samples of various biological CEOs (35 produced in 2003 and 31 produced in 2004) from the province of Messina (Sicily). This study evidenced that chronopotentiometry allowed the determination of concentrations of Mn(II), Se(IV), and Ni(II) of <0.06 ng g^{-1} in CEOs with a precision that ranged from 2.1% to 3.0%, expressed as mean relative standard deviation of the measurements.

In Calabrian bergamot oils, Mn was the most abundant element found (935–1550 ng g^{-1}), followed by Zn (110–807 ng g^{-1}), Ni (216–401 ng g^{-1}), Cu (76–440 ng g^{-1}), and Pb (40–347 ng g^{-1}); Se was present in very small amounts (max 29.2 ng g^{-1}) and Cd levels were lower than the detection limits in all the samples.

In biological CEOs produced in the province of Messina, Mn ranged between 123 and 1950 ng g^{-1}, Zn between 300 and 1350 ng g^{-1}, Ni between < 0.6 and 1099 ng g^{-1}, Cu between 58.5 and 251 ng g^{-1}, Pb between 26.9 and 201 ng g^{-1}, Se between < 0.05 and 64.3 ng g^{-1}, and Cd was <0.6 ng g^{-1} in all the samples.

Cautela et al. (2005) used ICP-OES to detect Pb, Ba, Co, Cu, Cr, Mn, Cd, Tl, Sb, Ag, Ni, and Be in orange (7 sweet, 3 bitter, and 7 blood samples), lemon (10 samples), and mandarin (10 samples) cold-pressed essential oils produced in Calabria and Sicily in the crop years 2004–2005. These authors found Ag only in one orange (20 ng g^{-1}) and in one lemon sample (40 ng g^{-1}), Cd was below the detection limits in all the samples, Co was present in one orange (130 ng g^{-1}) and in one lemon sample (110 ng g^{-1}), Pb and Sb was present only in one orange sample (320 and 290 ng g^{-1}, respectively), Ba (max 60 ng g^{-1} in a mandarin oil and 180 ng g^{-1} in a lemon oil) and Be (max 10 ng g^{-1} in a mandarin oil) were present in a few samples. Al was not detected in mandarin oils, but it reached values of 2920 and 5440 ng g^{-1} in orange and lemon oils, respectively. The same tendency was evidenced for Cr, Cu, and Fe. Cr was not detected in mandarin oils, but it reached values of 100 and 250 ng g^{-1} in orange and lemon oils, respectively. Cu was found only in one sample of mandarin oil (50 ng g^{-1}) and reached values of 2160 and 370 ng g^{-1} in orange and lemon oils. Fe was found only in one sample of mandarin oil (280 ng g^{-1}), but reached values of 1820 and 2880 ng g^{-1} in orange and lemon oils. Mn was found in many samples: the lowest values were in mandarin oil (max 20 ng g^{-1}) while they were higher in orange (max 610 ng g^{-1}) and lemon (max 450 ng g^{-1}) samples. Ni, Sn, and Zn were found in many samples too: Ni reached 650, 500, and 250 ng g^{-1} in orange, lemon, and mandarin samples, respectively; Sn reached 4530, 2430, and 3730 ng g^{-1} in orange, lemon, and mandarin samples; Zn reached 970, 1540, and 270 ng g^{-1} in orange, lemon, and mandarin samples.

Cautela et al. (2006) investigated the mineral components distribution of cold-pressed bergamot essential oils by three different atomic spectrometry techniques. Ag, Al, Ba, Be, Co, Cu, Cr, Fe, Mn, Ni, Tl, Sb, and Sn were quantified by ICP-OES, while GFAA and flow injection analysis-mercury hydride atomic absorption spectrometry (FI-M/H-AAS) were adopted for the determination of lead, cadmium, and mercury. Al, Sn, and Zn were the elements with greatest concentration present in the analyzed samples, with an average content of 955, 582, and 418 ng g^{-1}, while Fe, Ba, and Mn

TABLE 12.1
Mineral Components Found in Citrus Essential Oils

Reference	Essential Oils Examined (Number of Samples)	Mineral Components
La Pera et al. (2003)*,[2]	Lemon (1)	Cd (1.57), Cu (16.94), Pb (111.24), Zn (802.55).
	Mandarin (1)	Cd (1.81), Cu (277.71), Pb (174.80), Zn (1275.75).
	Sweet orange (1)	Cd (8.96), Cu (83.06), Pb (76.53), Zn (1640.30).
	Bergamot (1)	Cd (22.22), Cu (379.60), Pb (75.56), Zn (821.21).
La Pera et al. (2005)*,[1]	Bergamot (55)	Cd (n.d.), Cu (75.7–440), Pb (40.0–346.9), Zn (110–807), Mn (935–1550), Ni (216–401), Se (n.d.–29.2).
	Biological bergamot (8)	Cd (n.d.), Cu (77.2–203), Pb (26.9–107), Zn (650–1050), Mn (255–1950), Ni (100–600), Se (n.d.).
	Biological citron (4)	Cd (n.d), Cu (155–221), Pb (46.5–112), Zn (535–658), Mn (312–610), Ni (n.d.–375), Se (n.d.–21.6).
	Biological clementine (8)	Cd (n.d.), Cu (107–245), Pb (39.6–78.5), Zn (490–770), Mn (128–1195), Ni (130–170), Se (n.d.–64.3).
	Biological lemon (12)	Cd (n.d.), Cu (60.2–145), Pb (70.2–135), Zn (300–790), Mn (260–1403), Ni (33.1–156), Se (n.d.–16.2).
	Biological mandarin (12)	Cd (n.d.), Cu (116–251), Pb (33.4–85.1), Zn (970–1350), Mn (710–1114), Ni (150–1099), Se (n.d.–23.4).
	Biological blond orange (12)	Cd (n.d.), Cu (77–203), Pb (65.1–196), Zn (832–1235), Mn (458–1401), Ni (n.d.–217), Se (n.d.–13.6).
	Biological red orange (10)	Cd (n.d.), Cu (8.5–191), Pb (65.6–201), Zn (550–819), Mn (123–998), Ni (n.d.–768), Se (n.d.–8.1).
Cautela et al. (2005)**,[1]	Orange (10)	Ag (n.d.–0.02), Al (n.d.–2.92), Ba (n.d.), Be (n.d.), Cd (n.d.), Co (n.d.–0.13), Cr (n.d.–0.10), Cu (n.d.–2.16), Fe (n.d.–1.82), Mn (n.d.–0.61), Ni (n.d.–0.65), Pb (n.d.–0.32), Sb (n.d.–0.29), Sn (n.d.–4.53), Zn (n.d.–0.97).
	Lemon (10)	Ag (n.d.–0.04), Al (0.02–5.44), Ba (n.d.–0.18), Be (n.d.), Cd (n.d.), Co (n.d.–0.11), Cr (n.d.–0.25), Cu (n.d.–0.37), Fe (0.15–2.88), Mn (0.03–0.45), Ni (n.d.–0.5), Pb (n.d.), Sb (n.d.), Sn (n.d.–2.43), Zn (n.d.–1.54).
	Mandarin (10)	Ag (n.d.), Al (n.d.), Ba (n.d.–0.06), Be (n.d.–0.01), Cd (n.d.), Co (n.d.), Cr (n.d.), Cu (n.d.–0.05), Fe (n.d.–0.28), Mn (n.d.–0.02), Ni (n.d.–0.25), Pb (n.d.), Sb (n.d.), Sn (n.d.–3.73), Zn (n.d.–0.27).
Cautela et al. (2006)*,[2]	Bergamot (22)	Al (955), Sn (582), Zn (418), Fe (269), Ba (104), mn (88), Ni (17), Cu (29), Pb (46), Zn (418), Co (n.d.), Be (n.d.), Hg (n.d.), Cd (4).
Cautela et al. (2007)*,[2]	Lemon (15)	Ag (7.2), Al (5064.1), As (7.0), Ba (36), Be (4.12), Cd (1.96), Co (42.3), Cr (82.1), Cu (1043.5), Fe (2336.6), Hg (n.d.), Mn (189.9), Ni (194.2), Pb (5.9), Sb (4.1), Sn (1178), Zn (664.6).
	Mandarin (15)	Ag (0.3), Al (1.4), As (5.2), Ba (24), Be (4.37), Cd (0.59), Co (3.1), Cr (2.2), Cu (2.1), Fe (98.6), Hg (n.d.), Mn (13.8), Ni (182.3), Pb (12.2), Sb (147.4), Sn (3489), Zn (142.7).
	Orange (15)	Ag (7.3), Al (824.6), As (3.1), Ba (20), Be (20.48), Cd (0.62), Co (89.1), Cr (21.0), Cu (1371.4), Fe (1433.1), Hg (n.d.), Mn (308.7), Ni (294.4), Pb (23.2), Sb (59.2), Sn (3632), Zn (822.3).
	Bergamot (15)	Ag (4.1), Al (955.7), As (4.3), Ba (104), Be (0.83), Cd (4.42), Co (5.3), Cr (14.1), Cu (29.3), Fe (269), Hg (n.d.), Mn (88.2), Ni (17.2), Pb (46.1), Sb (63.3), Sn (582), Zn (418.4).

*ng g^{-1}; ** μg g^{-1}
Authors considered ranges[1] or means[2].
n.d.= not detectable.

assessed on average contents of 269, 104, and 88 ng g^{-1}. Ni and Cu assessed on average contents of 17 and 29 ng g^{-1}, while the average content of Pb and Zn was 46 e 418 ng g^{-1}, respectively. In all the analyzed samples, the Co, Be, and Hg concentrations resulted lower than limits of quantification, while Cd was present at an average concentration of 4 ng g^{-1}.

Cautela et al. (2007) used GFAA and FI-M/H-AAS to analyze Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sn, and Zn in 60 cold-pressed CEO samples extracted from mandarin, lemon, orange, and bergamot (15 samples each) produced in Calabria and Sicily during the crop years 2003, 2004, and 2005.

The results showed that Hg was not detected in all the samples (< 0.2 ng g^{-1}). Ag, As, Cd were in low concentrations (< 60 ng g^{-1}). Pb concentrations were usually < 50 ng g^{-1}, except in a few orange and bergamot oils. Other elements showed significant differences in the concentrations amongst the oils: Al was present in high concentrations in lemon oils (mean 5064 ng g^{-1}) but not in mandarin ones (mean 1.4 ng g^{-1}); Ba had a mean value of 36, 24, and 20 ng g^{-1} in lemon, mandarin, and orange oil, respectively, but it reached a mean of 104 ng g^{-1} in bergamot ones; Be had a mean value of 4.12, 4.37, and 0.83 ng g^{-1} in lemon, mandarin, and bergamot oil, respectively, but it reached a mean of 20.48 ng g^{-1} in orange ones; Co concentrations were low in mandarin and bergamot samples (mean 3.1 and 5.3 ng g^{-1}) but not in lemon and orange ones (mean 42.3 and 89.1 ng g^{-1}); Cr concentrations were low in mandarin, orange, and bergamot samples (mean 2.2, 21.0, and 14.1 ng g^{-1}), but not in lemon ones (mean 182.1 ng g^{-1}); Cu was present in high concentrations in lemon and orange oils (mean 1043 and 1371 ng g^{-1}), but not in mandarin and bergamot ones (mean 2.1 and 29.3 ng g^{-1}); Fe was present in high concentrations in lemon and orange oils (mean 2336 and 1433 ng g^{-1}), but not in mandarin and bergamot ones (mean 98.6 and 269 ng g^{-1}); Mn concentrations in mandarin oils were lower than those relative to lemon, orange, and bergamot ones (mean 13.8 ng g^{-1} with respect to 189.9, 308.7, and 88.2, respectively); Ni concentrations in bergamot oils were lower than those relative to lemon, mandarin, and orange ones (mean 17.2 ng g^{-1} respect to 194.2, 182.3, and 294.4, respectively); Sb concentrations in lemon oils were lower than those relative to mandarin, orange, and bergamot ones (mean 4.1 ng g^{-1} with respect to 147.4, 59.2, and 63.3, respectively); Sn concentrations in bergamot oils were lower than those relative to lemon, mandarin, and orange ones (mean 582 ng g^{-1} with respect to 1178, 3489, and 3632, respectively); Ni concentrations in mandarin oils were lower than those relative to lemon, orange, and bergamot ones (mean 142.7 ng g^{-1} with respect to 664.6, 822.3, and 418.4, respectively).

All the examined researches established that the elements concentrations in CEOs did not represent a risk for human health.

12.3 PESTICIDES

12.3.1 Organophosphorus Pesticides

In a previous review (Dugo and Di Bella, 2002), organophosphorus pesticides have been identified in essential oils from several species of citrus fruits. Unfortunately, this kind of contamination is still present in traditional and biological oils; the presence of these contaminants might be the result of an improper use of pesticide in citrus growing or maybe due to previous contamination of the extractors.

12.3.1.1 Analytical Methods

Fundamentally, no improvements in the methods have been done in recent years. Organophosphorus pesticides were analyzed in CEOs by gas chromatography (GC) with nitrogen phosphorus detector (NPD) or flame photometric detector (FPD), without extraction (Dugo and Di Bella, 2002).

12.3.1.2 Content in Citrus Oils

In Table 12.2 the results of the analyses of the organophosphorus pesticides in CEOs are reported.

Ten samples each of lemon and sweet orange essential oils industrially extracted from fruits grown under biological techniques and in parallel those extracted from fruits grown with traditional

TABLE 12.2
Organophosphorus Pesticides Found in Citrus Essential Oils

Reference	Essential Oils Examined (Number of Samples)	Pesticide Residues (Concentration, ppm)
Verzera et al. (2004)[1]	Biological sweet orange (10)	Chlorpyriphos ethyl (n.d.–0.09), Methidathion (n.d.–0.18).
	Traditional sweet orange (10)	Chlorpyriphos ethyl (0.06–0.15), Methidathion (1.15–1.21), Parathion ethyl (0.45–0.53), Parathion methyl (0.89–0.97).
	Biological lemon (10)	Methidathion (n.d.–0.29), Parathion ethyl (n.d.–0.15), Parathion methyl (0.17–0.30).
	Traditional lemon (10)	Azinphos ethyl (0.48–0.53), Chlorpyriphos methyl (0.04–0.10), Methidathion (0.82–0.86), Parathion ethyl (0.65–0.70), Parathion methyl (1.40–1.46), Quinalphos (0.13–0.20).
Di Bella et al. (2004)[1]	Bergamot (55)	No residues detected.
Di Bella et al. (2006)[1]	Biological lemon (16)	Azinphos methyl (n.d.–0.30), Methidathion (n.d.–0.99), Parathion ethyl (n.d.–0.08), Parathion methyl (n.d.–0.14).
	Biological orange (26)	Azinphos methyl (n.d.–0.16), Chlorpyriphos ethyl (n.d.–2.18), Chlorpyriphos methyl (n.d.–0.10), Methidathion (n.d.–2.62), Parathion ethyl (n.d.–0.06), Parathion methyl (n.d.–0.16), Quinalphos (n.d.–0.24).
	Biological clementine (3)	Azinphos methyl (n.d.–0.04), Chlorpyriphos ethyl (n.d.–0.02), Methidathion (n.d.–0.34), Parathion ethyl (n.d.–0.25), Parathion methyl (n.d.–0.09).
	Biological mandarin (5)	Azinphos methyl (n.d.–0.81), Chlorpyriphos ethyl (n.d.–0.05), Methidathion (n.d.–0.09), Parathion ethyl (n.d.–0.04), Parathion methyl (n.d.–0.07).
Gionfriddo and Postorino (2009)[1]	Mandarin (44)	Azinphos methyl (n.d.–1.31), Chlorfenvinphos (n.d.–2.61), Chlorpyriphos ethyl (n.d.–1.61), Chlorpyriphos methyl (n.d.–4.63), Diazinon (n.d.–0.16), Dichlofenthion (n.d.–0.01), Disulfoton (n.d.–1.40), Ethion (n.d.–0.01), Fenchlorfos (n.d.–0.02), Imidan (n.d.–0.92), Malathion (n.d.–1.04), Parathion ethyl (n.d.–0.50), Parathion methyl (n.d.–3.69), Prothiophos (n.d.–0.02), Terbufos (n.d.–0.04), Tetrachlorvinphos (n.d.–0.10), Tokuthion (n.d.–0.44), Trichloroate (n.d.–0.10).
Di Bella et al. (2009)[2]	Lemon (Italy, 30)	Azinphos methyl (0.55), Chlorpyriphos ethyl (0.20), Chlorpyriphos methyl (0.20), Methidathion (0.55), Pyridafenthion (0.35).
	Lemon (Brazil, 10)	Chlorpyriphos ethyl (2.22).
	Lemon (Argentine, 10)	Chlorpyriphos ethyl (1.99).
	Lemon (South Africa, 10)	Chlorpyriphos ethyl (0.28), Methidathion (1.50).
	Orange (Italy, 30)	Azinphos methyl (0.55), Chlorpyriphos ethyl (0.20), Chlorpyriphos methyl (0.20), Methidathion (0.55), Pyridafenthion (0.35).
	Orange (Brazil, 10)	Chlorpyriphos ethyl (0.60), Malathion (0.15), Methidathion (4.59).
	Green mandarin (Italy, 15)	No residues detected.
	Green mandarin (Brazil, 10)	Azinphos methyl (0.22), Chlorpyriphos ethyl (0.08), Methidathion (0.34), Pyridafenthion (0.19).
	Yellow mandarin (Italy, 15)	Chlorpyriphos ethyl (2.19), Chlorpyriphos methyl (0.94), Phentoate (0.20), Pyridafenthion (0.15).
	Yellow mandarin (Brazil, 10)	Chlorpyriphos ethyl (2.63), Malathion (4.80).
	Clementine (Spain, 10)	Chlorpyriphos ethyl (8.50), Fenitrothion (2.00), Fenthion (3.96), Methidathion (4.81).
	Bergamot (Italy, 30)	No residues detected.

Authors considered ranges[1] or means[2].
n.d.= not detectable.

methods (with the fruits picked in January 2002 from the province of Syracuse, Sicily) were analyzed by Verzera et al. (2004). Results showed that the sweet orange oils obtained from biological fruits were almost free of organophosphorus pesticides, and only methidathion and chlorpyriphos ethyl were detected, with a maximum total content of 0.27 mg L^{-1}. In traditional sweet orange oils, parathion methyl, parathion ethyl, methidathion, and chlorpyriphos ethyl were found, and the total content ranged from 2.55 to 2.86 mg L^{-1}. Parathion methyl, parathion ethyl, and methidathion were identified in lemon oils obtained from biological farming, with a maximum total content of 0.74 mg L^{-1}. In traditional lemon oils, parathion methyl, parathion ethyl, quinalphos, methidathion, chlorpyriphos methyl, and azinphos ethyl were found, and the total content ranged from 3.52 to 3.85 mg L^{-1}.

Di Bella et al. (2004) analyzed 55 bergamot essential oils produced in the Ionic Coast of the province of Reggio Calabria (Italy) during the crop years 1999–2000. All the samples resulted free of organophosphorus pesticides, confirming a trend found in previous studies.

Various CEOs from biological cultivation were analyzed by Di Bella et al. (2006). Oils were produced in 2003 (40 samples: 13 lemon, 21 orange, 2 clementine, and 4 mandarin) and 2004 (10 samples: 3 lemon, 5 orange, 1 clementine, and 1 mandarin). Methidathion was the most widely detected contaminant in lemon essential oil from 2003 (mean level 0.60 mg L^{-1}); the values were below the limit of detection in lemon oil samples from 2004. In orange oils, azinphos methyl (mean level 0.09 mg L^{-1}) and chlorpyriphos ethyl (0.53 mg L^{-1}) were widely recovered in samples of 2003, while chlorpyriphos ethyl and methidathion were found in samples from 2004. In the clementine samples from 2003, azinphos methyl was recovered (mean level 0.04 mg L^{-1}), while in the clementine essential oil sample from 2004, azinphos methyl was present (0.09 mg L^{-1}), as well as parathion ethyl (0.25 mg L^{-1}) and methidathion (0.34 mg L^{-1}). In the mandarin samples from 2003, parathion methyl (mean level 0.05 mg L^{-1}), chlorpyriphos ethyl (mean level 0.04 mg L^{-1}), parathion ethyl (mean level 0.03 mg L^{-1}), methidathion (mean level 0.07 mg L^{-1}), and azinphos methyl (mean level 0.30 mg L^{-1}) were recovered, while in the mandarin essential oil sample from 2004, the values were below the limit of detection. A chromatogram of a contaminated mandarin oil is reported in Figure 12.1.

Gionfriddo and Postorino (2009) analyzed 44 samples of industrial mandarin essential oil from Calabrian fruits obtained by cold press technology during the 2005–2006 season (25 samples) and 2006–2007 season (19 samples). The authors evidenced that all the samples contained organophosphorus pesticides. In particular, several 2005–2006 samples were contaminated by chlorfenvinphos (mean value 0.56 mg L^{-1}), chlorpyriphos ethyl (mean value 0.11 mg L^{-1}), chlorpyriphos methyl (mean value 1.61 mg L^{-1}), malathion (mean value 0.11 mg L^{-1}), terbufos (mean value 0.01 mg L^{-1}), and tokuthion (mean value 0.07 mg L^{-1}). In the 2006–2007 samples, chlorpyriphos ethyl (mean

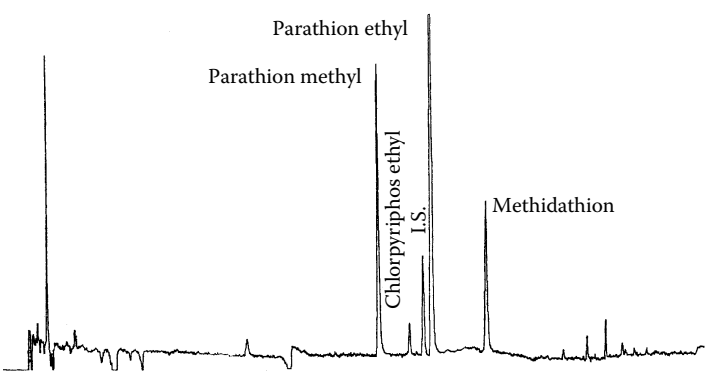

FIGURE 12.1 HRGC-FPD chromatogram of a contaminated Italian mandarin oil.

value 0.98 mg L^{-1}), malathion (mean value 0.36 mg L^{-1}), parathion methyl (mean value 0.68 mg L^{-1}), and tetrachlorvinphos (mean value 3.09 mg L^{-1}) were the pesticides found more frequently.

Di Bella et al. (2009) compared the contamination of 120 CEOs produced in Italy with 70 samples from other countries. Samples (from Italy: 30 lemon, 30 orange, 15 green mandarin, 15 yellow mandarin, 30 bergamot; from Spain: 10 clementine; from Brazil: 10 lemon, 10 orange, 10 green mandarin, 10 yellow mandarin; from Argentina: 10 lemon; from South Africa: 10 lemon) were produced in the 2006–2007 season.

Most of the Italian lemon oils were contaminated by organophosphorus pesticides: in particular, 80% of these oils showed a methidathion mean content of 0.55 mg L^{-1}, a chlorpyriphos methyl mean content of 0.20 mg L^{-1}, and a chlorpyriphos ethyl mean content of 0.20 mg L^{-1}. In the lemon oils from Brazil and Argentina, only chlorpyriphos ethyl was found (mean content 1.99 mg L^{-1}) while in the South Africa samples, chlorpyriphos ethyl (mean content 0.28 mg L^{-1}) and methidathion (mean content 1.50 mg L^{-1}) were found.

In the orange oils from Italy, chlorpyriphos ethyl and chlorpyriphos methyl were at high concentrations (mean 1.44 and 1.56 mg L^{-1}, respectively); azinphos methyl was present at smaller level (mean 0.24 mg L^{-1}), but in a great number of samples. In the Brazilian orange oils, methidathion was detected in high content (mean value 4.59 mg L^{-1}).

In the Italian yellow mandarin oils, chlorpyriphos ethyl was found in 20% of the samples with a mean concentration of 2.19 mg L^{-1}; in the oils from Brazil, chlorpyriphos ethyl (mean value 2.63 mg L^{-1}) and malathion (mean value 4.8 mg L^{-1}) were detected in 70%–80% of the samples.

Residues of organophosphorus pesticides were not found in the green mandarin oils from Italy. On the contrary, in the Brazilian samples it was possible to observe that methidathion was present in 40% of the oils (mean value 0.34 mg L^{-1}), azinphos methyl in 60% of the oils (mean value 0.22 mg L^{-1}) and chlorpyriphos ethyl in 60% of the oils (mean value 0.08 mg L^{-1}).

Italian bergamot oils showed no organophosphorus contamination.

The clementine oils from Spain had high level of contamination: 40% of the oils contained methidathion (mean level 4.81 mg L^{-1}), 70% contained fenthion (mean level 3.96 mg L^{-1}), and 80% contained chlorpyriphos ethyl (mean level 8.50 mg L^{-1}).

12.3.2 Organochlorine Pesticides

In a previous review (Dugo and Di Bella, 2002), organochlorine pesticides have been identified in essential oils from several species of citrus fruits. Unfortunately, this kind of contamination is still present in traditional and biological oils.

12.3.2.1 Analytical Methods

Analyses were carried out with a cleanup procedure and a detection with GC-ECD, sometimes using GC-MS to confirm the peak assignment (Dugo and Di Bella, 2002).

12.3.2.2 Content in Citrus Oils

In Table 12.3 the results of the analyses of the organochlorine pesticides in CEOs are reported.

Verzera et al. (2004) analyzed lemon and sweet orange essential oils (10 samples each of lemon and sweet orange oils industrially extracted from fruits grown under biological techniques, and 10 samples each of lemon and sweet orange oils extracted from fruits grown with traditional methods; the fruits were picked in January 2002 and came from the province of Syracuse in Sicily).

Dicofol was detected only in the lemon and sweet orange oils obtained from fruits grown under traditional agricultural methods, with maximum values of 1.0 mg L^{-1} and 1.2 mg L^{-1}, respectively.

Di Bella et al. (2004) analyzed 55 bergamot essential oils produced in the Ionic Coast of the province of Reggio Calabria in the crop years 1999–2000. Tetradifon (mean values 0.06 mg L^{-1} in

TABLE 12.3
Organochlorine Pesticides Found in Citrus Essential Oils

Reference	Essential Oils Examined (Number of Samples)	Pesticide Residues (Concentration, ppm)
Verzera et al. (2004)[1]	Biological sweet orange (10)	No residues detected.
	Traditional sweet orange (10)	Dicofol (n.d.–1.2).
	Biological lemon (10)	No residues detected.
	Traditional lemon (10)	Dicofol (n.d.–1.0)
Di Bella et al. (2004)[1]	Bergamot (55)	4,4'-Dichlorobenzophenone (n.d.–0.32), Dicofol (n.d.–0.58), Tetradifon (n.d.–0.11).
Di Bella et al. (2006)[1]	Biological lemon (16)	Dicofol (n.d.–2.14), Tetradifon (n.d.–0.27).
	Biological orange (26)	Dicofol (n.d.–7.92), Tetradifon (n.d.–1.70).
	Biological clementine (3)	Tetradifon (n.d.–0.02).
	Biological mandarin (5)	Tetradifon (n.d.–0.05).
Di Bella et al. (2009)[2]	Lemon (Italy, 30)	Dicofol (0.41).
	Lemon (Brazil, 10)	Dicofol (0.60).
	Lemon (Argentine, 10)	Dicofol (0.80).
	Lemon (South Africa, 10)	Dicofol (2.50).
	Orange (Italy, 30)	No residues detected.
	Orange (Brazil, 10)	Bromopropilate (0.39), Dicofol (3.88).
	Green mandarin (Italy, 15)	No residues detected.
	Green mandarin (Brazil, 10)	No residues detected.
	Yellow mandarin (Italy, 15)	Dicofol (0.61).
	Yellow mandarin (Brazil, 10)	No residues detected.
	Clementine (Spain, 10)	Bromopropilate (3.50), Dicofol (2.00).
	Bergamot (Italy, 30)	No residues detected.

Authors considered ranges[1] or means[2].
n.d.= not detectable.

1999 and 0.06 mg L^{-1} in 2000), dicofol (mean values 0.26 mg L^{-1} in 1999 and 0.20 mg L^{-1} in 2000), and its decomposition product, 4,4′-dichlorobenzophenone (mean values 0.16 mg L^{-1} in 1999 and 0.09 mg L^{-1} in 2000), were detected in many samples. The contamination by organochlorine pesticides in bergamot essential oils from different crop years appeared essentially constant.

Di Bella et al. (2006) analyzed various CEOs from biological cultivation. Oils were produced in 2003 (40 samples: 13 lemon, 21 orange, 2 clementine and 4 mandarin) and 2004 (10 samples: 3 lemon, 5 orange, 1 clementine, and 1 mandarin). In the 2003 lemon samples, there was a little contamination (7.7% of the oils) due to dicofol (mean value 2.14 mg L^{-1}) and a more diffuse, but less intense contamination (76.9% of the oils), due to tetradifon (mean value 0.16 mg L^{-1}). The 2004 lemon samples were virtually free of organochlorine pesticides. The percentage of the contaminated orange oils were lower than the lemon ones in 2003, but the mean levels of dicofol and tetradifon were higher (4.77 and 0.41 mg L^{-1}, respectively). In the 2004 orange oils, more samples were contaminated, but the concentrations decreased (mean value 0.84 and 0.03 mg L^{-1}, respectively). The clementine and mandarin samples showed only a very little tetradifon contamination.

Di Bella et al. (2009) investigated the contamination of 120 CEOs produced in Italy and 70 samples from other countries. Samples (from Italy: 30 lemon, 30 orange, 15 green mandarin, 15 yellow mandarin, 30 bergamot; from Spain: 10 clementine; from Brazil: 10 lemon, 10 orange, 10 green mandarin, 10 yellow mandarin; from Argentina: 10 lemon; from South Africa: 10 lemon) were produced in the 2006–2007 season. About 50% of the Italian lemon oils and 33% of the Italian

yellow mandarin oils were contaminated by dicofol (mean values 0.41 and 0.61 mg L^{-1}, respectively); dicofol was detected in 40% of the Brazilian lemon oils (mean concentration 0.60 mg L^{-1}), in 60% of the Argentinian lemon oils (mean concentration 0.80 mg L^{-1}) and in 50% of the South African lemon oils (mean concentration 2.50 mg L^{-1}). In Brazilian orange oils, 20% of the samples were contaminated by bromopropilate (mean concentration 0.39 mg L^{-1}) and 50% by dicofol (mean concentration 3.88 mg L^{-1}). Yellow and green mandarin oils showed no residues of organochlorine pesticides and clementine oils from Spain showed most intense contamination, in 50% of the samples by bromopropilate (mean concentration 3.50 mg L^{-1}), and in 70% of the samples by dicofol (mean concentration 2.00 mg L^{-1}).

12.4 PLASTICIZERS

The plasticizers are compounds used to improve the characteristic of the plastic materials; they are added to make more elastic plastics. Different kinds of plasticizers are triarylphosphates, chloroparaffins, phthalates, adipates, and sebacates. Essential oils can extract plasticizers coming into contact with plastic materials.

Table 12.4 summarizes the results reported for the analyses of plasticizers in CEOs.

12.4.1 Triarylphosphates

These plasticizers are usually composed of seven different classes of triarylphosphates: triphenylphosphate, diphenyltolylphosphates, phenylditolylphosphates, tritolylphosphates, ditolylxylylphosphates, tolyldixylylphosphates, and trixylylphosphates. These compounds are present only in some plastic materials (Dugo and Di Bella, 2002).

12.4.1.1 Analytical Methods

Essential oils are directly analyzed by GC with phosphorus-sensitive detectors such as NPD or FPD (Dugo and Di Bella, 2002).

12.4.1.2 Content in Citrus Oils

Three papers investigated the presence of triarylphosphate plasticizers in bergamot oils (Di Bella et al., 2004); biological lemon, orange, clementine and mandarin oils (Di Bella et al., 2006); and various CEOs from Italy, Brazil, Argentina, South Africa, and Spain (Di Bella et al., 2009). In these studies, no residues of triarylphosphates were detected in the samples (values < 0.01 mg L^{-1}).

12.4.2 Chloroparaffins

These compounds are complex mixtures of chloroalkanes, used as secondary plasticizers in plastic materials as PVC (Dugo and Di Bella, 2002).

12.4.2.1 Analytical Methods

Essential oils are previously purified by column chromatography and then analyzed by GC with electron capture detector (ECD) (Dugo and Di Bella, 2002).

12.4.2.2 Content in Citrus Oils

Di Bella et al. (2004) reported that in bergamot essential oils from Calabria, no residues of chloroparaffins were detected (<0.30 mg L^{-1}). The same results were obtained analyzing several different CEOs of different origin (Di Bella et al., 2009).

Chloroparaffins were found in biological CEOs from 2003 to 2004 by Di Bella et al. (2006). These plasticizers contaminated about 54% of the 2003 lemon oils, 67% of the 2004 lemon oils, and 25% of the 2003 mandarin oils, with mean concentrations of 1.97, 0.92, and 0.33 mg L^{-1}, respectively. No residues were found in orange and clementine oils.

TABLE 12.4
Plasticizers Found in Citrus Essential Oils

Reference	Essential Oils Examined (Number of Samples)	Plasticizer Residues (Concentration, ppm)
Di Bella et al. (2004)[1]	Bergamot (55)	Di-isobutyl phthalate (n.d.–2.40), Di-*n*-butyl phthalate (n.d.–4.45), Bis-(2-ethyl-hexyl) phthalate (n.d.–3.08). No residues of chloroparaffins and phosphorated plasticizers were detected.
Di Bella et al. (2006)[1]	Biological lemon (16)	Chloroparaffins (n.d.–3.09), Diethyl phthalate (n.d.–0.62), Di-isobutyl phthalate (n.d.–1.09), Di-*n*-butyl phthalate (n.d.–0.21), Di-*n*-octyl phthalate (n.d.–1.16), Di-*n*-butyl adipate (n.d.–0.21), Bis-(2-ethyl-hexyl) adipate (n.d.–0.10).
	Biological orange (26)	Diethyl phthalate (n.d.–0.38), Di-isobutyl phthalate (n.d.–1.69), Di-*n*-butyl phthalate (n.d.–0.25), Di-*n*-octyl phthalate (n.d.–2.27), Di-n-butyl adipate (n.d.–0.25), Bis-(2-ethyl-hexyl) adipate (n.d.–0.12).
	Biological clementine (3)	Diethyl phthalate (n.d.–0.10), Di-isobutyl phthalate (n.d.–2.42), Di-*n*-butyl phthalate (n.d.–0.22), Di-*n*-octyl phthalate (n.d.–2.10), Di-*n*-butyl adipate (n.d.–0.80).
	Biological mandarin (5)	Chloroparaffins (n.d.–0.33), Diethyl phthalate (n.d.–0.56), Di-isobutyl phthalate (n.d.–1.29), Di-*n*-butyl phthalate (n.d.–0.20), Di-*n*-octyl phthalate (n.d.–4.55), Bis-(2-ethyl-hexyl) adipate (n.d.–0.09).
Di Bella et al. (2009)[2]	Lemon (Italy, 30)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Lemon (Brazil, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Lemon (Argentine, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Lemon (South Africa, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Orange (Italy, 30)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Orange (Brazil, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Green mandarin (Italy, 15)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Green mandarin (Brazil, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Yellow mandarin (Italy, 15)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Yellow mandarin (Brazil, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Clementine (Spain, 10)	No residues of chloroparaffins and phosphorated plasticizers were detected.
	Bergamot (Italy, 30)	Chloroparaffins, 5.00 ppm in one sample of the 30 analyzed.

Authors considered ranges[1] or means[2].
n.d.= not detectable.

12.4.3 Phthalates, Adipates, Sebacates

Phthalates are the most common plasticizers. In this class of compounds, bis-(2-ethyl-hexyl)-phthalate is the more used compound (Dugo and Di Bella, 2002). Other substances used as plasticizers are the esters of the dicarboxylic acids adipic (hexanedioic) and sebacic (decanedioic).

12.4.3.1 Analytical Methods

Essential oils are directly analyzed by GC-MS in selected ion monitoring (SIM) mode (Dugo and Di Bella, 2002).

12.4.3.2 Content in Citrus Oils

Di Bella et al. (2004) investigated the presence of phthalates in bergamot oils in the crop years 1999–2000. Essential oils were contaminated by di-isobutyl phthalate, di-*n*-butyl phthalate, and bis-(2-ethyl-hexyl) phthalate in a homogeneous way; mean concentrations for the three phthalates were 1.22, 1.51, and 1.38 mg L^{-1} in the oils produced in 1999, and 1.25, 1.65, and 1.42 mg L^{-1} in the oils produced in 2000. In Figure 12.2 a chromatogram of a contaminated bergamot oil is reported.

In 50 different biological CEOs, Di Bella et al. (2006) looked for the phthalates, adipates, and sebacates contamination. In essential oils from 2003, diethyl phthalate (mean values: lemon 0.35 mg L^{-1}, orange 0.12 mg L^{-1}, clementine 0.10 mg L^{-1}, mandarin 0.49 mg L^{-1}), di-isobutyl phthalate (mean values: lemon 0.56 mg L^{-1}, orange 0.52 mg L^{-1}, mandarin 1.11 mg L^{-1}), and di-*n*-octyl phthalate (mean values: lemon 0.59 mg L^{-1}, orange 1.69 mg L^{-1}, clementine 0.49 mg L^{-1}, mandarin 2.76 mg L^{-1}) were very frequently found, while di-*n*-butyl adipate (mean values: lemon 0.30 mg L^{-1}, orange 0.18 mg L^{-1}, clementine 0.80 mg L^{-1}) and bis-(2-ethyl-hexyl) adipate (mean values: lemon 0.10 mg L^{-1}, orange

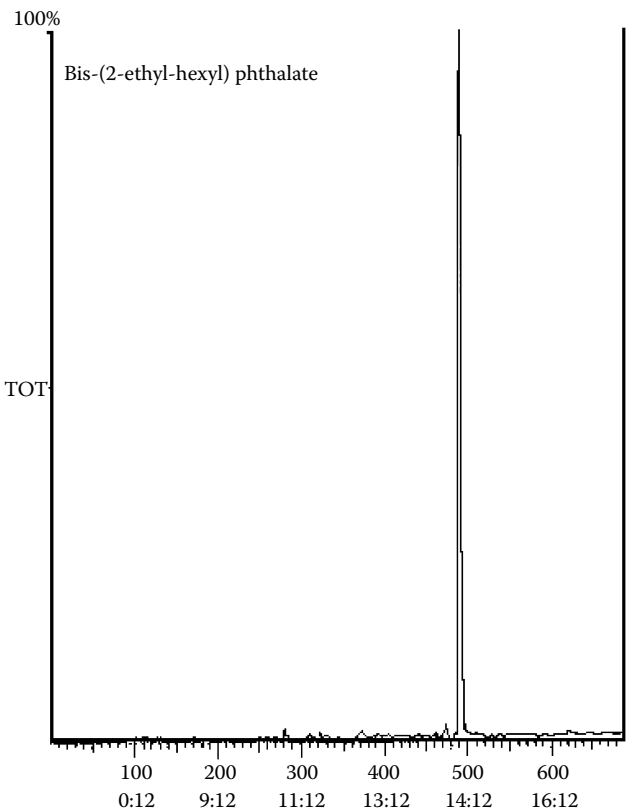

FIGURE 12.2 HRGC-MS chromatogram of a contaminated bergamot essential oil.

0.11 mg L^{-1}, mandarin 0.09 mg L^{-1}) were occasionally found. In essential oil samples from 2004, di-isobutyl phthalate (mean values: lemon 0.43 mg L^{-1}, orange 0.80 mg L^{-1}, clementine 2.42 mg L^{-1}) and di-n-octyl phthalate (mean values: lemon 0.71 mg L^{-1}, orange 1.23 mg L^{-1}, clementine 2.10 mg L^{-1}) residues were recovered, while di-*n*-butyl adipate residues were not found. The levels of sebacate esters were below the detection limits.

12.5 POLYCHLORINATED DIBENZO-*P*-DIOXINS, POLYCHLORINATED DIBENZO-*P*-FURANS, POLYCHLORINATED BYPHENYLS

PCDDs and PCDFs are organic environmental contaminants that are extremely stable and ubiquitous, usually produced during the combustion of municipal and industrial waste. Combustion sources emit large quantities of these compounds into the atmosphere, where they are dispersed as vapor and particulates, leading to their ubiquitous presence in the environment. Some of these pollutants are very toxic (2,3,7,8-chlorine substituted congeners), stable, and hydrophobic, so they accumulate in fatty animal tissues and, therefore, in the human food chain.

12.5.1 Analytical Methods

The analytical method used consists of three steps of extraction, cleanup, and quantification by gas chromatography with high-resolution mass spectrometry (GC-HRMS) (Cautela et al. 2007).

12.5.2 Content in Citrus Oils

Cautela et al. (2007) verified the PCDDs, PCDFs and PCBs contamination of 60 CEOs produced in Calabria and Sicily in 2003–2005. Results showed that the sum of toxic PCDDs (mean values) was 15.24 pg g^{-1} in the lemon samples, 14.39 pg g^{-1} in the mandarin samples, 12.16 pg g^{-1} in the orange samples, and 10.72 pg g^{-1} in the bergamot samples; the sum of toxic PCDFs (mean values) was 13.69 pg g^{-1} in the lemon samples, 12.58 pg g^{-1} in the mandarin samples, 8.81 pg g^{-1} in the orange samples, and 8.99 pg g^{-1} in the bergamot samples. The sum of PCBs (mean values) was 226 pg g^{-1} in the lemon samples, 410 pg g^{-1} in the mandarin samples, 387 pg g^{-1} in the orange samples, and 249 pg g^{-1} in the bergamot samples.

The PCB contamination was predominant with respect to the levels of PCDD and PCDF, but from a toxicological point of view, the PCDD concentrations were more important. Moreover, from a comparison of total mean concentrations of PCDDs and PCDFs found in the four different types of CEOs analyzed, it was clear that PCDD concentration was higher than PCDF contamination; these data and the relatively high concentrations of the not toxic octachloro dibenzo-*p*-dioxin indicated background contamination of rural areas.

12.6 CHLOROHYDRINS

These compounds can be produced by a reaction between D-limonene and chlorine-treated water used in the oil extraction plants. The reaction involves D-limonene and hypochlorous acid (HOCl) to produce 2-chlorohydrins and 2,9-dichlorohydrin. Potential sources of HOCl include chlorinated treatment water used in the oil-recovery process and sanitizers used in postharvest handling and process equipment cleaning.

12.6.1 Analytical Methods

Chlorohydrins were detected by direct analysis of the CEOs, without extraction and cleanup procedures, using a gas chromatograph equipped with on-column injector and electrolytic conductivity detector (ELCD). The column was a polar DB-WAX, and 2,3-dichloroaniline was used as internal

standard (Weiss et al., 2003a,b). Nuclear magnetic resonance analyses indicated that the major chlorohydrin present was the diequatorially substituted (1R,2R,4R)-2-chloro-8-*p*-menthen-1-ol. The other two compounds detected were the diaxial trans stereoisomer (1S,2S,4R)-2-chloro-8-*p*-menthen-1-ol and the dichlorohydrin (1R,2R,4R)-2,9-dichloro-8-*p*-menthen-1-ol (Weiss et al., 2003b).

12.6.2 Content in Citrus Oils

Weiss et al. (2003b) analyzed 52 commercial citrus oils for the presence of (1R,2R,4R)-2-chloro-8-*p*-menthen-1-ol. Of the oils tested, 35 were found to contain <2 ppm, 15 contained between 2 and 25 ppm, and one commercial folded orange oil contained 160 ppm. Other chlorohydrins were found in low concentrations.

In another study, Weiss et al. (2003a) tested 70 oils (grapefruit, lemon, and orange). Results show a range of <1 to 140 ppm of (1R,2R,4R)-2-chloro-8-*p*-menthen-1-ol. Fifty oils contained less than 2 ppm of this chlorohydrin. Only in a few oils were high concentrations of (1R,2R,4R)-2-chloro-8-*p*-menthen-1-ol found, and a Valencia orange oil contained 140 ppm of this compound. Pilot plant studies indicated that a total residual chlorine concentration of 30 ppm in oil-recovery water produced cold-pressed orange oils containing terpene chlorohydrins at threshold levels of approximately 0.5 ppm. No chlorohydrins were detected at potable water use levels.

Braddock and Goodrich (2005) verified a method to decrease the chlorohydrins content using a reaction with dilute solutions of KOH. Cold-pressed orange oils with low (1 to 5 ppm) and high chlorohydrin levels (20 to 30 ppm) showed, after the reaction, no residues of (1R,2R,4R)-2-chloro-8-*p*-menthen-1-ol and (1R,2R,4R)-2,9-dichloro-8-*p*-menthen-1-ol, while the treatment had very little effect on (1S,2S,4R)-2-chloro-8-*p*-menthen-1-ol. The authors affirmed that this process did not significantly alter the composition or sensory quality of the oils treated in this manner.

12.7 SPINOSAD

The spinosyns are compounds derived from a naturally occurring Actinomycetes bacterium, *Saccharopolyspora spinosa*; spinosad (a mixture of spinosyns A and D) is the common name of the active material that is derived from a fermentation broth. Spinosad is used for the management of insect pests in citrus crops and than residues can be found in CEOs.

12.7.1 Analytical Methods

Two different extractions are requested to purify the CEOs samples: the first step involves a silica solid phase extraction (SPE) procedure and the subsequent second step requires a cyclohexyl SPE tube. High performance liquid chromatography (HPLC) with UV detection is used to evaluate the spinosad residues (West and Turner, 2000), with OctaDecylSilyl-type columns and isocratic elution [44% CH_3OH, 44% CH_3CN and 12% of a mixture CH_3CN (2%): aqueous ammonium acetate (33:67)].

12.7.2 Content in Citrus Oils

West and Turner (2000) established the method for the determination of spinosad and its metabolites in orange oils, but no data on the residues of these compounds in real CEOs samples were available.

12.8 CONCLUSIONS

It is clear that researches are able to detect several types of contamination in CEOs, and new ones are to be expected in the coming years. The employment of state-of-the-art instruments guarantees very low detection limits and high precisions. From a qualitative point of view, CEOs actually appear more contaminated in respect to the past. Nevertheless, the reported data show that CEOs

are today less contaminated in respect to 10 years ago: pesticide and plasticizer concentrations have decreased; heavy metals, dioxins, and chlorohydrins are not in such quantity to justify alarmisms.

In conclusion, the investigations about the CEOs contamination evidenced a qualitative problem, but not a quantitative one.

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13 Biological Activities of Citrus Essential Oils

Giuseppe Bisignano, Francesco Cimino, Antonella Saija

CONTENTS

13.1 INTRODUCTION

Citrus essential oils extracted from the whole fruit and from the pulp-deprived peel of different species of citrus are widely used as flavoring agents in a number of food, beverage, and confectionery products and fragrance application.

In particular, lemon and bergamot essences are used in the cosmetic industry, for the production of perfumes, detergents, and body-care products, mostly because of their fragrance and solvent properties.

Citrus oils are present in various pharmaceutical preparations for gynecological, ophthalmic and surgical use, and also in dentistry, because of their well-known antiseptic properties. Apart from being used as disinfectants, recent studies point to the possibility of employing citrus oils and/or their active principles to prevent or treat several pathological conditions in humans, as preservatives in the food industry, and as alternative pesticides in integrated programs.

However, citrus oils are rarely utilized as such in the pharmacotherapeutic field, and much has been learned about the biological properties of the active principles isolated from these oils. As a result, some of these active principles (or compounds derived from them) are being successfully employed in therapy (e.g., 5-methoxypsoralen in the Psoralen-Ultraviolet A-Therapy (PUVA), used to treat psoriasis and vitiligo).

Finally, extensive toxicological studies have proved to be fundamental in regulating, on the basis of scientific criteria, the use of citrus oils and/or their active principles in several different fields, such as in the cosmetics industry.

This chapter represents an update of a previous paper (Bisignano and Saija, 2002) to exhaustively review the evidence of the biological and pharmacological activities of citrus oils, as reported in the international scientific literature during the last decade.

13.2 ANTIMICROBIAL ACTIVITIES OF CITRUS ESSENTIAL OILS

Citrus essential oils and their derivatives are mainly terpene mixtures. It is well known that many of these terpenes can have antimicrobial activities (Dorman and Deans, 2000). In fact, such molecules can interact with some cellular structures, causing the inhibition of cell growth or cell death.

13.2.1 Methodological Matters

A preliminary condition for these effects is the contact between the antimicrobial molecule and the target cells. The contact usually requires the molecules to be in their most hydrophobic state, that is, in their vapor phase, because this makes possible their solubilization in the cell membranes. It is well known that several procedures have been adopted to evaluate the antimicrobial activity of essential oils and that the data collected depend on the method used. In fact, each methodology can measure different aspects of microbial cell damage, and the screening conditions can considerably affect the bioactivity of these substances (Belletti et al., 2004; Sharma and Tripathi, 2008). Then, such discrepancies could be attributed to different assays employed.

Recently, Belletti et al. (2004) studied citrus essential oil antimicrobial activity by evaluating yeast-growth kinetics in solid medium in relation to oil concentration using a gas chromatographic method. This method is based on the detection of metabolic CO_2 released from the microbial cells in the headspace, containing the oils, of sealed vials. Although the headspace composition does not correspond to the whole-oil components, it gives a measure of the volatile composition of the oil, which, in turn, depends on the vapor pressure of the molecules. This indirect index of microbial growth has been extensively used to monitor microbial dynamics in food and beverages, as well as in model systems (Gardini et al., 1997). However, many authors reported that even the most effective oils showed a lower antimicrobial activity when added to the headspace; furthermore, the higher activity was observed when essential oils were added directly in to the medium (Belletti et al., 2004; Sharma and Tripathi, 2008). To explain the higher efficacy of the citrus oils in a liquid medium, several hypotheses had been proposed: (a) the solubilization of the active molecules in the aqueous phase of the solid medium does not allow their contact with the microbial cells located on the surface of the medium, (b) the molecules released faster into the headspace could not be the most effective, and (c) the surface exposure to the active molecules of cells superficially inoculated on the solid medium is lower as compared to that of cells suspended in a liquid medium. This latter effect may be enhanced by the inoculum procedure, which favors the clumping of cells, further reducing the amount of exposed surface.

These backgrounds suggest that standardizing the methods used to test the antimicrobial efficacy of essential oils or their derivatives is a prerequisite. Also, Suhr and Nielsen (2003) observed that appropriate screening procedures are needed to relate to the potential future applications.

Belletti et al. (2004) assayed the antimicrobial properties of different citrus oils, all derived from concentrations of the essential oils, on the strain *Saccharomyces cerevisiae*. Four of them were orange-based oils (blond and blood sweet oranges and bitter orange) while the others were derived from mandarin, lemon, and citron. All the citrus-based products tested in this research (characterized by high concentrations of limonene, β-thujone, β-myrcene, and linalol) showed a lower inhibiting effect against the target yeast. Moreover, all the oils were more bioactive when added directly to the liquid medium and not to the headspace. If added to the liquid medium, all the flavoring

agents were able to reduce yeast growth by about 20% when present at their lower concentrations, with the exception of the blood-orange oil. The growth reduction of *S. cerevisiae* caused by blond sweet orange was 50%; about 85% for blood orange; and 100% for bitter orange. Citron oil was the most effective. In fact, it completely inhibited the growth of *S. cerevisiae* when present at 500 ppm. Similar behaviors were observed when the citrus oils were added to the headspace of solid media inoculated with the target organism. Also, in these experimental conditions, citron was the most effective oil, followed by lemon. However, the yeast growth reduction was, at the same concentrations, noticeably lower than those observed in liquid media. In fact, citron oil completely inhibited yeast growth only when added in the headspace at a level of 3000 ppm.

13.2.2 Antimicrobial Properties and Human Infective Diseases

Romano et al. (2005) investigated the antifungal properties of the bergamot natural oil and its furocoumarin-free and distilled derivatives against vaginal isolates of several *Candida* species in vitro. They established that these preparations were effective agents mainly when tested in association with boric acid, which suggested that they are potentially active against filamentous fungi as well.

Recently, Sanguinetti et al. (2007) demonstrated the high in vitro activity of *Citrus bergamia* (bergamot) oil against a wide number of clinical isolates of various pathogenic dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*, *T. interdigitale*, *T. tonsurans*, *Microsporum canis*, *M. gypseum*, and *Epidermophyton floccosum*). In general, of the three preparations tested, natural oil of bergamot (NO), its distilled (DE) and furocoumarin-free (FF) derivatives, had low MICs. However, the two derivatives, DE and FF, were more active than NO against all of the species tested. This is of great importance in the light of the fact that the two derivatives are devoid (in part or completely) of nonvolatile residues, in particular, of the phototoxic bergaptene. These results give substantial support to popular beliefs in the effectiveness of treating skin and mucosal infections with bergamot oils.

Citrus plants from the *Rutaceae* family are the main source of the natural limonoids, and 39 limonoid aglycones and 21 glucosides have been isolated from citrus plants so far. Among these, limonin and nomilin are the most prevalent citrus limonoids (Kelly et al., 2003). Limonin and nomilin have been evaluated for their capacity to act as antiretroviral agents (Battinelli et al., 2003). The effect of the two compounds on the growth of human immunodeficiency virus-1 (HIV-1) was examined in a culture of human peripheral blood mononuclear cells (PBMC) and on monocytes/macrophages (M/M). Both compounds were found to inhibit viral replication in PBMC in the EC_{50} = 50–60 μM range. The limonoids were effective at inhibiting HIV-1 replication in infected M/M at concentrations ranging from 20 to 60 μM. Finally, the authors reported that the mechanism of action could be attributed to inhibition of in vitro HIV-1 protease activity.

Recently, Manners (2007) reviewed the activity of citrus limonoids. In particular, limonin and nomilin are able to effectively disrupt larvae development relative to corn earworm and fall armyworm. Field test results provide evidence that the insecticidal citrus limonoids can function as an important component of an integrated pest-management program to control insects populations.

13.2.3 Inhibition by Citrus Oil of Microbial Growth in Food

Sharma and Tripathi (2008) reported that *Citrus sinensis* essential oil caused complete growth inhibition of *Aspergillus niger* at 3.0 μg/ml on agar plates. This concentration was found to be lethal under the test conditions. The oil showed fungistatic activity at 1.5 μg/ml with about 79% growth inhibition after 7 days of incubation and a delay of conidiation compared to control. The essential oil significantly reduced the growth of *A. niger* in a dose-dependent manner. After calculating the percent inhibition with respect to radial growth and dry weight basis, the authors demonstrated also that the oil was more effective in liquid medium than solid medium (Sharma and Tripathi, 2008). Furthermore the thermostability of the oil was tested, and it was found that

its activity was not altered at temperature ranging from 40°C to 100°C and even after autoclaving (121°C for 15 minutes) the oil.

The chemicophysical and composite characteristics of food and soft drinks make these products susceptible to microbial spoilage. They are usually characterized by high C/N ratios and low pH, which allow the growth of specific microbial groups, such as acetic and lactic acid bacteria, molds, and yeasts (Battey et al., 2002). The addition of CO_2 in beverages further reduces growth possibilities, mainly favoring yeasts (Louriero and Querol, 1999). The stability of these beverages often depends on thermal treatments to which ingredients and intermediate and final products can be subjected. However, products packaged in polyethylene terephthalate (PET) bottles are often not thermally treated because of the susceptibility of plastic material to heat. For this reason, their stability relies upon the addition of preservatives, generally belonging to the weak acid group, such as sorbic and benzoic acids. The pressure from consumers for minimally processed products free from traditional preservatives has induced manufacturers to find new strategies for the stabilization of food (Rangan and Barceloux, 2009). In fact, consumers are inclined to consider these preservatives as extraneous and unsafe because they have no connection with the food matrix. This framework has recently been complicated by the information that in beverages a chemical reaction can induce the transformation of part of benzoic acid in benzene (Meadows, 2006).

In this scenario, the search for new strategies and new antimicrobials for stabilization of beverages (and other products) has become a central goal for producers. Aroma compounds and essential oils can be an interesting alternative. The principal limitations to an industrial use of these substances as preservatives are their organoleptic impact and the variable composition of the essential oils (which can be reflected in their antimicrobial activity) (Burt, 2004). In any case, a stabilization strategy based only on the use of these substances can be difficult to achieve without the addition of excessive concentrations of the flavoring agent, not compatible with an acceptable flavor profile.

Belletti et al. (2007) reported that the use of citron essential oil gave the most interesting responses in terms of microbial stability against *S. cerevisiae* in soft drinks. The inhibition of yeast growth in the presence of this essential oil has been attributed to the high percentage of citral (7.1%) found in the essential oil composition. In fact, citral is considered to be among the most interesting molecules with respect to the antimicrobial activity (Wuryatmo et al., 2003). However, other compounds with potential antimicrobial properties were present in the citron essential oil, such as β-pinene (20.1%), limonene (41.1%), γ-terpinene (8.3%), *p*-cymene (5.9%), α-pinene (3.4%), and linalol (1.7%) (Belletti et al., 2004). The inhibition of yeast growth in beverages was achieved at a concentration of essential oil containing amounts of citral that, if used alone, were not sufficient to allow yeast inhibition (500 ppm of citron essential oil contains about 35 ppm of citral). This could be due not only to the presence of other molecules active against microorganisms but also to their synergistic action. Some studies have concluded that whole essential oils have a greater antibacterial activity than do the major components mixed, which suggests that also the minor components could be critical to the bioactivity and may have a synergistic effect or a potentiating influence (Burt, 2004).

Belletti et al. (2008) demonstrated that citral and citron essential oil can also be used to prolong the microbial shelf life of fruit-based salads. Both citral (25 to 125 ppm) and citron essential oil (300, 600, and 900 ppm) were able to prolong the microbial shelf life of the fruit-based salads. The essential oil gave excellent results, doubling the time needed for the wild microflora to reach concentrations able to produce a perceivable spoilage in condition of thermal abuse (9°C). In particular, the use of the citron essential oil resulted in a pleasant flavor, compatible with the final products, and gave excellent results, avoiding the undesirable effects attributable to the cytotoxicity of pure citral.

However, active compounds and toxicity should also be clearly documented before being used within the food industry or clinical arena (Rios and Recio, 2005). As demonstrated recently by Dusan et al. (2006) high doses of some essential oils can have detrimental effects on intestinal cells and therefore the effect to the whole of the intestinal tract needs to be assessed before safe usage

can be achieved. Other studies have noted that higher concentrations of essential oils are needed to be effective when being used on food compared with in vitro studies (Fisher and Phillips, 2006). Furthermore, the use of essential oil vapors may be a potential way of combating the organoleptic effect brought about by direct contact between the food and oil. However, longer exposure to the vapor is required to produce a similar inhibitory effect (18 hours as against 60 seconds), which has cost implications for the food industry (Fisher and Phillips, 2006).

13.2.4 Antimicrobial Action Mechanisms of Citrus Essential Oils

The mechanisms by which essential oils bring about their antibacterial effect are incompletely understood, but there are a number of proposed mechanisms (Holley and Patel, 2005) (Figure 13.1). Some studies have found Gram-positive bacteria to be more sensitive to essential oils than Gram-negative bacteria, which, it has been suggested, may be due to the relatively impermeable outer membrane that surrounds Gram-negative bacteria (Smith-Palmer et al., 2001). Other studies suggest that there is only a time delay in the growth of Gram-negative bacteria. Therefore, over a longer time period, the essential oils would have the same effect on both Gram-negative and Gram-positive bacteria (Tassou et al., 2000). This differential sensitivity has been observed when using citrus oils/components in vitro, but not in on-food studies (Fisher and Phillips, 2006). Little research has been carried out on the mechanism of action of essential oils, including citrus oil or vapors, despite the potential ability of the latter to be used as an alternative antimicrobial against those bacterial strains developing antibiotic resistance.

Citrus fruits are characterized by a high percentage of neryl and geranyl acetates (Perez-Cacho and Rouseff, 2008). Many authors postulated a significant relationship between the presence of citral (the mixture of the isomers neral and geranial) in the peel of citrus fruits and inhibition of *Penicillium digitatum*, *P. italicum*, and *Geotrichum candidum*, the major fungi responsible for postharvest spoilage of citrus (Wuryatmo et al., 2003). Belletti et al. (2004) reported that citral was in a remarkable concentration (more than 7%) in citron oil, whose antimicrobial activity against *S. cerevisiae* was the highest among their tested mixtures. The inhibitory effects of citral against microorganisms are due to the presence of a carbonyl group adjacent to the α- and β-carbons in the α, β-unsaturated aldehydes neral and geranial (Cosentino et al., 1999). This makes the β-carbon positively polarized and the aldehyde can act as a direct alkylating agent able to bind cellular nucleophilic groups.

β-Pinene is found in high amounts (more than 20%) in the headspace of lemon and citron oils, but also, in sweet lime. The negative effect of α-pinene on the yeast membrane function has been

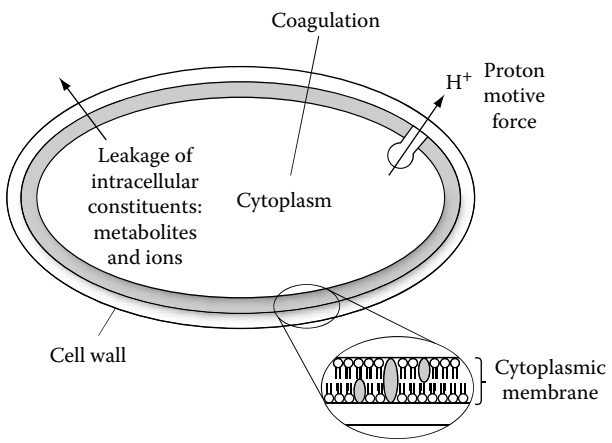

FIGURE 13.1 Mechanisms and targets of the antimicrobial activity of terpenes.

already studied. This monoterpene hydrocarbon was found to be particularly effective against *Escherichia coli* O157:H7 (Takikawa et al., 2002).

Fisher and Phillips (2009) studied the mechanism of action of a citrus-oil blend against *Enterococcus faecium* and *E. faecalis*. The findings of this investigation suggest that the antimicrobial action of a bergamot : orange (1 : 1 v/v) essential oil on *Enterococcus* spp. may be affected by the uptake of the oil into the cell. Vacuoles are observed within the cells, which were not present in the control cells. The lipophilic characteristic of the essential oils will allow the preferential partitioning from the aqueous phase into the membrane structure of the cell (Cox et al., 2000), and thus could result in the oil uptake into the cell. A novel point of view of oils mechanisms of action is the uptake of the citrus essential oil into the cell to form vacuoles and thus having an antimicrobial effect from the inside. Furthermore, after exposure of *E. faecalis* and *E. faecium* to the citrus blend, there is an increase in cell-membrane permeability with a twofold increase in the case of the essential oil and a 32 to 40 times increase in the case of exposure to vapor. This might suggest that the vapor might be bringing about its effect via a different mechanism or that because of the smaller molecular size or of specific particles evaporating from the oil, the vapor may be more effective at penetrating the membrane and thus causing damage to the cell. Although an increased permeability of the cell membrane is observed, the loss of ATP that occurs within *Enterococcus* cells does not leak into the surrounding fluid (Fisher and Phillips, 2009). This finding suggests that the loss of intracellular ATP may be due to the lack of synthesis or increased hydrolysis of ATP. The possible uptake of the oil/vapor into the cell may be interfering with normal cellular function rather than increased permeability of the cell membrane to ATP.

13.3 EFFECTS OF CITRUS ESSENTIAL OILS ON THE NERVOUS SYSTEM

Citrus essential oils have been utilized widely in traditional medicine, and there are various reports of their actions, such as effects on behavior and on pain. However, there is very little verified scientific evidence to support this use, also due to the few number of data concerning their effects on brain functions.

13.3.1 Neuroprotective Effects

Several studies report the neuroprotective effect of the essential oil of bergamot (BEO) in vitro and in vivo experimental models. Corasaniti et al. (2007) have demonstrated that BEO can reduce neuronal damage caused in vitro by excitotoxic stimuli and that this neuroprotection is associated with prevention of injury-induced engagement of critical death pathways. In fact, the protective effects of BEO on excitotoxic damage induced by exposure to *N*-methyl-D-aspartate (NMDA) in human SH-SY5Y neuroblastoma cells was investigated by means of in vitro experiments. BEO reduced death of SH-SY5Y cells caused by NMDA. In addition to preventing accumulation of reactive oxygen species (ROS) and activation of the calcium-activated protease calpain, BEO counteracted the deactivation of the prosurvival kinase Akt and the consequent activation of glycogen synthase kinase-3β (GSK-3β), induced by NMDA. Results obtained by using specific fractions of BEO suggested that monoterpene hydrocarbons are responsible for neuroprotection afforded by BEO against NMDA-induced cell death.

Furthermore Amantea et al. (2009) have investigated the effects of systemic pretreatment with BEO on brain damage following permanent focal cerebral ischemia (induced by occlusion of the middle cerebral artery, MCAo) in rats. BEO is able to significantly reduce infarct size following permanent MCAo throughout the brain, especially in the medial striatum and the motor cortex. BEO does not affect basal amino acid levels, whereas it significantly reduces aspartate and glutamate efflux in the frontoparietal cortex following MCAo. These early effects are associated with a significant increase in the phosphorylation and activity of Akt and in the phosphorylation of the deleterious downstream kinase GSK-3β, whose activity is negatively regulated via phosphorylation by Akt.

Finally, lemon pure essential oils were shown able to inhibit heat shock–induced apoptosis in the human astrocyte cell line CCF-STTG1 and in primary cultured rat astrocytes, by blocking caspase-3 activation, DNA fragmentation and poly-ADP-ribose polymerase fragmentation (Koo et al., 2002).

13.3.2 Anxiolytic and Sedative Properties

The word "aromatherapy" combines two words: *aroma* (a fragrance or sweet smell) and *therapy* (a treatment). Aroma and massage therapy are the practice of using essential oils for psychological and physical well-being via inhalation or massage. The term "aromatherapy" is used to describe a wide range of practices involving odorous substances, although only aroma delivery through inhalation for inducing psychological or physical effects can be correctly defined as aromatherapy. Nevertheless, the clinical use of essential oils and their volatile constituents via inhalation or massage has expanded worldwide (Edris, 2007).

A number of essential oils are currently in use as aromatherapy agents to relieve anxiety, stress, and depression. Popular anxiolytic oils include those from *Citrus sinensis*, *Citrus aurantium*, and *Citrus limon*, suggesting central nervous system action.

The anxiolytic, anticonvulsant, and sedative effects following oral administration of *C. aurantium* L. essential oil have been characterized in different behavioral models in mice (Carvalho-Freitas and Costa, 2002; Pultrini et al., 2006); these beneficial effects are not accompanied by deficits in general activity or motor coordination. Furthermore Komiya et al. (2006) and Ceccarelli et al. (2004) have demonstrated the antistress action of the essential oil of lemon by means of different behavioral models in mice and rats; these anxiolytic, antidepressant-like effects appeared to be mediated via suppression of dopaminergic activity related to enhanced serotoninergic neurons.

As to studies carried out in humans, Lehrner et al. (2000) reported that exposure to ambient odor of orange diffused in a dental office has a relaxant effect; in particular, women exposed to orange odor had a lower level of state anxiety, a more positive mood and a higher level of calmness. These data support the other evidences of sedative properties of the natural essential oil of orange. Conversely, no positive effect on anxiety levels of adults accompanying children to a pediatric emergency department was observed by Holm and Fitzmaurice (2008) when aromatherapy alone (neroli essential oil) or music in addition to aromatherapy diffused in the waiting area. These different results could be because of environmental conditions or application modalities of the aromatherapy.

13.3.3 Modulation of Neurotransmitter Functions

A large number of biological effects elicited by exposure to citrus essential oils may be related to their capability to modulate neurotransmitter functions.

BEO contains into its volatile fraction some monoterpene hydrocarbons able to stimulate glutamate release by transporter reversal and/or by exocytosis, depending on the dose administered (Morrone et al., 2007). In fact intraperitoneal administration of BEO in rats could significantly affect the extracellular concentration of aspartate, glycine, and taurine; furthermore, when perfused into the hippocampus, BEO produced a significant increase of extracellular aspartate, glycine, and taurine as well as of GABA and glutamate. These effects appeared to be dependent on the glutamate transporter blocker dl-threo-β-benzyloxyaspartic acid and on extracellular Ca^{2+}. More recently Rombolà et al. (2009) have described the systemic effects of this phytocomplex on gross behavior and EEG activity recorded from the hippocampus and cerebral cortex of the rat. Systemic administration of BEO produces dose-dependent increases in locomotor and exploratory activity that correlate with significant changes in the EEG spectrum.

Fukumoto et al. (2006) used brain-tissue slices to demonstrate the capability of R-limonene, γ-terpinene, and citral, major components of lemon essential oil, and of their metabolites on

monoamine release; interestingly, the metabolites of these monoterpenes have a stronger effect on monoamine release from brain tissue than the monoterpene compounds themselves.

The antidementia effects of (*S*)-(–)-limonene and (*S*)-(–)-perillyl alcohol have been reported by Zhou et al. (2009). In fact, these compounds showed strong ability to improve memory impaired by scopolamine in experimental animals; brain dopamine concentration of the scopolamine group was significantly lower than that of the control group, but this phenomenon was reversed by pretreatment with (*S*)-(–)-limonene or (*S*)-(–)-perillyl alcohol. Furthermore, these two lemon essential oil components could inhibit acetylcholinesterase (AChE) activity in vitro. Besides the lemon essential oil, the essential oils of *Citrus paradisi* (pink grapefruit) were also shown able to inhibit AChE activity, very likely because of the nootkatone and auraptene contained in them (Miyazawa et al., 2001).

Today there is a raising interest in studying the alterations on nervous system functions following inhalation of citrus essential oils. For example, essential oil from citrus lemon, inhaled by female rats experiencing a persistent nociceptive input, can affect and modulate the increase in hippocampal acetylcholine release induced by pain (Ceccarelli et al., 2002); however, this effect appeared to be gender dependent because it was evident only in female, and not in male, animals. Consistent with these findings, long-term exposure of rats to lemon essential oil can induce significant, at times sex-specific, changes in neuronal circuits involved in anxiety and pain (Ceccarelli et al., 2004).

In experimental animals, olfactory stimulation with scent of grapefruit oil (SGFO) enhances sympathetic nerve activities, suppresses gastric vagal nerve activity (GVNA) and increases plasma glycerol concentration; furthermore olfactory stimulation with SGFO or with limonene elevates renal sympathetic nerve activity (RSNA) and blood pressure and lowers GVNA in urethane-anesthetized rats (Tanida et al., 2005). The authors suggested that SGFO and its active component limonene affect autonomic neurotransmission and blood pressure through central histaminergic nerves and the suprachiasmatic nucleus. In agreement with data from experimental animals, fragrance inhalation of grapefruit oil in normal humans results in an increase of sympathetic activity (Haze et al., 2002).

The activation of sympathetic nerve innervating the white adipose tissue is known to facilitate lipolysis, resulting in a suppression of body weight gain. Olfactory stimulation with SGFO excites the sympathetic nerve innervating the white and brown adipose tissue and adrenal gland and inhibits the parasympathetic gastric nerve in rats (Shen et al., 2005). Limonene induces responses similar to those caused by SGFO. The capability of SGFO, and particularly of its primary component limonene, to affect autonomic nerves and enhance lipolysis is mediated through a histaminergic response. An increased sympathetic nerve activity to white adipose tissue in anesthetized rat was shown by Niijima and Nagai (2003) also following olfactory stimulation with the scent of lemon oil.

Finally, one has to mention that the essential oils of hakyul (*Citrus natsudaidai* Hayata), yuza (*Citrus junos* Sieb. ex Tanaka), and lemon have a significant lipolytic effect, as shown by Choi (2006) using an olive oil model solution. Among the authentic compounds relating to citrus-peel oils, octanal, γ-terpinene, limonene, terpinen-4-ol, nerol, *p*-cymene and geranyl acetate showed the highest lipolytic effect; thus monoterpene hydrocarbons having one or two double bonds would have stronger lipolytic effect than those having three double bonds.

13.4 ANTINOCICEPTIVE PROPERTIES OF CITRUS ESSENTIAL OILS

Due to the increasing use of aromatherapy oils, the antinociceptive properties of citrus essential oils have been investigated by several authors.

Sakurada et al. (2009) reported that the capsaicin-induced nociceptive response in mice is significantly reduced by intraplantar injection of BEO, while sweet orange essential oil is without effect. Among the monoterpene hydrocarbons found in BEO volatile fraction, linalol might be responsible for the antinociceptive effects of this phytocomplex. In fact, linalol possesses antinociceptive, antihyperalgesic, and anti-inflammatory properties in different experimental animals. Linalol effects

in neuropathic pain have been investigated by Berliocchi et al. (2009). Chronic administration of linalol is able to reduce mechanical allodynia (but not sensitivity to noxious radiant heat) following spinal nerve ligation as model of neuropathic pain in mice. Mechanisms other than an action on inflammatory processes may be supposed to mediate the protective effect of linalol in this model of neuropathic pain.

Also the polymethoxylated flavone 3′,4′,3,5,6,7,8-heptamethoxyflavone (HMF), found in citrus essential oils, possesses anti-inflammatory properties. In fact, intraperitoneal (but not oral) administration of HMF proved to induce an anti-inflammatory effect when studied in the bacterial lipopolysaccharide-challenge/tumor necrosis factor-α (TNF-α) response in mice and in the carrageenan/paw edema assay in rats (Manthey and Bendele, 2008). This effect appeared to be related to the different bioavailability of the intact HMF following oral and intraperitoneal administration, while the glucuronidated HMF metabolites seem to be inactive.

There is evidence about the ability of citrus essential oils to modulate (not only behavioral but also neuronal) responses related to nociception and pain also following inhalation and aroma-massage therapy.

Lemon essential oil inhaled in rats experiencing a persistent nociceptive input decreased behavioral responses related to the nociceptive stimulus, influencing c-Fos expression (used to test the degree of neuronal activation of areas belonging to the limbic system) in the arcuate and paraventricular nuclei of the hypothalamus and in the dentate gyrus of the hippocampus (Aloisi et al., 2002), with an effect dependent on animal gender. Furthermore, the aroma-massage therapy with citrus essential oils might have potential as an alternative/complementary method for short-term pain relief, as shown by Yip and Tam (2008), who carried out a double-blind, placebo-controlled study to assess the efficacy of the aroma massage (6 massage sessions for 3 weeks) with aromatic essential oils (*Zingiber officinalis* and *Citrus sinesis*) on older subjects with moderate to severe knee pain.

Specific formulations can improve the anti-inflammatory efficacy of citrus essential oils. For example the combination of Dead Sea magnesium chloride ($MgCl_2$) and citrus oils, tested in a subcutaneous chamber model in mice, resulted in lower levels of TNF-α and leukocyte migration without changes in IL-10 levels, in comparison with controls (Mizrahi et al., 2006).

One has to point out that, despite the anti-inflammatory effects, citrus essential oils do not affect prostaglandin basal levels. In fact, the essential oil from *Citrus aurantium* (that is used in traditional medicine to treat gastritis and gastric disorders) and its main component limonene provide, when orally administered in rats, effective gastroprotection against lesions induced by absolute ethanol and nonsteroidal anti-inflammatory drugs, increasing gastric mucus production and without interfering with gastric H^+ secretion, serum gastrin, or glutathione level in gastric mucosa (Moraes et al., 2009).

13.5 ANTIOXIDANT PROPERTIES OF CITRUS ESSENTIAL OILS

Reactive oxygen species, as well as reactive nitrogen species, play either harmful or beneficial role in biological systems. Beneficial effects of ROS include physiological roles in cellular responses against infectious agents and in several cellular signaling pathways. Harmful effects are due to high concentrations of ROS, which can damage biomolecules, including lipids, proteins, and nucleic acids. The harmful effects of ROS are counterbalanced by the antioxidant action of both antioxidant enzymes and nonenzymatic antioxidants; however, despite the presence of the cellular antioxidant system, oxidative damage accumulates during the life cycle and has been proposed to play a pivotal role in the development of age-dependent diseases such as atherosclerosis, arthritis, neurodegenerative disorders and cancer.

There is today increasing interest in the radical scavenging activities of some natural antioxidants, especially those found in edible plants, which might have a role in preventing various chronic pathologies and find applications in food and cosmetic industry. Essential oils and some of their

components are highly lipophilic and have been shown to possess antioxidant properties. However, little is known about the antioxidant properties of essential oils from edible plants and, in particular, of citrus essential oils.

Thirty-one kinds of citrus essential oils and their components were investigated for their radical scavenging properties against the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Choi et al., 2000), and appeared to have an antioxidant power similar to that of Trolox; the citrus volatile components geraniol, terpinolene, and γ-terpinene showed marked scavenging activity against DPPH. γ-Terpinene was also found to inhibit human low density lipoprotein (LDL) oxidation induced in vitro by Cu^{2+} or 2.2′-azobis(2-amidinopropane) dihydrochloride (AAPH; Edris, 2007). Also, essential oils from sweet orange peels have been investigated in terms of DPPH radical scavenging, β-carotene bleaching and nitrite scavenging activities (Anagnostopoulou et al., 2006).

Misharina and Samusenko (2008) demonstrated the good antioxidant properties of essential oils from lemon (*Citrus limon* L.) and especially from pink grapefruit (*Citrus paradisi* L.) by capillary gas-liquid chromatography against the oxidation of the aliphatic aldehyde hexanal to the carboxylic acid. Furthermore, the essential oil from *Citrus karna* Raf, containing D-limonene as major constituent, together with α-pinene and β-pinene as minor constituents, showed significant inhibition against the oxidation of linoleic acid in the β-carotene-linoleic acid system (Malhotra et al., 2009). The results indicate a main role for D-limonene in antioxidant activity. Finally, bergaptol, a bioactive compound isolated from grapefruit peel oil, showed radical scavenging activity using 2,2′-azobis(3-ethylbenz-thiazoline-6-sulfonic acid) and DPPH (Girennavar et al., 2007); this compound is also a potent inhibitor of debenzylation activity of CYP3A4 enzyme.

Oxidation of LDL has been implicated in atherogenesis for several years. Therefore many researchers are looking for potent antioxidants, which are able to inhibit LDL oxidation and thus lower the risk for atherosclerosis. The antioxidative action of citrus essential oil components was studied on LDL oxidation in vitro (Grassmann et al., 2001; Takahashi et al., 2003). Lemon oil and, among its volatile compounds, γ-terpinene showed the strongest antioxidative effect and inhibited both copper- and AAPH-induced oxidation of LDL. In particular the loss of carotenoids during LDL oxidation appeared to be strongly retarded by lemon oil and γ-terpinene (Grassmann et al., 2001).

Recently, it has been suggested that Lectin-like oxyLDL receptor-1 (LOX-1) is involved in smooth muscle cell (SMC) proliferation and neointima formation in injured blood vessels. The BEO nonvolatile fraction has a protective effect on LOX-1 expression and free-radical generation in common carotid-artery injury induced by balloon angioplasty in rats (Mollace et al., 2008). These results suggest that natural antioxidants may be relevant in the treatment of vascular disorders in which proliferation of SMCs and oxyLDL-related endothelial cell dysfunction are involved.

Besides terpenes, other components found in citrus essential oils have well-established antioxidant properties. For example, Tirkey et al. (2005) demonstrated the good protective effect of the citrus bioflavonoid hesperidinin against liver and kidney oxidative damage induced in rats by CCl_4 (a toxic agent which is metabolized to produce free radicals and widely used to experimentally induce acute and chronic hepatic and renal injuries in rodents).

Limonene is used as flavoring agent in a wide range of food. Since it is lipid soluble, limonene is often added to foods in oil-in-water emulsions, which are susceptible to both physical instability and oxidative degradation, leading to loss of aroma and formation of off-flavors. Whey protein isolate (WPI) could inhibit the oxidative deterioration of limonene in oil-in-water emulsions (Djordjevic et al., 2008), through the formation of a cationic emulsion-droplet interface and/or the ability of amino acids in WPI to scavenge free radical and chelate pro-oxidative metals. Also a sodium dodecyl sulfate-chitosan complex has appeared able to inhibit the oxidative deterioration of limonene because of the formation of a cationic and thick emulsion-droplet interface that could repel pro-oxidative metals (Djordjevic et al., 2007).

13.6 CHEMOPREVENTIVE ACTIVITY OF ACTIVE COMPONENTS OF CITRUS OILS

Extensive research during the last half century has identified various molecular targets that can potentially be used not only for the prevention but also for treatment of cancer. However, lack of success with targeted monotherapy resulting from bypass mechanisms has forced researchers to employ either combination therapy or agents that interfere with multiple cell-signaling pathways. Increasing attention is paid to the possibility of applying cancer chemopreventive agents for individuals at high risk of neoplastic development (Aggarwal and Shishodia, 2006; Grassmann, 2005; Nishino et al., 2000; Tsuda et al., 2004; Wagner and Elmadfa, 2003). Natural compounds have practical advantages for this purpose with regard to availability, suitability for oral application, regulatory approval and mechanisms of action. Recent studies have indicated that mechanisms underlying chemopreventive potential may be combinations of antioxidant, anti-inflammatory, immune-enhancing, and antihormone effects, with modification of drug-metabolizing enzymes, influence on the cell cycle and cell differentiation, induction of apoptosis and suppression of proliferation and angiogenesis playing roles in the initiation and secondary modification stages of neoplastic development (Figure 13.2). Accordingly, natural agents are advantageous for application to humans because of their combined action mechanisms (Wagner and Elmafda, 2003). However, while fruits and vegetables are recommended for prevention of cancer, their active ingredients (at the molecular level) and their mechanisms of action remain less well understood.

The monocyclic monoterpene limonene is a major constituent in several citrus oils (orange, lemon, mandarin, lime, and grapefruit). Because of its pleasant citrus fragrance, limonene is widely used as a fragrance and flavor additive in perfumes, soaps, foods, chewing gum, and beverages.

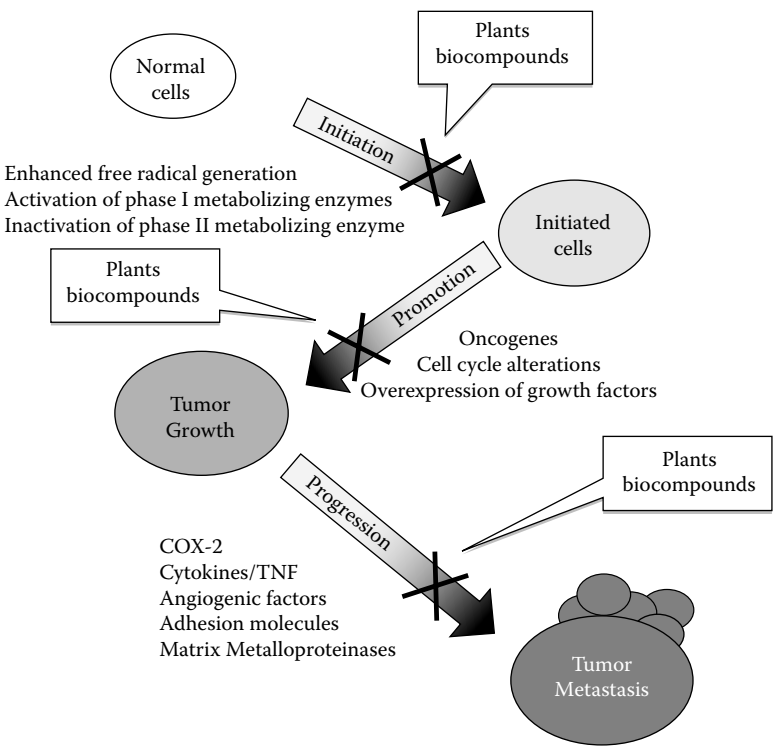

FIGURE 13.2 Effects of plants biocompounds on the multistage carcinogenesis process.

The Code of Federal Regulation lists D-limonene as generally recognized as safe (GRAS) for a flavoring agent. Dietary intake of D-limonene varies depending on the types of foods consumed. Hakim et al. (2002) designed a study, by self-administration of a citrus food–frequency questionnaire, to assess the D-limonene content of different citrus juices and beverages and to develop a dietary-assessment instrument to measure D-limonene intake. Mean intakes of D-limonene from citrus juices among consumers ranged between 13.0 and 13.2 mg/day.

D-Limonene has well-established chemopreventive activity against many types of cancers (Sun, 2007) and has demonstrated efficacy in preclinical models of breast and colon cancers. Hakim et al. (2000) carried out a case-control study to determine the usual citrus consumption patterns of an older Southwestern population and to evaluate how this citrus consumption varied with history of squamous cell carcinoma (SCC) of the skin. In this Arizona population, citrus-peel consumption was not uncommon, with 34.7% of all subjects reporting citrus-peel use. The authors found no association between the overall consumption of citrus fruits or juices and skin SCC, but the most striking feature was the protection purported by citrus-peel consumption, with a dose-response relationship. Concerning skin cancer, it is interesting to note that limonene and perillic acid were proven to inhibit the metastatic progression of B16F-10 melanoma cells in C57BL/6 mice (Raphael and Kuttan, 2003a).

Roberto et al. (2010) evidenced that limonene could protect normal lymphocytes from diseases related to oxidative stress, including cancer. In fact, limonene exerts antiproliferative action on a lymphoma cell line without modifying normal lymphocyte viability; in addition, it elicits a biphasic effect on proliferation of normal murine lymphocytes by decreasing H_2O_2 levels and increasing catalase and peroxidase activities, and protects murine lymphocytes against oxidative stress induced by exposure to H_2O_2.

Limonene has been reported to induce apoptosis on tumor cells. Hata et al. (2003) reported that essential oils of sweet orange, grapefruit, and lemon, induce apoptosis in HL-60 cells because of limonene contained in them. However, other components present in the essential oils of sweet orange and grapefruit may also be responsible for the observed apoptotic activity; in fact, the aldehyde compounds decanal, octanal, and citral present in the dichloromethane fraction proved to possess strong apoptotic activity. Furthermore, D-limonene has antiangiogenic and proapoptotic effects on gastric cancer, thereby inhibits tumor growth and metastasis, as shown in a metastatic model simulating human gastric cancer and established by orthotopic implantation of histologically intact human tumor tissue into gastric wall of nude mice (Lu et al., 2004).

Other mechanisms may be involved in the chemopreventive effect of limonene. For example, Parija and Das (2003) demonstrated the involvement of the YY1 (Yin Yang 1) transcription factor in *N*-nitrodiethylamine-induced hepatocarcinogenesis, also showing that D-limonene mediated chemoprevention in this hepatocarcinogenesis model might be regulated by c-myc oncoprotein. Consistent with these data, the chemopreventive effect of orally administrated orange oil (which contains 90% to 95% of limonene) was demonstrated on *N*-nitrosodiethylamine-induced hepatic preneoplasia in rats, together with restoration of the normal liver phenotype and upregulation of junctional complexes (Bodake et al., 2002). Limonene inhibits also hepatocarcinogenesis induced by N-nitrosomorpholine in male rats (Kaji et al., 2001); this effect may be clearly related to its effect in inhibiting cell proliferation and in enhancing apoptosis, but not through ras oncoprotein plasma membrane association.

D-Limonene induces phase I and phase II carcinogen-metabolizing enzymes (cytochrome p450), which metabolize carcinogens to less toxic forms and prevent the interaction of chemical carcinogens with DNA. It has been shown to enhance gastrointestinal UDP-glucuronosyltransferase activity in rats (Van der Logt et al., 2004).

Combination of D-limonene with cytotoxic agents or with other therapeutic approaches may be more effective than the employment of the monoterpene alone. For example, D-limonene could improve the treatment outcome of hormone-refractory prostate cancer with docetaxel (Rabi and Bishayee, 2009), without being toxic to normal prostate epithelial cells and through the modulation

of proteins involved in mitochondrial pathway of apoptosis. Furthermore the downregulation of the COL8A1 (collagen type VIII, α-1 gene) expression in the mouse hepatocarcinoma cell line Hca-F (which has a highly metastatic potential in the lymph nodes) also sensitized cells to the action of D-limonene (Zhao et al., 2009).

Furthermore D-limonene modulates the immune response with significant potential for clinical application. In fact, D-limonene increased the survival of lymphoma-bearing mice, and delayed hypersensitivity reaction to 2,4-dinitrofluorobenzene; furthermore it increased nitric oxide (NO) production in peritoneal macrophages obtained from these animals (Del Toro-Arreola et al., 2005). Furthermore, limonene demonstrated antiproliferative action also on a lymphoma cell line (BW5147), exerting a decrease in cell viability, that was related to apoptosis induction and increase in NO levels at long incubation times, and to cell arrest in different phases of the cell cycle at short times (Manuele et al., 2009). Finally, administration of various monoterpenes, including limonene, in Balb/c mice significantly increases the total antibody production, antibody producing cells in spleen, bone marrow cellularity and α-esterase positive cells, when compared to normal animals (Raphael and Kuttan, 2003b).

The chemopreventive effect of D-limonene is not accompanied by significant toxicity; in particular, it is not genotoxic. For example, limonene failed to increase the mutant frequency in the liver or kidney of male Big Blue rats exposed for 10 consecutive days to this monoterpene (Turner et al., 2001). Although male rats experienced an increased incidence of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney, no evidence of carcinogenic activity was observed in female rats or male or female mice (National Toxicology Program, 2007). Furthermore, no induction of chromosomal aberrations or sister chromatid exchange in cultured Chinese hamster ovary cells was observed (National Toxicology Program, 2007). Finally, D-limonene elicited no mutagenicity in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537). Similarly, the antigenotoxic effects of essential oil from *Citrus aurantium* L. peels in combination with mutagenic metals and alkylating agents were evidenced by Demir et al. (2009) using the wing spot test of *Drosophila melanogaster* exposed to potassium dichromate, cobalt chloride, ethylmethanesulfonate, and *N*-ethyl-*N*-nitrosourea as reference mutagens; the essential oil alone was not mutagenic and did not enhance the mutagenic effect of the reference mutagens, having also antigenotoxic effects in chronic cotreatments with all four mutagens.

Besides limonene, also other compounds contained in citrus essential oils, such as flavonoids and coumarins, are known for their chemopreventive properties.

Flavonoids are a ubiquitous family of phytochemicals that display a variety of biological effects, both beneficial and adverse depending on the individual compound. Polymethoxylated flavones (PMFs) from citrus inhibit production of TNF-α and other pro-inflammatory cytokines. As TNF-α also modulates Natural Killer (NK) cell activity, a mixture of PMFs purified from orange peel oil (consisting of nobiletin, tangeretin, trimethylscutellarein, sinensetin, 5-demethyl-nobiletin, hexa-*O*-methylquercetagetin, 5-demethyl-tetramethylscutellarein, and 5-hydroxy-3,3′,4′,6,7,8-hexamethoxyflavone) was evaluated by Delaney et al. (2001) to assess its potential to suppress, if orally administered, humoral, and innate immune functions in female B(6)C(3)F(1) mice sensitized to sheep red blood cells. Long-term, high-dose exposure to this citrus PMF mixture caused a mild suppression of NK cell activity; however, humoral immunity was not sensitive to suppression at the same exposure levels. Certain flavonoids are genotoxic, while others inhibit the genotoxicity of mutagenic agents. However Delaney et al. (2002) excluded the mutagenicity of the citrus PMF mixture described before by means of five bacterial tester strains (*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537) either in the absence or presence of S9 activation. Finally, the citrus coumarins isopimpinellin and imperatorin have chemopreventive effects when orally administered in SENCAR mice on skin-tumor initiation following topical application of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, by blocking DNA adduct formation (Kleiner et al., 2002).

13.7 CITRUS ESSENTIAL OILS AND SKIN

Citrus essential oils and/or their isolated components are efficiently used in dermatology. As also reviewed in the second section of this chapter, one of their main applications concerns prevention and treatment of cutaneous infective diseases, due to the well-established antimicrobial properties of essential oils. In addition, PUVA therapy continues to be the treatment of choice for patients with vitiligo, psoriasis, and other inflammatory skin diseases (Morison, 2004).

As to the employment of citrus monoterpenes, such as limonene, in cosmetic and pharmaceutical industry, there is today increasing evidence that these compounds are suitable enhancer(s) for improving transdermal permeation of poorly absorbed drugs and may be useful for designing and discovering innovative transdermal drug systems. In fact, chemicals offer tremendous potential in overcoming the skin barrier to enhance transport of drug molecules. Individual chemicals are, however, limited in their efficacy in disrupting the skin barrier at low concentrations and usually cause skin irritation at high concentrations. Multicomponent synergistic mixtures of chemicals (such as solvent mixtures, microemulsions, eutectic mixtures, complex self-assembled vesicles, inclusion complexes) have been shown to provide high skin permeabilization potency as compared to individual chemicals (Karande and Mitragotri, 2009). However, limonene, that is well known for its strong potential to act as skin penetration enhancer, and its oxidation products are also recognized as able to elicit allergic and irritative contact dermatitis.

13.7.1 Limonene as Enhancer of Percutaneous Absorption

Using skin as a port for systemic drug administration, transdermal drug delivery has expanded greatly over the last two decades. The main advantage of this route is that it avoids the hepatic first-pass effect. It is also recommended for some drugs in order to avoid problematic side effects. However, transcutaneous delivery is heavily limited by the permeation characteristics of the stratum corneum, and for many drugs it is insufficient to obtain and maintain efficacious systemic levels. Penetration enhancers are also a classical means for improving transdermal drug delivery when incorporated in transdermal therapeutic systems. Much interest is at the moment focused on the use, as penetration enhancers, of molecules of natural origin, such as D-limonene and, in general, terpenes. For example, D-limonene proved as good chemical enhancer by increasing the skin permeability of 6-mercaptopurine (Chandrashekar and Hiremath, 2008), and hydroxypropyl cellulose gel drug reservoir systems containing limonene act as optimal formulations for use in the design of membrane-controlled transdermal therapeutic system of ondansetron hydrochloride (Krishnaiah et al., 2008). Limonene may be efficiently used as penetration enhancer to improve skin permeation of carvedilol (Gannu et al., 2008a); furthermore, 8% v/w of D-limonene as a penetration enhancer and 20% v/w of dibutylphthalate as a plasticizer were used for the preparation of new, efficient, monolithic matrix-type transdermal drug-delivery systems for carvedilol, prepared using a film casting technique involving hydroxypropyl methylcellulose, hydroxypropyl cellulose, Eudragit RS 100, and Eudragit RL 100 as matrix-forming polymers (Gannu et al., 2008b). Güngör et al. (2008) developed a matrix-type transdermal patches of verapamil hydrochloride with pectin as a matrix polymer, where nerolidol and limonene appeared to be the most promising enhancers.

It is well known that enhancers permeate into the skin and reversibly decrease the barrier resistance through different mechanisms. The permeation enhancement of D-limonene and L-limonene to ligustrazine hydrochloride is due to multiple (very likely stereoselective) mechanisms including disordering and extracting the stratum corneum lipids (Zhang et al., 2006). Limonene is useful for enhancing the skin permeability of nimodipine and nicardipine from transdermal therapeutic systems containing hydroxypropyl methylcellulose gel or hydroxypropyl cellulose gel as reservoir respectively (Krishnaiah et al., 2002, 2004a,b), by inducing a partial extraction of lipids in the stratum corneum.

Besides limonene, that can deliver haloperidol at a sustained percutaneous rate if incorporated in an organogel comprised of gelator GP1 and propylene glycol (Lim et al., 2006), also the oxide monoterpene limonene oxide can enhance the in vitro permeation of haloperidol through the human epidermis (Vaddi et al., 2003). The mode of interactions of this terpene with stratum corneum is different depending on the employed solvent systems. In fact, limonene oxide in 50% v/v ethanol extracted stratum corneum lipids, disrupted the bilayer packing and partially fluidized the lipids, while, if dissolved in 100% v/v propylene glycol, disrupted the lipid bilayer and leaved the overall bilayer structure intact.

Besides to be employed as penetration enhancers for systemic drug administration, essential oils may be efficiently used for treating skin diseases by topical application. In this case, percutaneous absorption of essential oils and oil components is of great interest. So, the question is raised whether all components of a complex composed essential oil are equivalent with respect to their human skin permeation. Schmitt et al. (2009) investigated the cooperative effect of monoterpenes and phenylpropanoids on *in vitro* permeation through heat-separated human skin epidermis, clearly showing that cooperative effects of single essential oil components may influence percutaneous essential oil absorption. In particular limonene showed an enhancing effect on the permeation of citronellol and eugenol.

13.7.2 Limonene and Contact Dermatitis

Limonene is one of the most commonly used fragrance compounds in western countries today. When exposed to air, it autoxidizes, forming hydroperoxides that are strong contact allergens. Several papers support to the European classification of R-(+)-limonene, containing oxidation products, as a skin sensitizer (Matura et al., 2002, 2003). However not only oxidized R-(+)- but also S-(–)-limonene is a common cause of allergic dermatitis contact (Matura et al., 2006). Furthermore, autoxidation of linalol and R-limonene appeared to have also an irritative effect, being oxidized linalol less irritating than oxidized R-limonene (Bråred Christensson et al., 2009).

To cause allergic contact dermatitis, the hydroperoxides are considered to bind covalently to proteins in the skin via a radical mechanism. When the radical formation from, and sensitizing potential of, allylic hydroperoxides found in the oxidation mixture of limonene, were examined, Johansson et al. (2008) demonstrated that both carbon- and oxygen-centered radicals are important intermediates in the formation of hapten-protein complexes and that the sensitizing potency of the hydroperoxides is related to their structures. Epidermal treatment with antioxidants, such as ascorbic acid or α-tocopherol, may have a protective effect on the allergenic effect of haptens that form full antigens via a radical mechanism, including limonene-2-hydroperoxide (Gäfvert et al., 2002).

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Natural Products Chemistry

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The Boeing Company now uses citrus-based metal polishes and paint solvents, the American Princess Cruise Line uses citrus as a heavy duty degreaser, and when the Exxon Valdez spilled oil over Prince William Sound, citrus cleaners were used to clean up the oily rocks. Citrus oils are even being tested for use in cleaning silicon chips, thus replacing ozone-damaging CFCs. Presenting new and timely information, **Citrus Oils: Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity** explores the evolution of knowledge on the composition of essential oils, possible contaminants of different origin, and the development of analytical techniques.

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