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Carbohydrates in Sustainable Development I

Renewable Resources for Chemistry and Biotechnology

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Aims and Scope

The series *Topics in Current Chemistry* presents critical reviews of the present and future trends in modern chemical research. The scope includes all areas of chemical science, including the interfaces with related disciplines such as biology, medicine, and materials science.

The objective of each thematic volume is to give the non-specialist reader, whether at the university or in industry, a comprehensive overview of an area where new insights of interest to a larger scientific audience are emerging.

Thus each review within the volume critically surveys one aspect of that topic and places it within the context of the volume as a whole. The most significant developments of the last 5–10 years are presented, using selected examples to illustrate the principles discussed. A description of the laboratory procedures involved is often useful to the reader. The coverage is not exhaustive in data, but rather conceptual, concentrating on the methodological thinking that will allow the non-specialist reader to understand the information presented.

Discussion of possible future research directions in the area is welcome.

Review articles for the individual volumes are invited by the volume editors.

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Preface

Global warming and the increasing demand for raw materials make the transition to a sustainable era of energy and materials a necessity. The development of new chemical processes for intermediates not based on coal and oil is becoming essential [1]. Our economic system is currently built on finite resources, for which we will sooner or later need a substitute. Biomass is considered one of the possible alternatives to develop in the near future, which could contribute in the immediate to increase the lifetime of available fossil forms of carbon. The term "biomass" means any plant-derived organic matter available on a renewable basis, including energy crops and trees, agricultural food and feed crops, agricultural crop wastes and residues, wood waste and residues, aquatic plants, animal waste, municipal waste, and other waste materials [2]. Biomass resources can be used to produce an array of energy-related products including electricity, liquid, solid, and gaseous fuels, heat, chemicals, and other materials of high-added value. Biomass renewable energy is also considered relatively "sustainable," since the related environmental and social impact is normally more benign than that of fossil or nuclear fuels. However, large areas of land are necessary to grow energy crops, which introduce some concerns about the impact of using land for this purpose when it should first be used to produce food. Only the left-overs and wastes from agriculture should be exploited as a fuel source for the manufacture of carbon-based materials (e.g., commodities so much needed by our civilization such as cloths, detergents, paints, pesticides, herbicides, vehicles, and drugs). This observation should stimulate the use of solar energy and sea water to produce electricity and water suitable to irrigate desert land for food and more biomass production. Such projects would provide work for many people in poor regions of the world.

Combustion of biomass and biomass-derived fuels can produce air pollution; how serious this impact is will depend on how carefully the resource is managed. Today, a wide variety of production and conversion methods exist, each with different environmental impacts. However, renewable energy sources can be considered sustainable in the sense that they cannot come to an end, although often, as is the case of biomass, they require an "intelligent" management (adequate use of water and readily degradable pesticides) if they are to be used sustainably.

By integrating the production of higher-value chemicals into fuels production, the overall efficiency of all energy-related products will be improved, thus matching the goals of ecological restoration and economic development (for a discussion of this topic, see http://www.nrel.gov/biomass/biorefinery.html) [3]. Producing fuels and chemicals from biomass is not a new concept. Vegetable oil, cellulose (wood), ethanol, methanol, and many other biomass-based chemicals have been in use since more than two centuries to make solvents, paints, adhesives, synthetic cloths and so on. By the late 1960s, when petrochemicals began to dominate the market, many of these bio-derived products were displaced by petroleum derivatives. The energy crisis of the 1970s generated a renewed interest in the synthesis of fuels and materials from bioresources, but this interest decreased immediately in the subsequent decade as the oil price abated. Presently, economic, geopolitical, and environmental concerns are responsible for the renewed interest in biomass exploitation. In addition, it is noteworthy that new advances in biotechnology and chemical processes have significantly reduced the costs of producing biochemicals; many biochemicals can already compete economically with petrochemicals. When environmental benefits are considered, biochemicals may have even lower production, handling, use and risk management costs than their petroleum-based counterparts. Today, biomass can substitute petroleum feedstocks in the production of most fuels and chemicals. Nevertheless, different processes must be developed; fossil fuels are hydrocarbons, i.e., various combinations of carbon and hydrogen; biomass components are mainly carbohydrates, i.e., various combinations of carbon, hydrogen, and oxygen. The shift from petroleum hydrocarbons to oxygenated bio-based feedstocks may create new opportunities in the chemical and biochemical industries. Important building blocks may be produced from sugars and these can be transformed into new families of useful molecules [4]. The presence of oxygen makes it more challenging to synthesize some products and easier to prepare others; considering the wide range of types of biomass should make it possible to make new and valuable products not made from petrochemicals. However, to avoid recreating the environmental problems arising from many of the processes in use today, it is essential that the use of renewable biomass is complemented by the application of green chemical processes and technologies to generate environmentally compatible products based on green and sustainable supply chains. Currently, there is growing international interest in the development of safer alternatives to problematic chemicals, materials, and products. For example, a central goal of the European Community, as expressed by the new regulation on Registration, Evaluation, and Authorization of Chemicals (REACH), is the substitution of problematic chemicals with safer alternatives (http://www.reachcentrum.org). Clean technology concerns the reduction of waste from an industrial chemical process to a minimum, and it requires the rethinking and redesign of many current chemical processes.

The two books, Vols. 294 and 295, are based on the lectures presented at the Carbohydrates as Organic Raw Materials (CORM) V conference held in Lisbon (January 20–23, 2009), organized and chaired by Amélia Pilar Rauter (University of Lisboa) and Yves Queneau (University of Lyon, INSA Lyon), with the collaboration of Frieder Lichtenthaler (Technische Universität Darmstadt) as Honorary Chairman, who has also started these meetings in 1990. A series of conferences took place until

1997. This led to three monographs "Carbohydrates as Organic Raw Materials" by VCH-Weinheim: Vol. I edited by F.W. Lichtenthaler (1991), Vol. II edited by G. Descotes (1993), Vol. III edited by H. van Bekkum, H. Röpper, and A.G.J. Voragen (1996), and to a fourth volume (Vol. IV) by WUV-Univ. Vienna edited by W. Praznik and H. Huber (1998). After 12 years, the relaunch of CORM in Lisbon brought new insights into the role of carbohydrates for sustainable development.

The contributions presented in this first volume entitled "Carbohydrates and Sustainable Development, Part 1, Renewable Resources for Chemistry and Biotechnology" demonstrate that new knowledge has been collected during the last decade opening many opportunities for better biomass exploitation. Sucrose extracted from sugarcane and sugar beat can be much more than a source of biofuels (ethanol). It can be converted into valuable synthetic intermediates such as bioethylene, 1,2-propyleneglycol, 5-(hydroxymethyl)furfural, and levulinic acid and can be used as glycosyl donor in the enzyme-catalyzed glycosidation to efficiently produce biopolymers and antigenic carbohydrates (Sucrose: A Prospering and Sustainable Organic Raw Material and Sucrose-Utilizing Transglucosidases for Biocatalysis). Natural or synthetic fructose-based oligomers are promising compounds toward functional foods (food additive for better health) (Difructose Dianhydrides (DFAs) and DFA-Enriched Products as Functional Foods). Trees, seaweeds, cereal production left-overs such as straw and brans, and olive pomace constitute the most abundant source of carbon as alternative to oil and coal. Celluloses and hemi-celluloses found in these raw materials can be converted into a large variety of chemicals and materials, some of them with high-added value (Development of Agriculture Left-Overs: Fine Organic Chemicals from Wheat Hemicellulose-Derived Pentoses, Cellulose and Derivatives from Wood and Fibers as Renewable Sources of Raw-Materials, and Olive Pomace, a Source for Valuable Arabinan-Rich Pectic Polysaccharides); seaweeds are now a source of green sur-factants (Oligomannuronates from Seaweeds as Renewable Sources for the Development of Green Surfactants), and polysaccharides from diverse origins can be shaped to new materials for catalysis, absorption, and remediation (From Natural Polysaccharides to Materials for Catalysis, Adsorption, and Remediation). The second volume, which shows the diversity of reactions and synthons based on available carbohydrate-based resources, is entitled: "Carbohydrates and Sustainable Development, Part 2, A Mine for Functional Molecules and Materials".

Lausanne, Summer 2010

Alberto Marra, University of Ferrara
Pierre Vogel, Swiss Institute of Technology Lausanne

References

1. Climate Change (2007) The physical sciences basis. Report of the Intergovernmental Panel on Climate Change (February 5, 2007)
2. Biomass. IUPAC compendium of chemical terminology, Internet edition. http://goldbook.iupac.org/index.html (September 7, 2009)

3. Clark JH, Budarin V, Deswarte FEI, Hardy JJE, Kerton FM, Hunt AJ, Luque R, Macquarrie DJ, Milkowski K, Rodriguez A, Samuel O, Taverner SJ, White RJ, Wilson AJ (2006) Green Chem 8:853
4. Werpy T, Petersen G (eds) (2004) Top value added chemicals from biomass, vol 1. US Department of Energy, Oak Ridge

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Sucrose: A Prospering and Sustainable Organic Raw Material

Siegfried Peters, Thomas Rose, and Matthias Moser

Abstract Sucrose (α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside) is an inexpensive chemical produced by sugar cane and sugar beet cultivation. Chemical and/or biochemical transformations convert it into highly valuable synthetic intermediates such as 5-hydroxymethylfurfural (HMF), bioethylene, 1,2-propylene glycol and levulinic acid. Sucrose can also be converted into biodegradable polymers such as polyesters and polyurethanes, as well as into novel carbohydrates such as isomaltulose, trehalulose, inulin, levan, Neo-amylose, and dextran, highly valuable additives for food and cosmetics and materials for separation and purification technologies.

Keywords 1,2-Propylene glycol, 5-Hydroxymethylfurfural, Bioethylene, Dextran, D-Fructose, D-Glucose, Esters, Inulin, Isomalt, Isomaltulose, Levan, Levulinic acid, Neo-amylose™, Polyurethanes, Sucralose, Trehalulose

Contents

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1 Introduction

Sucrose or in general sugar is produced on the industrial scale with an annual production of about 167 million metric tons (data for 2007/2008) [1]. Two important sugar crops predominate: sugarcane (*Saccharum* spp.) and sugar beet (*Beta vulgaris*), in which sugar can account for 12–20% of the plant's dry weight. Sucrose is obtained by extraction of these crops with hot water. Purification and concentration of the extract gives syrups, from which solid sucrose can be crystallized.

A quarter of the total sugar produced in the world is derived from sugar beet, three quarters from sugar cane. Whilst the beet sugar amount has been quite constant during the last few decades, sugar production from cane has increased from the 1960s on [1].

Considering the figures given above, sucrose is without doubt the most available low molecular weight carbohydrate. Until now it has mainly been used for nutrition purposes and in the fermentation industry – above all for the production of bioethanol, citric acid, and lactic acid – and only to a smaller extent as chemical feedstock [2–4]. Many approaches to utilize sucrose as raw material for different organic chemicals have been attempted [2], but only a few are successfully implemented today. However, due to the predicted shortage of fossil resources within the next few decades [5], sucrose is amongst the predestined renewable organic compounds and alternative feedstock for many still petrochemically derived products or product applications. Although the total amount of sugar produced has increased dramatically, today's world market price of sugar is on the whole comparable to that of 20 years ago. Although the price in the past few decades has been fluctuating dramatically between 100 and 400 USD/ton for raw sugar and 160–500 USD/ton for white sugar, a definite price trend for the future cannot be stated, neither to the advantage nor to the disadvantage of sucrose with regard to its price competitiveness as an organic raw material.

2 Sustainability Efforts in Industrial Sugar Production

Any prospect for sucrose (or any other agro-based chemical) as an organic raw material is based on its sustainable supply and long-term availability.

The sugar industry has impressively progressed in the last few decades towards more sustainable conditions, particularly regarding its impact on natural resources like, e.g., energy and materials, its environmental impact, and the sustainability of the agricultural production base.

Continuous efforts are made in the breeding and cultivation in particular of sugar beet to increase the sugar yield, to reduce the nonsugar compounds in the crop, and to decrease the environmental impact. Intense breeding programs and improved advice to the farmers have contributed significantly to the continuous reduction of the specific mineral fertilizer consumption in the last few decades for sugar beet cultivation as shown in Fig. 1.

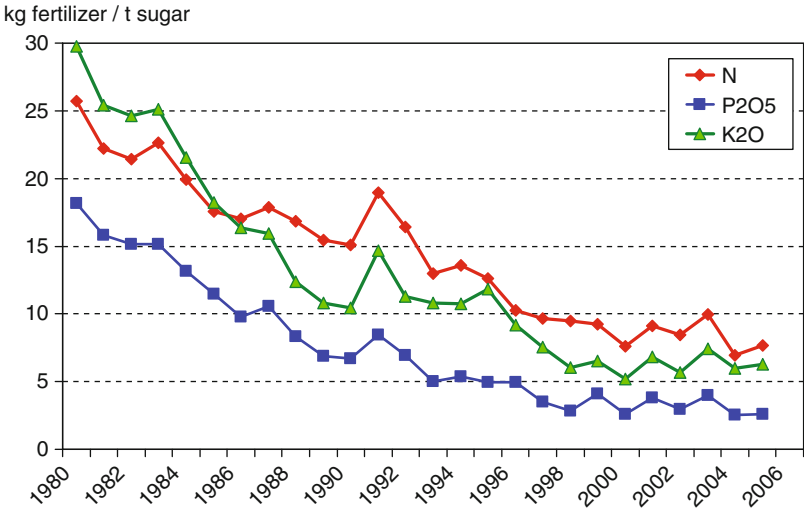


Fig. 1 Trend of the consumption of mineral fertilizer for the sugar beet cultivation

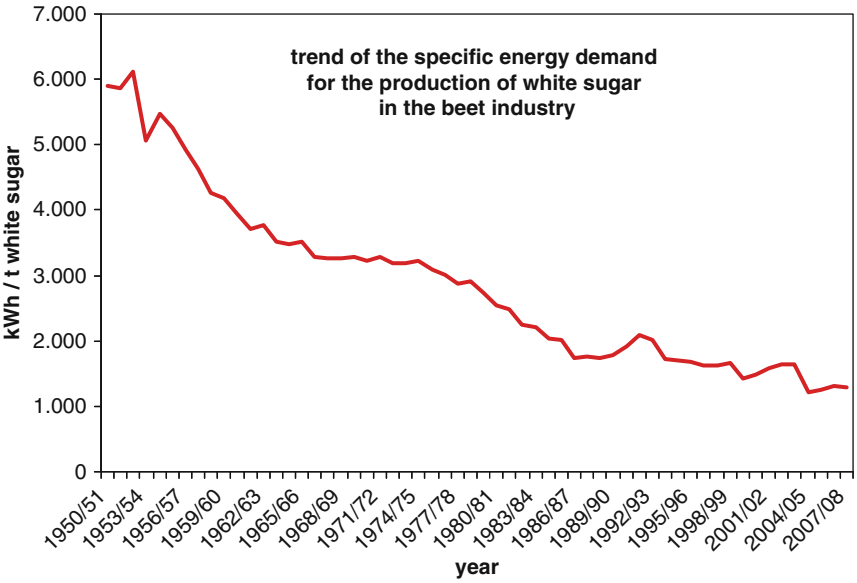


Fig. 2 Specific energy demand for the production of white sugar in the beet industry

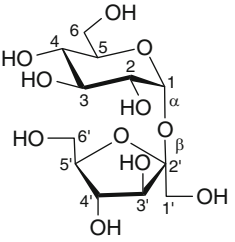
The application of herbicides for the cultivation of sugar beet could be reduced significantly, too, from 2.0 kg/ton sugar in 1975 to 1980 to 0.7 kg/ton sugar in 2007.

In spite of this, the beet sugar industry has significantly improved the overall energy efficiency for white sugar production within the last decades as illustrated in Fig. 2 by more than three times.

Further improvements, e.g., further reduction of application of chemicals and primary energy, will be achieved by using new beet varieties combined with the potential of green biotechnology. Promising research perspectives include approaches to increase the sucrose yield through expanding the vegetation period and thus the light interception of sugar beet.

3 Reaction Pathways of Sucrose Towards Organic Chemicals

In the nonreducing disaccharide sucrose (α-D-glucopyranosyl-(1→2)-β-D-fructofuranose) D-glucose and D-fructose are linked together at their anomeric carbon atoms. The interglycosidic bond is acid-sensitive; hence, under acidic conditions, hydrolysis, known as inversion of sucrose, occurs leading to the two building blocks of sucrose, D-glucose and D-fructose.



Sucrose
(α-D-glucopyranosyl-(1→2)-β-D-fructofuranose)

There are three main material-transformation-pathways under which sucrose could be valorized to promising organic chemicals and intermediates [6]:

1. Degradation of the sucrose framework
2. Derivatization by maintaining the sucrose skeleton
3. Reactions and rearrangements maintaining the carbohydrate structure

The first one of these converts sucrose into valuable noncarbohydrate compounds by degradation of the sucrose skeleton itself. Thereby, hydrolysis of sucrose (the usual first step) is subsequently followed by further degradation under elevated temperature and pressure conditions in the presence of certain catalysts. Chemical reactions or reaction cascades, e.g., dehydration/rehydration, ring opening/closure, bond scission/formation, lead to noncarbohydrate structures which are of known value from petrochemically-derived compounds. One of the most prominent representatives of these sucrose degradation products is 5-hydroxymethylfurfural (HMF).

In the second pathway the functional groups of sucrose could be utilized for chemical reactions with other compounds, thereby preserving the original sucrose structure as such. The only sucrose functionalities of immediate access for chemical reactions are the eight hydroxyl groups. Even though three of them are primary OH-groups (6, 1′, 6′) and five of them secondary OH-groups, the chemical

differentiation is difficult and selectivity of sucrose could not simply be differentiated by the different reactivity order of primary and secondary OH-groups. Due to hydrogen bond connections between OH-2 and OH-1′/OH-3′ in aqueous solutions, the reactivity of these OH-groups is influenced by electron-withdrawing effects. As a consequence, the OH-2 group is the most acidic one. Considerable impact on the selectivity, regiochemistry, and degree of substitution is also made by the reaction type and the conditions used [2, 7].

Focus is also put on the third pathway which includes reactions of sucrose which lead to carbohydrate structures different from the starting carbohydrate sucrose. This includes the inversion of sucrose to the monomers D-glucose and D-fructose but also enzymatic rearrangements of the two sucrose building blocks to other carbohydrates, e.g., isomaltulose and trehalulose.

In the following the examples mentioned should give an overview of possible chemical modifications of sucrose according to these three pathways. Thereby valuable chemical intermediates for industrial bulk-scale productions are addressed, some of them already industrially implemented.

3.1 Degradation of the Sucrose Framework

The application of elevated temperature and pressure conditions to sucrose provides many different chemical compounds. Some of them are well known from petrochemical feedstocks, other at least resemble petrochemically derived compounds and therefore carry the potential to replace them [8].

3.1.1 Bioethylene Based on Sucrose

Examples of a successful effort to utilize sucrose as feedstock for the manufacture of bulk organic chemicals are the announcements of several companies of the production of bioethylene from sugar via bioethanol. Based on such “green” ethylene, Crystalsev and Dow Chemicals are planning to start the production of 350,000 tons of polyethylene in 2011 [9]. Braskem has announced a smaller production of 200,000 tons of “the first certified linear polyethylene in the world made from 100% renewable raw material” but for 2010 [10].

Solvay Indupa has published the intention to expand their polyvinyl chloride (PVC) manufacturing plant in Santo Andre (Brazil) to a total amount of 360,000 tons/year. For the capacity extension the required monomer feedstock vinyl chloride should be based finally on sucrose and salt. Despite the ongoing financial crisis, Brazilian companies continue with their investments in “green” plastics [11–13].

In total the planned bioethylene production capacity seems low compared to the annual ethylene production of 100 million tons (2000) but it is enough to show the capability of utilizing sucrose as feedstock for organic chemicals. The door towards the whole C2-chemistry being based on renewable resources is opened.

3.1.2 Access to 1,2-Propylene Glycol and Other Polyhydric Alcohols

Many different diols, triols, and polyols are accessible from sucrose either chemically by partial hydrogenation or by biocatalysis [14–17]. Several attempts to utilize sucrose as feedstock for the chemical production of polyols have been made since the 1930s [15–17]. Under catalytic hydrogenation conditions, aqueous and/or alcoholic sucrose solutions are described to be converted into alcohol mixtures consisting essentially of 1,2-propyleneglycol, glycerol, ethylene glycol, and other higher alcohols. Especially the first one of these, 1,2-propyleneglycol, is known as a valuable organic chemical which is used for the manufacture of unsaturated polyesters and is utilized in deicer, antifreeze, and brake fluids, as well as in cosmetics and pharmaceutical products [18].

A process claimed by BASF describes the use of a cobalt/copper/manganese catalyst for the hydrogenation of an aqueous sucrose solution, thereby showing the opportunity of influencing the composition of the product mixture by properly maintaining reaction temperature and hydrogen pressure [19, 20]. The optimum temperature window is 230–280°C and the hydrogen pressure range is preferably 250–300 bar. Lower temperature and pressure favor the production of mannitol and sorbitol, higher temperature and pressure conditions leading to the formation of primary alcohols. Under optimal conditions the reaction favors essentially the formation of 1,2-propylene glycol (50–65 wt%) and ethylene glycol (20–25 wt%) accompanied by lesser amounts of 1,2-butylene glycol (5–7 wt%) and hexane-1,2,5,6-tetraol (3–10 wt%) (Fig. 3). The amount of glycerol is negligible. In order to prevent a decrease in catalyst selectivity, basic substances (e.g., calcium oxide, sodium carbonate) were added to the reaction mixture.

Until now, 1,2-propylene glycol has been chemically manufactured only by the petrochemical pathway via hydration of propylene oxide with excess water in the presence of acid or base catalyst. But the mentioned manufacture process based on the renewable feedstock sucrose might become interesting on depletion of the petrochemical feedstock.

Furthermore, 1,2-propyleneglycol and other polyols containing vicinal OH-groups could be reacted to valuable aldehydes and ketones. Thereby an acid catalyzed dehydration is subsequently followed by an intramolecular rearrangement according

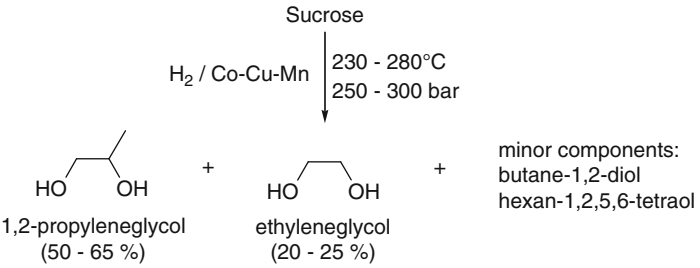


Fig. 3 Conversion of sucrose to 1,2-propyleneglycol, ethylene glycol and other di- and polyhydric alcohols [19, 20]

to the pinacol–pinacolone-rearrangement (conc. sulfuric acid) or more preferably under improved mild conditions which use catalytic amounts of acetic acid in water at high temperature and pressure [14].

3.1.3 5-(Hydoxymethyl)furfural and Its Derivatives

HMF is regarded as a secondary basic chemical derived from renewable resources [14]. Thus it is evaluated as a potential replacer of existing petrochemically derived organic compounds (see also [21]). Due to its chemical structure, HMF takes a unique position between petrochemical and carbohydrate chemistry [22]. The furanic compound carrying an alcoholic and an aldehyde functionality does not immediately reveal its carbohydrate origin. A petrochemical root seems to be much more presumable, but at least feedstocks for HMF are hexoses.

Under elevated temperature conditions and acidic catalysis, hexoses eliminate 3 moles of water to form HMF (see [21] for possible reaction mechanisms). But in aqueous solution, reaction does not stop at this stage (Fig. 4). The dehydration reaction is subsequently followed by a rehydration step leading to levulinic and formic acid. In addition, this reaction sequence is accompanied by intermediates, side products, and at least the formation of colored soluble or insoluble polymeric compounds [23, 24].

Within the last few decades many attempts for a most successful synthesis have been made. D-Fructose has proven to be the feed of choice. For the dehydration of D-fructose towards HMF, many variations in conditions (catalyst, temperature, time, concentration, and solvent) have been investigated and carefully balanced out. The aim was to overcome the by-product formation and thus an optimized HMF-yield [14, 23, 24].

Higher yields, almost quantitative, are reported when the solvent DMSO is used either in combination or without ion-exchange resins [23–27]. But from the ecological point of view the use of DMSO is unfavorable and furthermore the separation of the solvent DMSO from the desired product HMF is difficult. A different solvent approach utilizing a solvent with higher volatility and thus easier separation from HMF is the use of a sub- and supercritical organic solvent like acetone. In a 9:1 mixture of acetone/water – water is needed due to the low solubility of D-fructose in pure acetone – and sulfuric acid catalysis at a temperature of 180°C and a pressure of 20 MPa, high fructose conversion and HMF selectivity (up to 77%) could be achieved without the formation of insoluble humic acids [28–31]. The tremendous increase in selectivity for the acetone/water solvent system could be

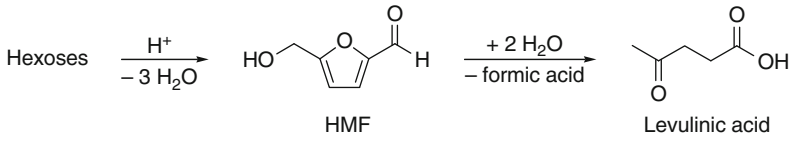


Fig. 4 Formation of HMF and rehydration to levulinic acid

explained by the tautomeric equilibrium shift in subcritical acetone towards the furanoid forms [14]. Using sucrose directly instead of D-fructose lowers the selectivity to 56%, when using D-glucose even to 48%.

In another approach, the dehydration has been conducted in ionic liquids (1-alkyl-3-methylimidazolium chloride) under acid or metal chloride catalysis yielding HMF up to more than 80% nearly without any formation of by-products like levulinic acid [32]. When using chromium(II) chloride as catalyst even D-glucose could be used as feedstock since this catalyst is effective for the in situ isomerization of D-glucose to D-fructose before dehydration takes place to produce HMF in 70% yield. The catalyst system N-heterocyclic carbene/$CrCl_2$ in 1-butyl-3-methyl imidazolium chloride has been developed for the selective conversion of D-fructose (96% yield) and D-glucose (81% yield) [33].

The production of HMF was even driven forward to a pilot plant scale including the work up and isolation of the pure product [34]. It consists essentially of the following steps: (1) dehydration of fructose to HMF in aqueous solution (0.5 M) under sulfuric acid catalysis at 150°C; (2) cooling and subsequent neutralization (CaOH or $CaCO_3$) and filtration; (3) chromatographic separation of the filtrate on calcium-loaded strong acidic cation exchanger resin; and (4) cooling crystallization of the HMF fraction with overall HMF yields of 40–50%.

Taking into account that fructose loses three molecules of water during dehydration towards HMF, from 1 kg of fructose calculating the theoretical maximum yield only 0.7 kg of HMF could be obtained. When considering a favorable average molar yield of 70% for the dehydration, from 1 kg of fructose only 490 g of HMF could be obtained.

From HMF, organic chemicals with large industrial potential are available by basic chemical operations [5, 35]. In particular the HMF-derived 1,6-diol 1,6-diamin and the 2,5-dicarboxilic acid (FDCA) have to be mentioned (Fig. 5). These structures are comparable to petrochemically derived alkyldiols, hexamethylene diamines, and adipic and terephthalic acid, and have proved to be suited to

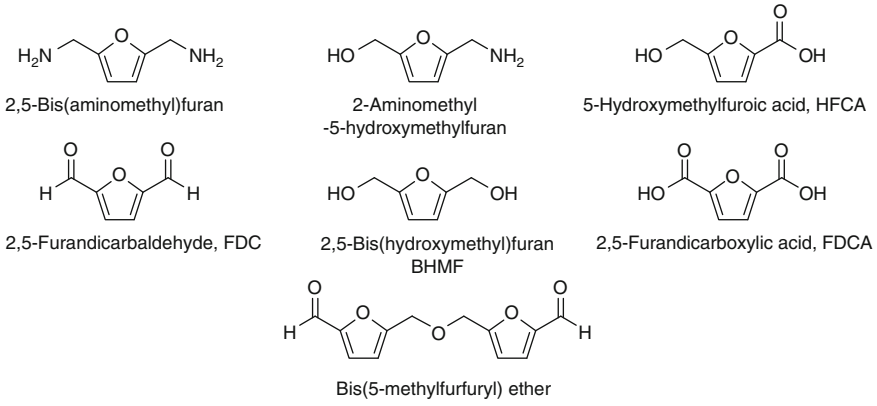


Fig. 5 Organic chemicals derived from HMF [5, 35, 36]

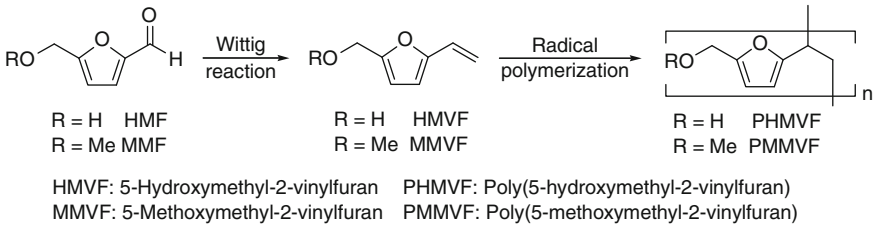


Fig. 6 HMF-derived vinyl polymers [38]

producing polyesters and polyamines; thus they might have the potential to replace petrochemical derived polymers [7, 37].

Another attempt shows the synthesis of HMF-derived vinyl polymers (Fig. 6) [38]. By the Wittig reaction, HMF and the methylated derivative 5-(methoxymethyl)furfural (MMF) could be converted into vinyl derivatives. Radical polymerization leads to the corresponding vinyl polymers which might become substitutes for "commodity polymers" like polyethylene, poly vinyl chloride, and polystyrene.

Despite the tremendous efforts in the elaboration of pathways for optimized reactions towards HMF and the possibilities of gaining a broad variety of HMF-based derivatives, all being estimated as potential replacers of existing petrochemically-based chemicals, and to some extent the proof of their ability for preparing value added products out of them, to date HMF has not been industrially manufactured. This seems to be mainly driven by economic reasons. Even on a large-scale the costs for HMF-production is estimated to be at least at 2.50 €/kg. Therefore HMF could not compete with petrochemical bulk chemicals [3, 4, 29].

3.1.4 Levulinic Acid

The rehydration product of HMF, levulinic acid, is also regarded as a potential biomass derived organic compound [35, 39, 40]. Levulinic acid could be utilized as feedstock for several large volume chemicals (Fig. 7), e.g., methyltetrahydrofuran and levulinate esters (fuel additives), delta-amino-levulinic acid (herbicide), and diphenolic acid (replacer for bisphenol A for polycarbonates).

3.2 Derivatization by Maintaining the Sucrose Skeleton

By chemical reactions sucrose can be modified to several interesting products for food and nonfood application, thereby preserving the sucrose skeleton as such.

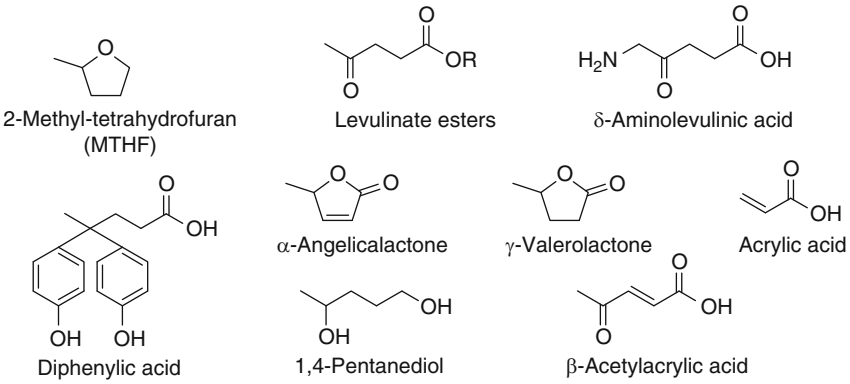


Fig. 7 Levulinic acid derivatives of industrial potential [35, 39, 40]

3.2.1 Sucrose Esters

As nonionic surface-active compounds, sucrose esters have proved their utilization as emulsifiers and fat replacers [41, 42]. They consist of the hydrophilic sucrose part and lipophilic fatty acid compounds which are linked together by esterification. Lower substituted sucrose esters are easily accessible by the reaction of the more reactive three primary OH-groups of sucrose with fatty acids, thus leading to mono-, di-, and triesters. These esters are well suited for application as emulsifiers. One classification for emulsifiers/surfactants is the hydrophilic–lipophilic-balance (HLB) ranging from 1 to 20. For sucrose esters a low HLB value indicates its applicability as stabilizer for water-in-oil emulsions, whereas high HLB-value sucrose esters are suited to stabilize oil-in-water emulsions. By changing the ester composition and the degree of substitution, sucrose esters with an HLB value ranging from 1 to 16 could be generated. Functional properties evolving therefrom are beneficial for food texture due to interaction with proteins and starch, for the control of sugar crystallization, and for stabilizing foam. Therefore sucrose esters are suited for use in many different cosmetic and food applications like dressings and sauces, confectionery, bakery, and icings and fillings. One application limitation is the narrow favored pH range of 4–8. At a pH lower than 4 the sucrose inversion becomes effective and under basic conditions saponification of the ester bond occurs.

Sucrose esters with a higher degree of substitution, so-called sucrose polyesters (Fig. 8), have characteristic properties of fats and oils. At the same time they are only partly digestible or even nondigestible by the body's lipases and are not absorbed by the human digestive system [7, 22, 42, 43]. Thus, their use as fat replacers appears predetermined and indeed sucrose polyesters with 6–8 acyl moieties were approved by the US Food and Drug Administration (FDA) in 1996 for certain use as fat replacers [43]. Known brand names are Olestra® and Olean®. They are used in food applications, e.g., in the preparation of dietary fried snacks [2, 42].

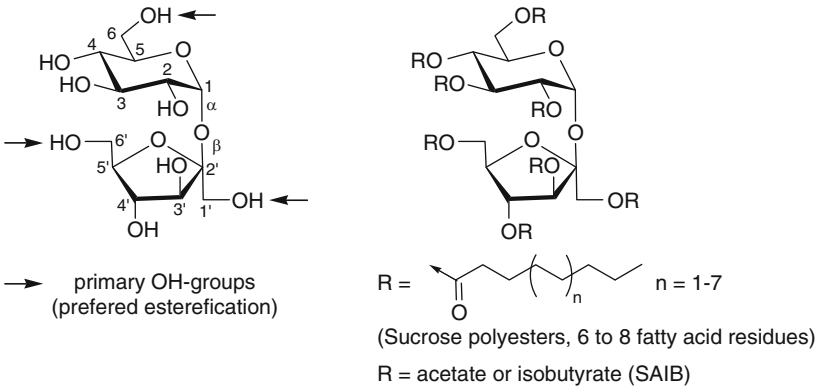


Fig. 8 Preferred substitution positions for sucrose fatty esters and examples for sucrose polysaccharides

Sucrose fatty esters were first prepared by the Hass–Snell reaction [44] where sucrose is treated with fatty acid esters under basic conditions in a solvent process with DMF, leading through interesterification to the desired sucrose fatty esters [41, 42, 45, 46]. DMSO and other solvents proved to be suited for this process too, but all of those were not food approved. In an improved process an emulsion of sucrose in propylene glycol could be reacted with fatty acid esters under basic conditions. The most interesting process – because of the avoidance of hazardous solvents – is the melt process [41] where sucrose and the fatty acid ester (e.g., methyl) are reacted together without any solvent. By these esterification reactions, preferably the three primary OH-groups of sucrose could be addressed. Although reactivity of the OH-groups in sucrose decreases in the order 6-OH, 6′OH, 1′-OH, and secondary-OH (Fig. 8), mixtures of different substitution patterns are obtained. Due to being more specific, enzymatic esterifications became attractive, leading to better defined products [41, 47].

One further example of a sucrose polyester is sucrose acetate isobutyrate (SAIB, Fig. 8) which is a mixed sucrose polyester prepared by reaction of sucrose with acetic and isobutyric anhydrides [46]. It is by far the widest industrially utilized sucrose ester and covers food as well as nonfood applications [48, 49]. SAIB shows a very high viscosity and is insoluble in water. In beverages it is used as a weighting agent which prevents the separation of essential oils during storage and stabilizes the appearance of cloudy beverage products. In the nonfood area, among others, SAIB improves gloss and adhesion in lipstick and nail polish and proves to be suited for pigment dispersion.

3.2.2 Polyurethanes

Polyurethanes are formed by polyaddition of diisocyanates and dihydroxy components. When, instead of diols, polyhydric alcohols are used, more rigid cross linked polyurethanes are formed. The more OH-functionalities present in the hydroxyl

compound the bigger the impact on rigidity. Therefore sucrose with its eight hydroxyl groups leads to rigid polyurethane foams [46, 50–53]. To improve the physicochemical properties of sucrose-based polyurethane foams, sucrose is first alkoxylated with alkylene oxide (ethylene or propylene oxide) and then the resulting polyether polyol is used as polyhydric component for the polyurethane formation reaction. Under hydroxyl catalysis the alkylene oxide is reacted with sucrose. By the amount of alkylene oxide the molecular weight of the resulting polyether polyol can be adjusted. Afterwards the reaction mixture is neutralized by acid. The polyurethane products so obtained are not well characterized concerning their cross linking pattern and the reactive positions in the sucrose moiety.

The preparation of well defined sucrose polyurethanes has been investigated in studies with partially protected sucrose esters [54]. Regioselectivity observations on the carbamoylation of sucrose in DMF showed in the case of alkylisocyanates a clear preference for the reaction position at the 2-OH in the glucose part. When phenylisocyanate is used the most favored reaction position is the 6-OH-group followed by the 2-OH-group [55, 56]. Similar regioselectivities could also be found for the reaction of the respective diisocyanates with sucrose [56].

3.2.3 Sucralose

In the 1970s studies on the selective replacement of hydroxyl groups in the sucrose molecule for halogen by Tate & Lyle in cooperation with Queen Elizabeth College, revealed a variety of halogenated sucrose derivatives which partly showed a remarkably enhanced sweetness compared to that of sucrose itself [57–60]. From all of those compounds finally 4,1′,6′-trichloro-4,1′,6′-trideoxy-galactosucrose (nowadays better known as sucralose) was evaluated as showing the most convenient physico-chemical properties. This contains the quality and intensity of sweet taste, good solubility in water (for use in beverages), and attractive stability of the glycosidic linkage under acidic conditions. Sucralose has a sweetness of about 400–800 times that of sucrose (depending on the field of application) and has been commercialized by Tate & Lyle under the Splenda® brand name [61]. Nowadays it is used in the food industry to the extent of about 1,000 tons/year.

The selective chlorination of the desired positions is maintained by efficient protection group chemistry in three basic steps. First, a protection of selective OH-groups, second the chlorination, and last the deblocking of the protection groups to yield sucralose which on crystallization could be obtained in high purity [60].

Since its first synthesis, many investigations on the optimization of the reaction sequence have led to a bundle of patents covering different executing possibilities for each reaction step [62–80]. Out of those patents a rather straightforward sucralose synthesis could be assembled (Fig. 9). A dibutyltin oxide supported selective acylation (acetate or benzoate) of the 6-OH-group in the glucose part starts the reaction sequence [62–67]. Alternatively, other selective approaches are reported, like acetylation at low temperature [68], using orthoester formation [69], or enzymatic techniques [70–73]. High efficiency chlorination with regioselectivity

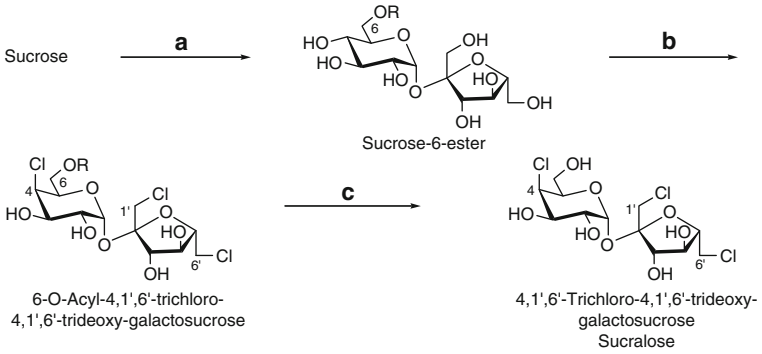


Fig. 9 Straightforward and efficient reaction pathway from sucrose to sucralose. R = Ac or Bz. (**a**) Organotin-mediated selective acylation: 1. Stannoxane ester formation, 2. Acylation. (**b**) Selective chlorination by Vilsmeier type reaction: DMF/acid chloride/ΔT. (**c**) Deacylation: basic condition (e.g., NaOMe/MeOH)

at the desired positions (4-OH, 1′-OH, and 6′-OH) could be conducted by use of acid chlorides like $COCl_2$, $SOCl_2$, $POCl_3$, SO_2Cl_2, or PCl_5 in DMF according to a Vilsmeier type of reaction [68, 74, 75]. During chlorination, inversion of the configuration at OH-4 occurs, thereby leading to a galactosucrose. After removal of the solvent DMF the 6-*O*-ester group is removed under basic conditions yielding sucralose which, on crystallization, is purified [76–80]. By this reaction cascade sucralose could be obtained in overall yields of about 40%.

3.3 Reactions and Rearrangements Maintaining the Carbohydrate Structure

Sucrose is also a valuable feedstock for other promising carbohydrate components of technical interest. By cleavage of the linkage between the two sucrose building blocks, D-glucose and D-fructose in a first step are separated and afterwards linked together at a different position. These rearrangements are catalyzed by enzymes.

The driving force of all these enzymatic rearrangements is the high free standard enthalpy of the glycosidic linkage between the D-glucose and the D-fructose moiety ($\Delta G^\circ = -27$ kJ/mol). This enthalpy value is in the same dimension as the free standard enthalpy of one phosphoanhydride bond within adenosine triphosphate (ATP, $\Delta G^\circ = -31$ kJ/mol), the universal energy carrier in all biological systems [81]. Thus, sucrose is not only the feedstock for the enzymatic rearrangements but also delivers the necessary reaction enthalpy. Therefore no additional energy supply for the rearrangement is required.

Furthermore, biotransformations in general show several advantages over classic chemical reactions. The solvent system is simply water at ambient temperature, pressure, and pH [82]. The enzymatic reactions are often highly specific, thus

providing nearly no by-products [83]. For the following examples, high sucrose concentrations in the feedstock up to 50 weight% are also feasible, thereby reducing the risk of microbial contamination.

3.3.1 Inversion of Sucrose

The easiest obtainable carbohydrates from sucrose are D-glucose and D-fructose (Fig. 10). Thereby acidic or enzymatic hydrolysis leads to these two building blocks [84]. The name of the resulting invert sugar syrup is based on the fact that the prefix of the optical rotation value of sucrose syrup changes ("inverts") during the hydrolysis from plus to minus. The annual invert sugar syrup production for the German market is in the 350,000–400,000 ton scale which is mainly used for food, e.g., beverages and dairy products.

The homogenous acid catalyzed hydrolysis of sucrose uses food approved mineral acids (sulfuric acid, hydrochloric acid) at elevated temperature. The degree of inversion could be adjusted by the point of neutralization (sodium or potassium hydroxide). This step leads inevitably to the formation of the respective salts, thereby causing high ash contents in the product. A further disadvantage of this method is the applied elevated temperature in combination with the low pH, thus causing by-product formation.

The obstacles mentioned could be avoided by the heterogeneously acid catalyzed process which is established as a state of the art process for sucrose inversion. Thereby the acid catalyst is an immobilized acid like a strong acid cation ion exchanger in protonated (H^+) form in a fixed bed column. The resins are legally approved for food production. In a continuous bulk production sucrose solutions with 50–60 wt% can be completely hydrolyzed at an inversion temperature of 30–45°C. This inversion product, a mixture of D-glucose and D-fructose, could be separated chromatographically. Thereby the semicontinuous or continuous simulated moving bed (SMB) technology yielding D-glucose and D-fructose fractions with a purity of minimum 95% proved to be advantageous over the batch-wise process.

A highly specific hydrolysis of sucrose could also be achieved by use of invertase from *Saccharomyces cerevisiae* (β-D-fructofuranosidase). Nevertheless high enzyme costs and the batch-wise operation mode proved economically disadvantageous over the ion exchange process.

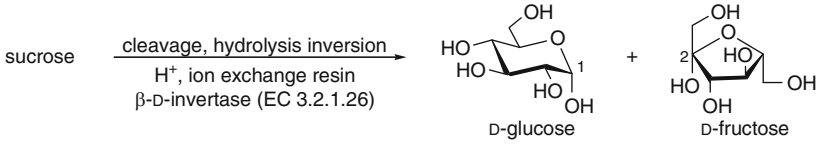


Fig. 10 Inversion of sucrose

3.3.2 Novel Carbohydrates, e.g., Isomaltulose (Palatinose™) and Trehalulose

Isomaltulose (α-D-glucopyranosyl-(1→6)-D-fructofuranose) and the related trehalulose (α-D-glucopyranosyl-(1→1)-D-fructofuranose) are both accessible from sucrose by enzyme-catalyzed rearrangements (Fig. 11).

Isomaltulose is commercialized as Palatinose™ which has achieved certain commercial relevance [85–93]. It is produced on the industrial scale by enzymatic conversion of sucrose using the strain of *Protaminobacter rubrum*, which was originally isolated in 1957 from sugar beet raw juice [88, 90, 94–98, 147]. It is valorized itself as a sugar replacer or used as precursor for the production of the sugar replacer isomalt, an approximate 1:1 mixture of 1-*O*-α-D-glucopyranosyl-D-mannitol (1,1-GPM) and 6-*O*-α-D-glucopyranosyl-D-sorbitol (1,6-GPS) (Fig. 12). Palatinose also showed potential as feedstock for the production of biodegradable tensides since, by reductive amination, the free carbonyl group of isomaltulose could react with long chain aliphatic amines, e.g., *n*-dodecylamin, thereby providing a surfactant compound [99–101].

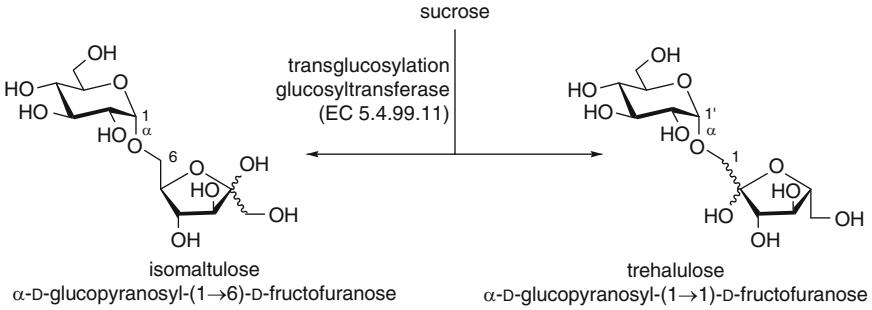


Fig. 11 Enzyme-catalyzed rearrangements of sucrose towards isomaltulose and trehalulose

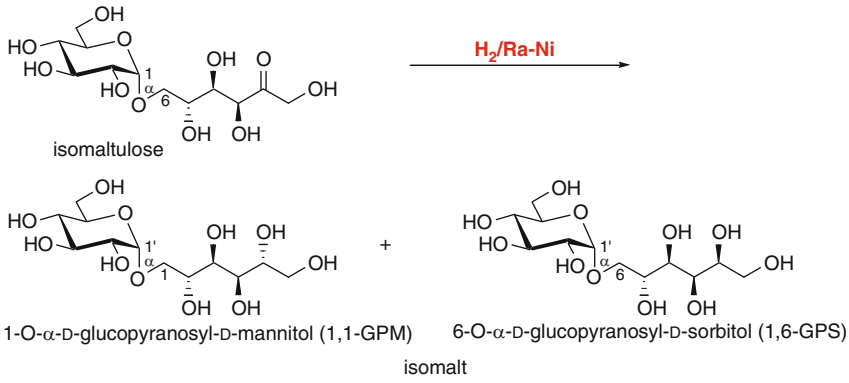


Fig. 12 Hydrogenation of isomaltulose to isomalt

Isomaltulose is a reducing, free-flowing, nonhygroscopic crystalline substance, crystallizing easily from water with 1 mole crystal water. The resulting crystalline isomaltulose melts at 123–124°C [86, 91, 92, 102]. Crystal structure [103] and C^{13}-NMR spectrum [104, 105] were reported. Like sucrose, isomaltulose shows a neutral sweetness without any aftertaste, but it is only 42% as sweet as sucrose [86, 91, 106, 107]. However, in contrast to sucrose, isomaltulose is noncariogenic [86, 108–115] and has a low glycemic index; therefore the insulin release is reduced compared to other sugars [86, 116, 117]. In humans, isomaltulose is hydrolyzed by isomaltase and absorbed as glucose and fructose; thus, like sucrose, its caloric value is 4 kcal/g [86, 91, 114]. It is suggested that isomaltulose has favorable effects on mental performance in humans [118]. The mentioned outstanding properties and the higher acid stability compared to sucrose substantiate its use as sweetener in comestibles and for sport food and drinks [86, 114, 119, 120]. After comprehensive verification of its safety, isomaltulose is approved as human nutrition [85, 120–124].

Trehalulose could be obtained by the enzymatic rearrangement of sucrose by *Pseudomonas mesocacidophila* [125–127]. Most characteristics of trehalulose are similar compared to those of palatinose. One main difference is the higher solubility of trehalulose which offers better opportunities regarding beverage applications [87, 125].

Due to their reducing properties, isomaltulose and trehalulose are technically used as mild reducing agent. Both compounds as such or in mixtures could be used for the gentle reduction of sulfur and vat dyes in aqueous alkaline medium [86, 128].

3.3.3 Inulin, Fructooligosaccharides and Levan, Neo-Amylose, and Dextran

Fructans are natural occurring polymers of D-fructose, which exist as two different types, known as inulin and levan, characterized by a different linkage pattern. In both types one end of the strain is terminated by a D-glucose moiety (Fig. 13).

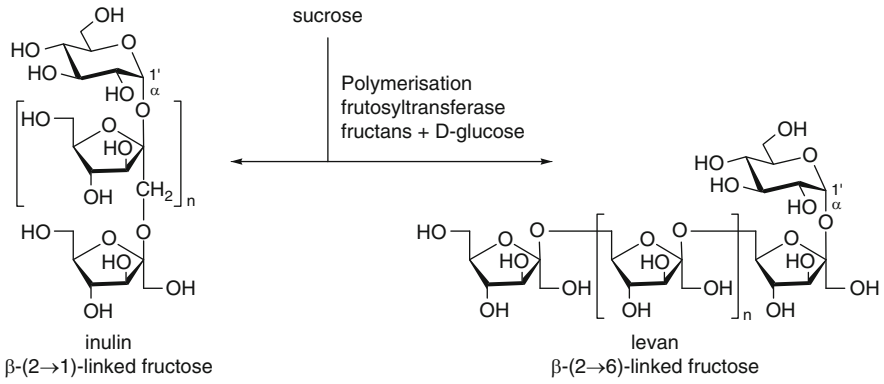


Fig. 13 Inulin and levan formation by fructosyltransferase from sucrose

Inulin is characterized by a β-(2→1) linked backbone and is generally found as reserve carbohydrate in plants such as chicory (up to 20%), Jerusalem artichoke, and onion, and also in some bacteria. Plant inulin has a degree of polymerization (DP) with a maximum up to 200, which depends on the plant species and their life-cycle. Bacterial inulin has a much higher DP (from 10,000 to more than 100,000) but is also highly branched (≥15%). Both DP and the presence of branches are important properties since they influence the functionality of the inulin. Many possible applications demand a high molecular weight inulin like bacterial inulin, but without branches.

This obvious structural difference between plant and bacterial inulin has its origin in the individual synthesis related system. Feedstock for both inulins is sucrose. However, the plant inulin production is a two-step reaction, starting with a sucrose-1-fructosyltransferase (1-SST). One sucrose molecule acts as donor and a second one as acceptor of a fructosyl unit. This leads to the formation of the trisaccharide 1-kestose. Catalyzed by a fructan-fructan-1-fructosyltransferase (1-FFT), fructosyl units are shuffled between the 1-kestose and higher polymeric β-(2→1) linked fructan molecules in the second step. Repetition of this step results in inulin with β-(2→1) linkages only [129–132].

Only the fructosyltransferase (FTF, EC 2. 4. 1. 9) is required in bacteria for the synthesis of bacterial inulin. The enzyme shuffles fructosyl units from one sucrose molecule (acting as donor) to another sucrose molecule, 1-kestose, and higher polymeric β-(2→1) linked fructan molecules, respectively (acting as acceptor). This enzyme partly leads to β-(2→6) linkages, which results in branches within the inulin molecule [130, 133].

Inulin is produced on a large scale for food applications including functional food, but it could also be used for manufacturing of furan-based and other chemicals [130, 131, 134–136].

Fructooligosaccharides (FOS) are enzymatically accessible from sucrose. The resulting products are known as short chain FOS (scFOS). Alternatively, FOS could be obtained via endo-inulinases by degradation of inulin. Fructooligosaccharides are used as prebiotic functional ingredients mainly in dairy products. Long chain inulin (about DP 25) provides food with a fat-like structure and taste. The blend of fructooligosaccharides and long chain inulin improves calcium absorption [137]. This combination of technological and nutritional properties has created an extremely successful market for inulin and FOS over the last 20 years.

Levan has a backbone of β-(2→6) linked D-fructose and occurs as high molecular weight polysaccharide in microorganisms. It is accessible from sucrose by use of the enzyme levansucrase (sucrose 6-fructosyltransferase (FTF, EC 2.4.1.10)). Either culture broth of bacterial strains like *Bacillus* or *Zymomonas* or the cell-free supernatant can be used for the enzymatic reaction with sucrose. The molecular weight and the viscosity of levan depend on the strain used and the reaction conditions [131, 134]. Even though levan has interesting properties, it has never gained extensive industrial use up to now [134].

Neo-amylose^{TM} is an unbranched water insoluble poly-1,4-α-D-glucan with a chain length of up to 35–100 glucose units (Fig. 14). It is analogous to amylose but

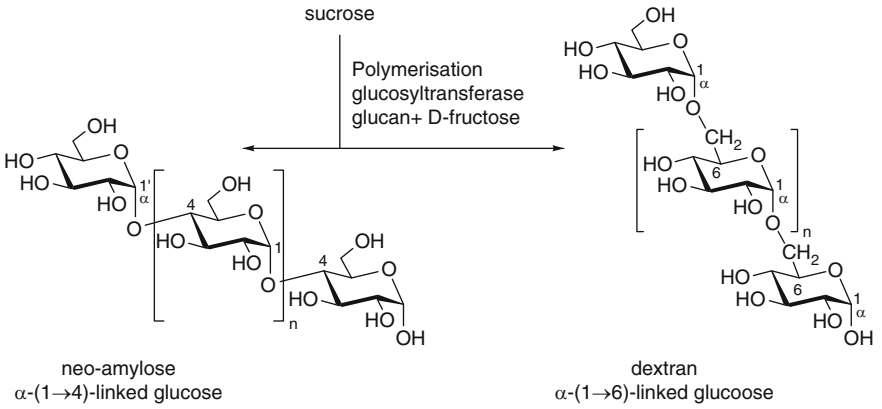


Fig. 14 Formation of Neo-amylose and dextran by action of glycosyltransferase on sucrose

can be synthesized via biocatalytical polymerization of sucrose. As biocatalyst for the synthesis of the glucan the enzyme amylosucrase is used (AmSu; sucrose–glucan Glycosyltransferase/E.C. 2.4.1.4) which is derived from the nonpathogenic bacterium *Neisseria polysaccharea*, originally isolated from the throat of a healthy child [138]. Amylosucrase splits sucrose into D-glucose and D-fructose and adds subsequently the glucose moiety to the nonreducing end of an α-1,4-D-glucan. Thereby, D-fructose is the by-product of this enzymatic reaction. The advantage of the process is that no activated α-D-glycosyl nucleoside diphosphate substrate is needed. This enzymatically derived polymer can be further processed by a heat-moisture treatment. Thereby a type 3 amylase resistant starch with a degree of resistance of >90% (RS_3) is obtained.

Neo-amylose is suited for food and non-food applications. Due to its resistant starch properties it is suited for use as dietary fiber [139]. Beside this food application, smooth spherical microparticles with a size of 10–100 μm are accessible by recrystallization of Neo-amylose in dimethylsulfoxide (DMSO). These are suited as cosmetics additive in creams, lotions, or as UV-reflectors [140]. Furthermore, Neo-amylose has proved to be an advantageous constituent in hard or soft films in order to generate gelatin free capsules [139].

Like Neo-amylose, *dextran* is a linear homopolysaccharide of glucose but composed of α-(1→6)-linked α-D-glucose backbone with only occasional branches at *O*-2, *O*-3, or *O*-4. The detailed structure of dextran depends on the specific dextransucrase and the microbial strain (Fig. 14). Several strains produce more than one type of dextran [141, 142]. The dextransucrase (EC 2.4.1.5) is an extracellular glucosyltransferase (GTF), catalyzing the cleavage of sucrose and transferring the D-glucopyranosyl residue to dextran, thereby releasing D-fructose. Industrially used dextran is mainly produced by growing cultures of *Leuconostoc mesenteroides* and was utilized in many different applications. In the early 1940s dextran was introduced as a blood-plasma volume expander. Since then, other applications based on native dextran, partially degraded dextran, and derivatives thereof have followed [142–146].

Among them, the use in the separation and purification of biochemically important macromolecules in molecular sieves (known as Sephadex®) [145], applications in the medical field (e.g., heparin analogs) [143], and due to its moisturizing properties and proven high stability, also in cosmetic formulations and for texture improvement in food applications [141, 142].

4 Outlook

Sucrose is amongst the predestined renewable organic compounds and alternative feedstock for many still petrochemically derived products or product applications. However, we have to remain realistic: from a technical feasibility perspective, renewable resources will not be able to replace petrochemistry in the foreseeable future. New products and processes need to be at least equal or better in terms of quality and economic performance. With regard to price competitiveness, the worldwide boom in the use of biomass for industrial purposes outside the food sector has very quickly revealed biomass limitations. The rise in the price of renewable resources themselves and particularly of foods, and even shortages in certain sectors, have illustrated that renewables are not inexhaustible.

Global challenges require the saving and more efficient use of fossil resources in the coming decades. In this respect, renewable resources like sucrose are very promising sources of energy and raw material. We need to explore its potential further.

References

1. Bartens A (2009) Zuckerwirtschaft Europa. Verlag Dr Albert Bartens KG, Berlin
2. Queneau Y, Jarosz S, Lewandowski B, Fitremann J (2007) Adv Carbohydr Chem Biochem 61:217
3. Peters D (2007) Nachwachsende Rohstoffe in der Industrie Fachagentur Nachwachsende Rohstoffe, 2. Aufl, Gülzow
4. Jahresbericht 2005/2006 (2006) Fachagentur Nachwachsende Rohstoffe, Gülzow
5. Lichtenthaler FW (2006) In: Kamm B, Gruber PR, Kamm M (eds) Biorefineries – industrial processes and products, vol 2. Wiley-VCH, Weinheim, p 3
6. Frenzel S, Peters S, Rose T, Kunz M (2009) In: Höfer R (ed) Sustainable solutions for modern economics. RSC Publishing, p 264 –>
7. Lichtenthaler FW, Peters S (2004) CR Chimie 7:65
8. Chheda JN, Huber GW, Dumesic JA (2007) Angew Chem Int Ed 119:7298
9. Press release from 04.06.2008. http://www.marketwatch.com (retrieved 28.07.2008)
10. Press release from 28.03.2008. http://www.braskem.com.br (retrieved 31.07.2008)
11. Press Release from 14.12.2007. http://www.solvinpvc.com (retrieved 25.02.2008); http://desarrolloydefensa.blogspot.com/2008/04/solvay-indupa-firma-contrato-con.html (retrieved 27.11.2008)
12. Denis N, Meiser A, Riese J, Weihe U (2008) Setting the right course in biobased bulk chemicals 1:22

13. Press release from 15.07.2009. http://www.icis.com (retrieved 12.11.2009)
14. Claus P, Vogel GH (2006) Chem Ing Tech 78:991
15. Weidenhagen R, Wegner H (1938) Chem Ber 71:2712
16. Ling GV, Driessen AJ, Piet AC, Vligter JC (1970) Ind Eng Chem Prod Res Dev 9:210
17. Wright LW (1976) US 3 965 199 (CI America Inc)
18. Weissermel K, Arpe H-J (2003) Industrial organic chemistry. Wiley-VCH, Weinheim
19. Schuster L, Himmele W (1989) DE 38 18 198 (BASF AG)
20. Schuster L (1991) DE 39 28 285 (BASF AG)
21. De Oliveira K, Vigier FJ (2010) Heterogeneously-catalyzed conversion of carbohydrates. Top Curr Chem. doi:10.1007/128_2010_55
22. Schiweck H, Munir M, Rapp K, Vogel M (1991) In: Lichtenthaler FW (ed) Carbohydrates as organic raw materials. VCH, Weinheim, p 57
23. Lewkowski J (2001) ArkiVoc i:17
24. Kuster BFM (1990) Starch/Stärke 42:314
25. Gaset A, Rigal L, Paillassa G, Salome J-P, Fleche G (1985) FR 2 551 754 (Roquette Freres)
26. Brown DW, Floyd AJ, Kinsmann RC, Roshan-Ali Y (1982) J Chem Technol Biotechnol 32:920
27. Musau RM, Munawu RM (1987) Biomass 13:67
28. Hirth J (2002) Dissertation, TU Darmstadt
29. Bicker M, Hirth T, Vogel H (2003) Green Chem 5:280
30. Bicker M (2005) Stoffliche Nutzung von Biomasse mit Hilfe überkritischer Fluide. Dissertation, TU Darmstadt
31. Bicker M, Kaiser D, Ott L, Vogel H (2005) J Supercrit Fluids 36:118
32. Zhao H, Holladay JE, Brown H, Zhang ZC (2007) Science 316:1597
33. Yong G, Zhang Y, Jing JY (2008) Angew Chem Int Ed 47:9345
34. Rapp KM (1987) EP 230 250 (Südzucker AG)
35. Kamm B, Kamm M, Schmidt M, Hirth T, Schulze M (2006) In: Kamm B, Gruber PR, Kamm M (eds) Biorefineries – industrial processes and products, vol 2. Wiley-VCH, Weinheim, p 97
36. Lichtenthaler FW (2002) Ullmann's encyclopedia industrial chemistry, 6th edn, vol 6. Wiley-VCH, Weinheim, p 237
37. Gandini A, Belgacem MN (1997) Prog Polym Sci 22:1203
38. Yashida N, Kasuya N, Haga N, Fukuda K (2008) Polym J 47:1164
39. Werpy T, Petersen G http://www1.eere.energy.gov/biomass/pdfs/35523.pdf (publication is dated August 2004)
40. Manzer LE (2006) ACS Symp Ser 921:40
41. Nelen BAP, Cooper JM (2004) In: Whitehurst RJ (ed) Emulsifiers in food technology. Wiley-Blackwell, UK, p 131
42. Wang Y-J (2004) In: Tomasik P (ed) Chemical and functional properties of food saccharides. CRC, Boca Raton, p 35
43. Bimal C, Guonong Z (2006) Food Rev Int 22:245
44. Hass HB (1968) Sugar esters. Noyes Development Corporation, Park Ridge, NJ, p 1
45. Osipow L, Snell FD, York WC, Finchler A (1956) Ind Eng Chem 48:1459
46. Hough L (1991) In: Lichtenthaler FW (ed) Carbohydrates as organic raw materials. VCH, Weinheim, p 33
47. Karmee SK (2008) Biofuels Bioprod Bioref 2:144
48. Brockway BE (2005) Cosmetics & Toiletries Magazine 120:93
49. Godshall MA (2001) Int Sugar J 103:1233
50. Le Maistre JW, Seymour RB (1948) J Org Chem 13:782
51. Kollonitsch V (1970) Sucrose chemicals. The International Sugar Research Foundation Inc, Washington
52. Frich KC, Kresta JE (1977) In: Hickson JL (ed) Sucrochemistry. ACS symposium series, vol 41. ACS, Washington, p 238

53. Vohrspohl K, Hinz W, Güttes B (1993) In: Eggersdorfer M, Warwel S, Wulff G (eds) Nachwachsende Rohstoffe – Perspektiven für die Chemie. VCH, Weinheim/New York, p 383
54. Jhurry D, Deffieux A (1997) Eur Polym J 33:1577
55. Chauvin C, Aczko K, Plusquellec D (1993) J Org Chem 58:2291
56. Kohlstrung R (2001) Polyurethan – Präpolymere auf der Basis von Sacchariden und Saccharid-Derivaten-Von der Selektivität zur anwendungstechnischen Prüfung. Dissertation, TU Darmstadt
57. Fairclough PH, Hough L, Richardson AC (1975) Carbohydr Res 40:285
58. Hough L, Phadnis SP, Khan RA, Jenner MR (1977) GB 1 543 167 (Tate & Lyle)
59. Molinary SV, Jenner MR (1999) Food Food Ingredients J Jpn 182:6
60. Molinary SV, Quinlan ME (2006) In: Mitchell H (ed) Sweeteners and alternatives in food technology. Blackwell Publishing, Oxford, UK, p 130
61. Sham CW (2005) Nutritional bytes 10/2 article 5. http://repositories.cdlib.org/uclabiolchem/nutritionbytes/vol10/iss2/art5
62. Navia JL (1990) US 4 950 746 (Noramco Inc)
63. Neiditch DS, Vernon NM, Wingard RE (1991) US 5 023 329 (Noramco Inc)
64. Walkup RE, Vernon NM, Wingard RE (1992) US 5 089 608 (McNeill PPC Inc)
65. Sankey GH, Vernon NM, Wingard RE (1995) US 5 470 969 (McNeill PPC Inc)
66. White JE, Bradford BA (1997) EP 776 903 (McNeill PPC Inc)
67. Clark JD, LeMay RR (2003) US 6 939 962 (Tate & Lyle)
68. Mufti KS, Khan RA (1983) US 4 380 476 (Talres Dev)
69. Simpson PJ (1989) US 4 889 928 (Tate & Lyle)
70. Dordick JS, Hacking AJ, Khan RA (1992) US 5 128 248 (Tate & Lyle)
71. Dordick JS, Hacking AJ, Khan RA (1993) US 5 270 460 (Tate & Lyle)
72. Bornemann S, Cassells JM, Combes L, Dordik JS, Hacking AJ (1992) US 5 141 860 (Tate & Lyle)
73. Palmer DC, Terradas F (1995) US 5 445 951 (McNeill PPC Inc)
74. Walkup RE, Navis JL, Vernon NM (1990) US 4 980 463 (Noramco Inc)
75. Khan RA, Sankey GH, Simpson PJ, Vernon NM (1992) US 5 136 031 (Tate & Lyle)
76. Navia JL, Walkup RE, Neiditch DS (1996) US 5 530 106 (McNeill PPC Inc)
77. Navia JL, Walkup RE, Vernon NM, Neiditch DS (1996) US 5 498 709 (McNeill PPC Inc)
78. Navia JL, Walkup RE, Vernon NM, Wingard RE (1994) US 5 298 611 (McNeill PPC Inc)
79. Catani SJ, Leinhos DA, O'Connor T (1999) US 5 977 349 (McNeill PPC Inc)
80. Vernon NM, Micinski E, Catani SJ, Navia JL (2003) US 6 890 581 (McNeill PPC Inc)
81. Seibel J, Beine R, Moraru R, Behringer C, Buchholz K (2006) Biocatal Biotransformation 24:157
82. Vasik-Racki D (2006) In: Liese A, Seelbach K, Wandrey C (eds) Industrial biotransformations. Wiley-VCH, Weinheim, p 29
83. Buchholz K, Kasche V (1997) Biokatalysatoren und Enzymtechnologie. VCH, Weinheim, p 11
84. Wach W (2004) Ullmann's encyclopedia of industrial chemistry, 7th edn. Wiley-VCH, Weinheim. doi:10.1002/14356007.a12.047
85. Bruhns J (1991) Zuckerind 116:736
86. Kowalczyk J (2007) In: Rosenplenter K, Noehle U (eds) Handbuch Süßungsmittel. Behr's Verlag, Hamburg, p 194
87. Kowalczyk J (2007) In: Rosenplenter K, Noehle U (eds) Handbuch Süßungsmittel. Behr's Verlag, Hamburg, p 295
88. Liese A, Seelbach K, Wandrey C (2006) Industrial biotransformations. Wiley-VCH, Weinheim, p 512
89. Nakajima Y (1988) Denpun Kagaku 35:131
90. Rose T, Kunz M (2002) In: Prüsse U, Vorlop KD (eds) Practical aspects of encapsulation technologies, vol 241. Federal Agricultural Research Centre (FAL), Braunschweig, p 75
91. Schiweck H, Munir M, Rapp KM, Schneider B, Vogel M (1990) Zuckerind 115:555
92. Straeter PJ (1987) Zuckerind 112:900

93. Taniguchi H (2005) In: Hou CTJ (ed) Handbook of industrial catalysis, vol 20. CRC, Taylor & Francis Group, Boca Raton, pp 1–25
94. Lorenz S (1958) Zuckerind 8:490
95. Lorenz S (1958) Zuckerind 8:535
96. Nakajima Y (1984) Proc Res Soc Jpn Sugar Refin Technol 33:55
97. Weidenhagen R, Lorenz S (1957) Angew Chem 69:641
98. Weidenhagen R, Lorenz S (1957) Zuckerind 7:533
99. Cartarius R, Krause T, Vogel H (2001) Chem Ing Tech 73:118
100. Cartarius R, Krause T, Vogel H (2002) Chem Ing Tech 74:869
101. Cartarius R (1999) Bioabbaubare Tenside durch reduktive Aminierung von Isomaltulose – Katalysator und Verfahrensentwicklung. Dissertation, TU Darmstadt
102. Schiweck H (1980) Alimenta 19:5
103. Dreissig W, Luger P (1973) Acta Crystallogr B 29:514
104. Jarell HC, Conwell TF, Moya P, Smith ICP (1979) Carbohydr Res 76:45
105. Seymour FR, Knapp RD, Zweig JE, Bishop SH (1979) Carbohydr Res 72:57
106. Cheetham PSJ, Imber CE, Isherwood J (1982) Nature 299:628
107. Huang JH, Hsu LH, Su YC (1998) J Ind Microbiol Biotechnol 21:22
108. Baer A (1989) Lebensm Wiss Technol 22:46
109. Minami T, Fujiwara T, Ooshima T, Nakajima Y, Hamada S (1990) Oral Microbiol Immunol 5:189
110. Ooshima T, Izumitani A, Sobue S, Okahashi N, Hamada S (1983) Infect Immun 39:43
111. Ohata K, Takazoe I (1983) Bull Tokyo Dent Coll 24:1
112. Sasaki N, Topitsoglou V, Takazoe I, Frostell G (1985) Swed Dent J 9:149
113. Takazoe I (1985) Int Dent J 35:58
114. Takazoe I, Frostell G, Ohta K, Topitsoglou V, Sasaki N (1985) Swed Dent J 9:81
115. Topitsoglou V, Sasaki N, Frostell G (1984) Caries Res 18:47
116. Kawai K, Okuda Y, Yamashita K (1985) Endocrinol Jpn 32:933
117. Kawai K, Yoshikawa H, Murayama Y, Okuda Y, Yamashita K (1989) Horm Metab Res 21:338
118. Kashimura J, Nagai Y, Ebashi T (2003) J Nutr Sci Vitamino (Tokyo) 49:214
119. Krastanov A, Yoshida T (2003) J Ind Microbiol Biotechnol 30:593
120. Wilson R (2007) Sweeteners, 3rd edn. Wiley-Blackwell, Oxford, UK
121. Asquith J, Pickering K, Trenchard-Morgan S, Sangster S (1986) Completion of bacterial reverse mutation test on isomaltulose. Toxicol Lab Ltd, Ledbury, Herefordshire
122. Jonker D, Lina BAR, Kozianowski G (2002) Food Chem Toxicol 40:1383
123. Lina BAR, Smits-van Prooji AE, Waalkens-Berendsen DH (1997) Food Chem Toxicol 35:309
124. Lina BAR, Jonker D, Kozianowski G (2002) Food Chem Toxicol 40:1375
125. Nagai Y, Sugitani T, Tsuyuki K, Yamada K, Ebashi T, Kishihara S (2002) Proc Res Soc Jpn Sugar Refin Technol 50:57
126. Nagai Y, Sugitani T, Yamada K, Ebashi T, Kishihara S (2003) J Jpn Soc Food Sci Technol 50:488
127. Ravaud S, Robert X, Watzlawick H, Haser R, Mattes R, Aghajari N (2007) J Biol Chem 282:28126
128. Gruell DR, Haji Begli A, Kubadinow N, Kunz M, Munir M (1998) EP 943 030 (Suedzucker AG)
129. Edelman J, Jefford TG (1968) New Phytologist 67:517
130. Franck A, de Leenheer L (2002) In: Steinbüchel A (ed) Biopolymers, vol 6. Polysaccharides II: polysaccharides from eukaryotes. Wiley-VCH, Weinheim, p 439
131. Iizuka M, Minamiura N, Ogura T (2000) In: Ohnishi M (ed) Glycoenzymes. Tokyo, Karger
132. van Laere A, van den Ende W (2002) Plant Cell Environ 25:803
133. Seibel J, Moraru R, Goetze S, Buchholz K, Na'amnieh S, Pawlowski A, Hecht HJ (2006) Carbohydr Res 341:2335
134. Han YW (1990) Adv Appl Microbiol 25:171

135. Pontis HG (1990) In: Dey PM (ed) Methods in plant biochemistry: carbohydrates, vol 2. Academic, New York, p 353
136. Rhee SK, Song KB, Kim CH, Park BS, Jang EK, Jang KH (2002) In: Vandamme EJ, de Baets S, Steinbüchel A (eds) Biopolymers, vol 5. Polysaccharides I: polysaccharides from prokaryotes. Wiley-VCH, Weinheim, p 351
137. Abrams S, Griffin I, Hawthorne K, Liang L, Gunn S, Darlington G, Ellis K (2005) Am J Clin Nutr 82:471
138. Anand CM, Ashton F, Shaw H, Gordon R (1991) J Clin Microbiol 29:22434
139. Bengs H, Brunner A (2000) WO 0038537 (Aventis Res & Tech GmbH)
140. Bengs H, Schneller A (2000) WO 0038623 (Aventis Res & Tech GmbH)
141. Leathers TD (2002) In: Vandamme EJ, de Baets S, Steinbüchel A (eds) Biopolymers, vol 5. Polysaccharides I: polysaccharides from prokaryotes. Wiley-VCH, Weinheim, Germany, p 299
142. Naessens M, Cerdobbel A, Soetaert W, Vandamme EJ (2005) J Chem Technol Biotechnol 80:845
143. Alsop RM (1983) In: Bushell ME (ed) Microbial polysaccharides. Elsevier, Amsterdam, p 1
144. de Belder AN (1996) In: Dumitriu S (ed) Polysaccharides in medical applications. Marcel Dekker, New York, p 505
145. Robyt JF (1985) In: Kroschwitz JI (ed) Encyclopaedia of polymer science, vol 4. Wiley-VCH, New York, p 753
146. Yalpani M (1985) CRC Crit Rev Biotechnol 3:375
147. Kaga T, Mizutani T (1985) Proc Res Soc Jpn Sugar Refin Technol 34:45

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Sucrose-Utilizing Transglucosidases for Biocatalysis

Isabelle André, Gabrielle Potocki-Véronèse, Sandrine Morel, Pierre Monsan, and Magali Remaud-Siméon

Abstract Sucrose-utilizing transglucosidases are valued tools in chemistry to generate glycodiversification. Not only do these enzymes use as substrate an abundant agroresource, sucrose, but they also share a remarkable versatility regarding the acceptor substrate, allowing the structurally-controlled synthesis of diverse glucosylated products. Latest research has demonstrated the potential of enzyme engineering to tailor novel sucrose-utilizing transglucosidases that give access to original carbohydrate-based structures. This chapter gives an overview of the recent achievements in biocatalysis using these enzymes.

Keywords Enzymatic glucosylation, Enzyme engineering, Glucansucrase, Glycochemistry, Neo-enzyme, Sucrose phosphorylase, Transglucosidase

Contents

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1 Introduction

Carbohydrates and glycoconjugates find more and more applications in the food, feed, pharmaceutical, or cosmetic industries as stabilizers, bulking agents, immunostimulating agents, or prebiotic compounds able to stimulate the growth of beneficial bacteria in the human and animal gut [1]. There is thus an increasing demand for novel structures synthesized from renewable resources through environmentally friendly processes. At the crossroad of many disciplines, biocatalysis relying on natural or artificial neo-enzymes can provide innovative and efficient routes for the production of high value carbohydrate-based molecules. In particular, the selectivity and regiospecificity of carbohydrate-active enzymes can be profitable to overcome the difficulties encountered in glycochemistry, which are principally due to the high reactivity of the numerous hydroxyl groups found in carbohydrates. Indeed, this reactivity necessitates many steps of protection and de-protection which often reduce chemical synthesis yields. Despite accomplished advancements, chemical approaches towards specific usable carbohydrates still need considerable effort [2–5].

In this context, the use of enzymes has emerged as a practical alternative to chemical synthesis [6–13]. Several examples have been reported that are based on the use of Leloir type-glycosyltransferases, which are mostly membrane-associated and act on nucleotide-activated sugars as donor substrates. However, the use of these enzymes *in vitro* still remains limited by the difficulty of enzyme purification and by their need for expensive sugar-nucleotides [6]. Re-engineering of microbial cells producing these proteins appears to be promising for the synthesis of specific carbohydrate structures [14].

Another possible enzymatic approach utilizes the transglycosylation capability of glycoside-hydrolases. Among these enzymes, many are considered really poor transglycosidases as they mostly transfer the glycosyl moieties from a donor onto the water molecule. Enzyme engineering approaches have been proposed to generate the so-called "glycosynthases" from glycoside-hydrolases [15–19]. These glycosynthases use fluoride-activated sugars as the donor substrate and are unable to hydrolyze the transfer reaction product. Although elegant, this approach does not open the route to the direct use of renewable material as substrate. Conversely, in the class of glycoside-hydrolases, some enzymes deserve the true denomination of transglycosidase as they naturally display very efficient transferase activities, i.e., cyclodextrin glucosyltransferase amylomaltase, branching enzymes. They are known to function with diverse non-nucleotide sugar donors and preferentially transfer the glycosyl moiety in a regio- and stereoselective way onto a broad variety of acceptors distinct from water to synthesize a wide range of glycosides.

Among these enzymes, transglucosidases that use sucrose as the sole donor substrate to catalyze glucosyl transfer reactions have emerged as attractive synthetic tools. The relaxed and broad substrate specificity displayed by these enzymes towards many acceptor substrates has been extensively used for the stereo- and regioselective synthesis of original glucoconjugates, oligo- and polysaccharides.

Performances of native sucrose-utilizing transglucosidases are often not optimal for many applications, especially for industrial developments. Protein engineering can today bring solutions that have proven to be useful to improve the performance characteristics of these enzymes. The high degree of functional plasticity exhibited by these enzymes also suggests that they can be further adapted to perform the desired biotransformation. In particular, enzyme engineering techniques can be successfully used to tailor enzymes able to perform novel specific transformations or become adapted to perform under specific conditions. In particular, recent developments have provided more straightforward access to well-defined carbohydrates using enzymes designed on purpose to enter in chemo-enzymatic pathways.

The latest developments in this field and the potential of sucrose-utilizing transglucosidases as synthetic tools to access carbohydrate-based derivatives will be illustrated in the following.

2 Potential of Native Sucrose-Utilizing Transglucosidases for Glucoside Synthesis

Sucrose-utilizing transglucosidases catalyze the transfer of a glucosyl group from a sucrose donor substrate onto an acceptor molecule. These enzymes are ideal biocatalysts for glucoside synthesis *in vitro* since they do not require specially activated substrates but directly employ the free energy of sucrose cleavage, an abundant and low-cost substrate. In that sense, sucrose is quite a unique disaccharide due to the unusually high energy of its glycosidic bond (~27.6 kJ/mol). Like nucleotide-activated sugars (e.g., UDP-glucose or ADP-glucose), sucrose keeps D-glucose in a protected and activated form to play its role in plant metabolism [20].

Two main classes of enzymes enter into this definition: glucansucrases (E.C.2.4.1.4, E.C.2.4.1.5, and 2.4.1.140) that catalyze the conversion of sucrose for the synthesis of high-molecular weight glucans, and sucrose phosphorylases (E.C.2.4.1.7) which catalyze the phosphorolysis of sucrose to produce glucose-1-phosphate. They both utilize sucrose as the sole source of energy to synthesize their products.

Sucrose-utilizing transglucosidases are classified into Glycoside Hydrolase (GH) families 70 and 13 [21]. Most glucansucrases belong to GH family 70 with the exception of amylosucrases which belong to GH family 13, a class of catalysts that mainly contains enzymes involved in starch transformation. However, instead of degrading starch, amylosucrases use sucrose to synthesize an amylose-like polymer. With more than 6,000 entries and 23 distinct activities, GH family 13 is a large group which also contains hydrolases, such as α-amylases and α-glucosidases, and glycosyl-transferring enzymes (transglucosidases), like cyclodextrin glucanotransferases (CGTases), α-1,4-glucanotransferases, and sucrose phosphorylases (SPases) which share high structural similarities with amylosucrases.

Both glucansucrases and sucrose phosphorylases will be discussed herein, however, we will mostly focus on glucansucrases, on which extensive work has been done over recent years.

2.1 A General Mechanism

Sucrose-utilizing transglucosidases share many common mechanistical and structural features. Both glucansucrases and sucrose phosphorylases are α-retaining transglucosidases. They follow the same double displacement mechanism as retaining glycosidases which involves the participation of two catalytic residues, located approximately 5.5 Å apart on the opposite side of the glucopyranosyl plane. In a first step (glucosylation step), the nucleophile residue (aspartate) exerts a nucleophilic attack onto the anomeric carbon of the glucosyl ring in sucrose and, simultaneously, the general acid/base (glutamic acid) protonates the glycosidic oxygen of the sucrose donor. This leads to the formation of a β-D-glucosyl enzyme covalent intermediate and the release of fructose illustrated in Fig. 1 for *N. polysaccharea* amylosucrase [22]. In a second step (de-glucosylation step), the glucosyl moiety is transferred to an acceptor activated by the deprotonated acidic carboxylate to attack the covalent bond between the anomeric C1 of the glucosyl ring and the aspartate. A third aspartate residue is assumed to be involved in the mechanism by ensuring the correct positioning of the glucosidic bond via the distortion of the glucosyl ring.

The main reaction catalyzed by sucrose-utilizing transglucosidases is the formation of soluble oligosaccharides and α-glucan polymers for glucansucrases and α-D-glucose-1-phosphate formed from orthophosphate for sucrose phosphorylases (Fig. 2). These reactions are accompanied by the minor production of byproducts: glucose resulting from sucrose hydrolysis and sucrose isomers (turanose, trehalulose, isomaltulose, or leucrose, which result from the transfer of the glucosyl moiety onto fructose released during the reaction).

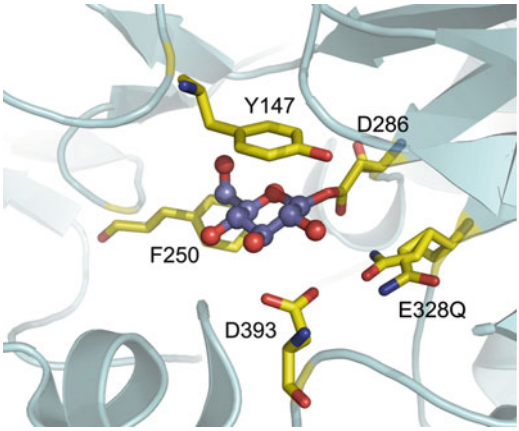

Fig. 1 Covalent intermediate structure of inactive E328Q mutant of amylosucrase from *N. polysaccharea* (PDB: 1 S46) [22]. Glucosyl moiety covalently bound to the catalytic nucleophile (D286) is shown in *purple*. E328 is the catalytic general acid/base which has been mutated into an inactive Q328 to trap the covalent intermediate form. D393 is involved in the stabilization of the glucosyl ring through hydrogen bonding interactions. Y147 and F250 provide stabilizing stacking interactions to the glucosyl moiety

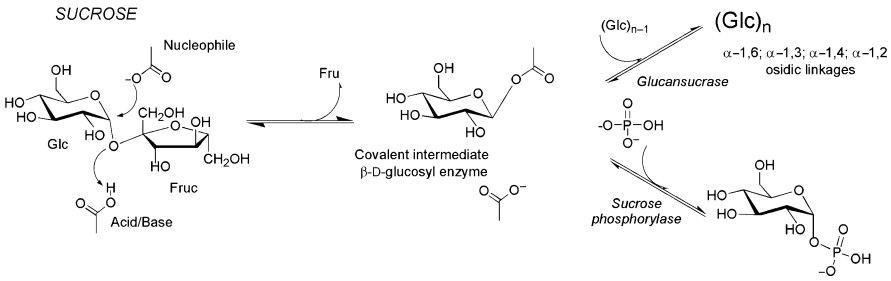

Fig. 2 Main reactions catalyzed by sucrose-utilizing transglucosidases. The synthesis of α-glucans $(Glc)_n$ linked through either α-1,6 α-1,3, α-1,4, or α-1,2 osidic linkages depending on the enzyme specificity is obtained by glucansucrases. The formation of α-D-glucose-1-phosphate from ortho-phosphate is obtained using sucrose-phosphorylases. The mechanism involves a double-inversion at the anomeric center via the formation of a covalent β-D-glucosyl enzyme intermediate

In addition to these reactions, when a hydroxylated acceptor, such as a saccharide, an alcohol, a polyol, or a flavonoid is added to the reaction mixture, the enzymatic activity of glucansucrases and sucrose phosphorylases can be re-directed from its natural reaction toward the transglucosylation of the exogenous acceptor if it is well-recognized by the enzyme [23, 24]. Such examples of transglucosylation reactions will be given later in the manuscript.

On the structural level, amylosucrases and sucrose phosphorylases from the GH13 family share the same catalytic machinery, adopting a common architecture [25, 26]. The only structures available are those of the amylosucrase from *Neisseria polysaccharea* [22, 27–29] and the sucrose phosphorylase from *Bifidobacterium adolescentis* [30, 31] (Fig. 3). Comparison of these structures revealed that these enzymes share important structural similarities, in particular with the amylases from the GH family 13, the oligo-1,6-glucosidase (an exo-acting enzyme specific for the cleavage of α-1,6 glucosidic bond [32]), and the *Aspergillus oryzae* α-amylase (TAKA-amylase) [33]. Their architecture adopts a $(\beta/\alpha)_8$-barrel which consists of eight parallel β-strands forming the inner β-barrel surrounded by eight α-helices so that the individual β-strands and α-helices alternate and are interconnected by loops. In spite of these strong structural similarities, the topology of the active site shows significant variations. Whereas α-amylases present an active site in the form of a cleft, both amylosucrase and sucrose phosphorylase active sites adopt a deep pocket topology of ~15 Å, with the catalytic triad located at the bottom (Fig. 4). This particular topology is conferred by two loops from the catalytic barrel, the so-called B-domain (β3-α3 loop) and the B' domain (β7-α7 loop), which cover up the active site to form a pocket shielded from the outside environment.

Regarding glucansucrases from the GH70 family, it is believed that they share a common ancestor with GH13 members, but they could adopt a circularly permutated $(\beta/\alpha)_8$-barrel (Fig. 5) [34].

The large size (between 155 and 200 kDa) of glucansucrases from the GH family 70 has been a challenge for crystallographers for many years, and only very recently

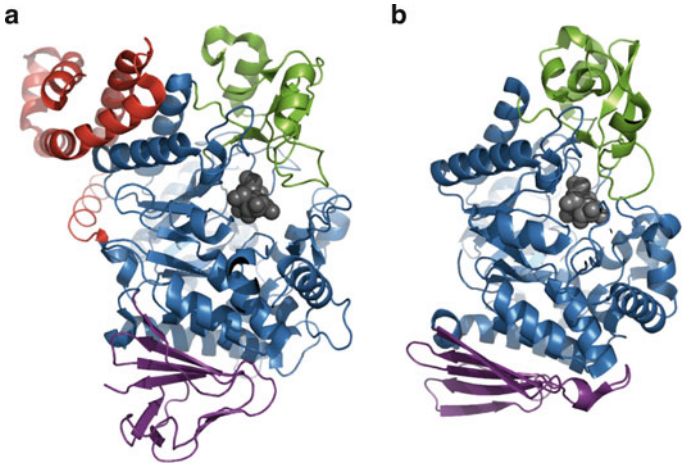

Fig. 3 Overall fold of sucrose-utilizing transglucosidase from GH13 family. (**a**) Amylosucrase from *N. polysaccharea* (PDB: 1G5A) [22, 27–29]. (**b**) Sucrose phosphorylase from *B. adolescentis* (PDB: 2GDU) [30, 31]. Both enzymes adopt a $(\beta/\alpha)_8$-barrel architecture. Sucrose is shown as *grey spheres* to indicate the active site; The catalytic $(\beta/\alpha)_8$-barrel domain (A-domain; *blue*), the C-domain (*purple*), and the B-domain (*green*), N-domain (*red*)

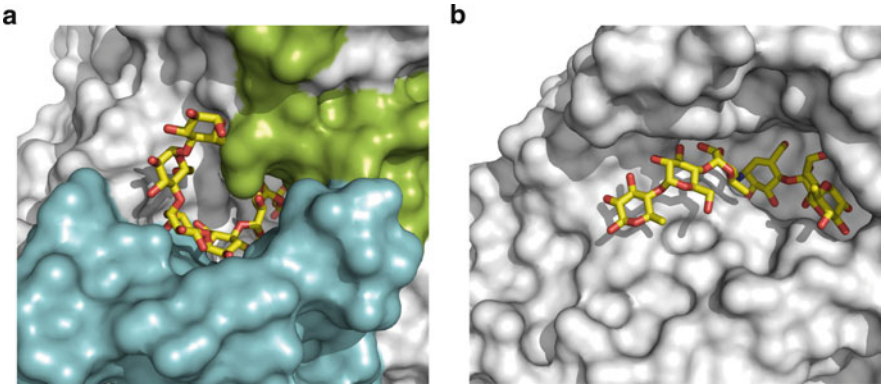

Fig. 4 Comparison of active site topologies of GH13 members. (**a**) Pocket topology for the amylosucrase from *N. polysaccharea* shown with a bound maltoheptaose molecule (PDB: 1G5A) [22, 27–29]. The B-domain (β3-α3 loop) is colored in green whereas the B'-domain (β7-α7 loop) is colored in cyan. **(b)** Cleft topology for the *Aspergillus oryzae* α-amylase (TAKA-amylase) in complex with acarbose (PDB: 7TAA) [33]

have Dijkstra et al. succeeded in obtaining the crystal structure of *Lactobacillus reuteri* 180 glucansucrase (GTF-180) which revealed an unexpected three-dimensional organization in a "U-like" shape, only partially resembling other α-amylase family enzymes, and confirming the earlier predicted $(\beta/\alpha)_8$-circular permutation [35, 36].

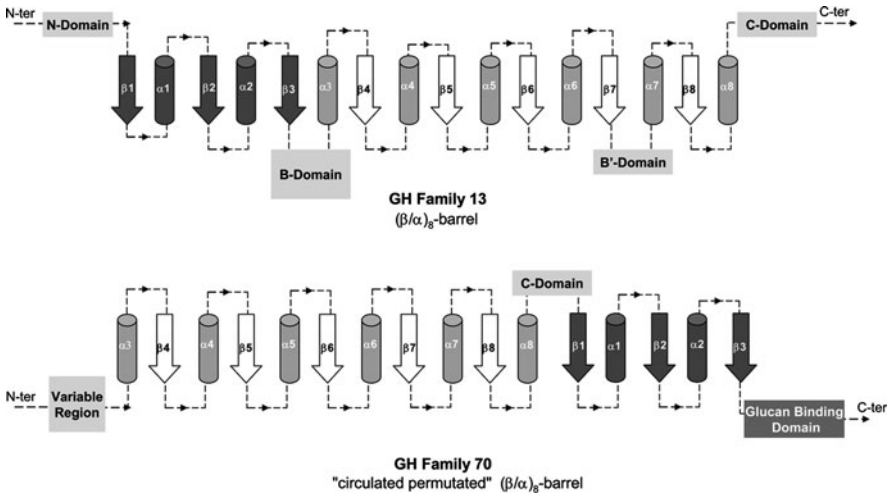

Fig. 5 Topology diagrams of GH13 and GH70 sucrose-utilizing transglucosidases. *Cylinders* represent α-helices and *arrows* β-sheets constituting the $(\beta/\alpha)_8$ barrel. The permutated elements are shown in *dark grey*

2.2 Natural Promiscuity Is at the Heart of Large Product Diversity

Enzyme catalytic promiscuity, where enzymes catalyze not only reactions with their natural substrates but also with non-natural substrates, has begun to be recognized as a valuable research and synthesis tool [6–10, 12, 13]. In particular, the catalytic promiscuity displayed by sucrose-utilizing transglucosidases toward a wide range of acceptor molecules has started to be exploited to provide novel synthesis pathways that are currently not available.

Glucansucrases accept a limited number of glucosyl donors other than sucrose. These enzymes show a limited promiscuity toward the glucosyl donor molecule. There has been only a few reports of the use by some glucansucrases of activated donors such as *p*-nitrophenyl glucosides [37] or glucosyl fluorides [38] which are rapidly and irreversibly cleaved. In contrast, glucansucrases have revealed a tremendous promiscuity toward the acceptor substrate which gives rise to large product diversity. The nature of the acceptor molecule recognized by the enzyme and the product formed will determine the enzyme specificity.

2.2.1 Polysaccharide Synthesis

The main reaction catalyzed by these enzymes is the synthesis of extracellular bacterial polysaccharides, α-glucans linked through different types of linkages and harboring distinct sizes and structures depending on the enzyme specificity and producing strain (Tables 1 and 2). Polymer elongation has been shown to proceed

Table 1 Regiospecificity of glucansucrases

Glucansucrase	Glucan	Osidic linkage
Dextransucrase (E.C.2.4.1.5)	Dextran	α-1,6 (>50%)
Mutansucrase (E.C.2.4.1.5)	Mutan	α-1,3 (>50%)
Reuteransucrase (E.C.2.4.1.5)	Reuteran	α-1,4 (>50%)
Alternansucrase (E.C.2.4.1.140)	Alternan	Alternating α-1,3/α-1,6
Amylosucrase (E.C.2.4.1.4)	Amylose	α-1,4 (100%)

through glucosyl unit transfer at the non-reducing end of the acceptor molecules (glucose, sucrose, or other primers initially produced) [69, 70]. Reaction conditions such as substrate concentration, pH, and temperature were also shown to have an undeniable influence on the structure of products synthesized by glucansucrases, in particular the degree of branching and the polymer size [41, 71–75].

Five distinct types of glucansucrases have been identified which produce different types of polysaccharides of molecular weights that can reach over 10^6 Da, and are constituted by D-glucosyl units connected by various types of osidic linkages, i.e., α-1,6, α-1,3, α-1,4, or α-1,2 linkages, the latter being relatively rare in nature (Fig. 6). Polysaccharides mainly linked through α-1,6 linkages are called dextrans and they are produced by dextransucrases (DSR), mainly found in *Leuconostoc* spp. Noteworthy, depending on the *L. mesenteroides* producing strain, varying amounts of branched glucosyl units (up to 50%) have also been found on these dextrans which can be α-1,3, α-1,4, and α-1,2 linked. Mutansucrases, mostly isolated in *Streptococcus* spp., synthesize α-1,3 linked glucans, called mutans, which play an essential role in cariogenesis by enhancing the attachment and colonization of teeth surface by streptococcal bacteria [76]. Reuteransucrases, mostly found in *Lactobacillus* spp., produce glucans linked by α-1,4 and α-1,6 bonds. Alternansucrases (ASR), essentially produced in *Leuconostoc* spp., synthesize atypical glucans of alternated α-1,3 and α-1,6 bonds, called alternans. Amylosucrases (AS) from GH family 13, found in *Neisseria, Deinococcus,* and *Alteromonas* spp., produce a linear polymer composed of α-1,4 glucopyranosyl residues highly similar to amylose [40–42, 72]. The morphology and structure of the produced amylose vary depending on the initial sucrose concentration used. In particular, α-glucan showing an exceptional crystallinity can be synthesized demonstrating the potentiality of amylosucrase for the design of amylose with controlled morphology, structure, and physicochemical properties [72].

Sucrose-utilizing transglucosidases are produced by bacteria belonging to various genera: *Leuconostoc*, *Lactobacillus*, *Weissella*, *Streptococcus*, *Deinococcus*, *Neisseria, Alteromonas, Pseudomonas*, and *Bifidobacterium* (Table 2).

2.2.2 Exogenous Acceptor Glucosylation

Glucansucrases have been known to catalyze the transglucosylation of exogenous acceptors since the 1950s [77] (Fig. 7). Short gluco-oligosaccharides (GOS) are formed by elongation of disaccharides such as maltose, isomaltose, nigerose, and

Table 2 Main sucrose-utilizing transglucosidases cloned and characterized to date

Organism	Enzyme	Size (aa)	Osidic linkage specificity				References
			α-1,6	α-1,4	α-1,3	α-1,2	
GH family 13							
Neisseria polysaccharea	Amylosucrase	637		100			[39]
Deinococcus radiodurans	Amylosucrase	644		100			[40]
Deinococcus geothermalis	Amylosucrase	650		100			[41]
Alteromonas addita	Amylosucrase	649		100			[42]
Alteromonas macleodii	Amylosucrase	649		100			[42]
Bifidobacterium longum	Sucrose phosphorylase	508					[43]
Bifidobacterium adolescentis	Sucrose phosphorylase	504					[31]
Leuconostoc mesenteroides	Sucrose phosphorylase	490					[44–46]
Streptococcus mutans	Sucrose phosphorylase	481					[47]
GH family 70							
Leuconostoc mesenteroides B-512F	DSR-S	1,527	95		5		[48]
Leuconostoc mesenteroides B-1299	DSR-E	2,835	68		3	29	[49]
Leuconostoc mesenteroides B-1299	DSR-A	1,290	85		15		[50]
Leuconostoc mesenteroides B-1299	DSR-B	1,508	95				[51]
Leuconostoc mesenteroides B-1355	DSR-C	1,477	96				[52]
Leuconostoc mesenteroides Lcc4	DSR-D	1,527	Major				[53]
Streptococcus mutans GS 5	GTF-D	1,430	70		30		[54]
Streptococcus oralis	GTF-R	1,575	86				[55]
Streptococcus downei Mfe 28	GTF-S	1,365	90				[56]
Streptococcus sobrinus OMZ176	GTF-T	1,468	79		21		[57]
Streptococcus sobrinus B13N	GTF-U	1,554	69		31		[58]

(*continued*)

Table 2 (continued)

Organism	Enzyme	Size (aa)	Osidic linkage specificity				References
			α-1,6	α-1,4	α-1,3	α-1,2	
Streptococcus salivarus ATCC25975	GTF-K	1,599	100				[59]
Streptococcus salivarus ATCC25975	GTF-M	1,577	95				[59]
Streptococcus gordonii CH1	GTF-G	1,577	75		23		[60]
Lactobacillus reuteri 180	GTF-180	1,772	61		39		[61]
Lactobacillus sakei Kg15	GTF-Kg15	1,561	90		10		[61]
Lactobacillus fermentum Kg3	GTF-Kg3	1,595	92		8		[61]
Lactobacillus parabuchneri 33	GTF-33	1,463	81		19		[61]
Streptococcus salivarus ATCC25975	GTF-L	1,449	50		50		[59]
Streptococcus salivarus ATCC25975	GTF-J	1,517			90		[59]
Streptococcus mutans GS 5	GTF-B	1,475	12		88		[62]
Streptococcus mutans GS 5	GTF-C	1,375	15		86		[63]
Streptococcus downei Mfe 28	GTF-I	1,597	12		88		[64]
Streptococcus sobrinus 6715	GTF-Ia	1,592			Major		[65]
Lactobacillus reuteri ML1	GTF-MLI	1,772	35		65		[61]
Lactobacillus reuteri 121	GTF-A	1,781	43		57		[66]
Lactobacillus reuteri BioGaia	GTF-Bio	1,781	22	78			[67]
Leuconostoc mesenteroides B-1355	ASR	2,057	45		55		[52]
Weissella cibaria	rDSRWC	1,472	Major				[68]

gentiobiose. The glucosylation yield is dependent on both the enzyme recognition of the acceptor molecule and the reaction conditions. The regiospecificity of the reaction is controlled by the topology of the enzyme active site and thus the positioning of the acceptor in a catalytically productive conformation. Synthesis thus proceeds by successive transfers of glucosyl units onto oligosaccharides, which can be either the product or the acceptor substrate. In particular, DSR-S

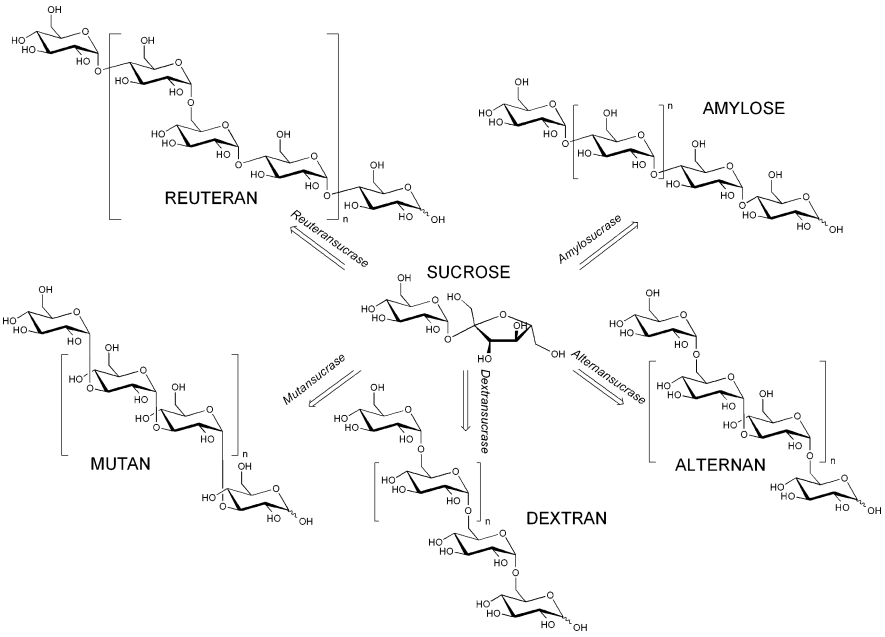

Fig. 6 Schematic representation of the α-glucan polymers synthesized by glucansucrases from sucrose donor substrate

synthesizes a series of GOS of the isomaltosaccharide type, which are partially non-digestible, and are of similar structure to the isomaltooligosaccharides (IMOs) produced at the industrial scale by enzymatic transformation of maltooligosaccharides obtained from starch degradation. These products are the main prebiotics present on the Japanese market [78, 79]. DSR-E is a dextransucrase from *L. mesenteroides* NRRL B-1299 which synthesizes a dextran containing about 29% α-1, 2 linkages (Table 2). This specificity is maintained when using maltose as an acceptor, resulting in the production of a series of oligodextrans that may or may not contain an α-1, 2 linkage at the non-reducing end [80]. Such GOS are not hydrolyzed by digestive enzymes from humans and animals [81, 82]. Studies carried out in animal models showed that when they are metabolized by bacteria from the human gut microbiota, and particularly by bacteroides bacteria [79], they induce the production of a wide range of glycolytic enzymes in the cecum without any increased production of gases [83]. It was demonstrated that disaccharides containing an α-1, 2 linkage are particularly selective from the point of view of intestinal bacteria [84]. Such GOS are produced on the industrial scale and used for dermo-cosmetic applications to improve the equilibrium of the skin microbiota [85].

By analogy with maltose and isomaltose, alternansucrase from *Leuconostoc mesenteroides* NRRL B-21297 was found to synthesize from gentiobiose as acceptor a series of gentiooligosaccharides that could be used as potential prebiotics, without the bitterness imparted by gentiobiose [86]. Alternansucrase also catalyzes

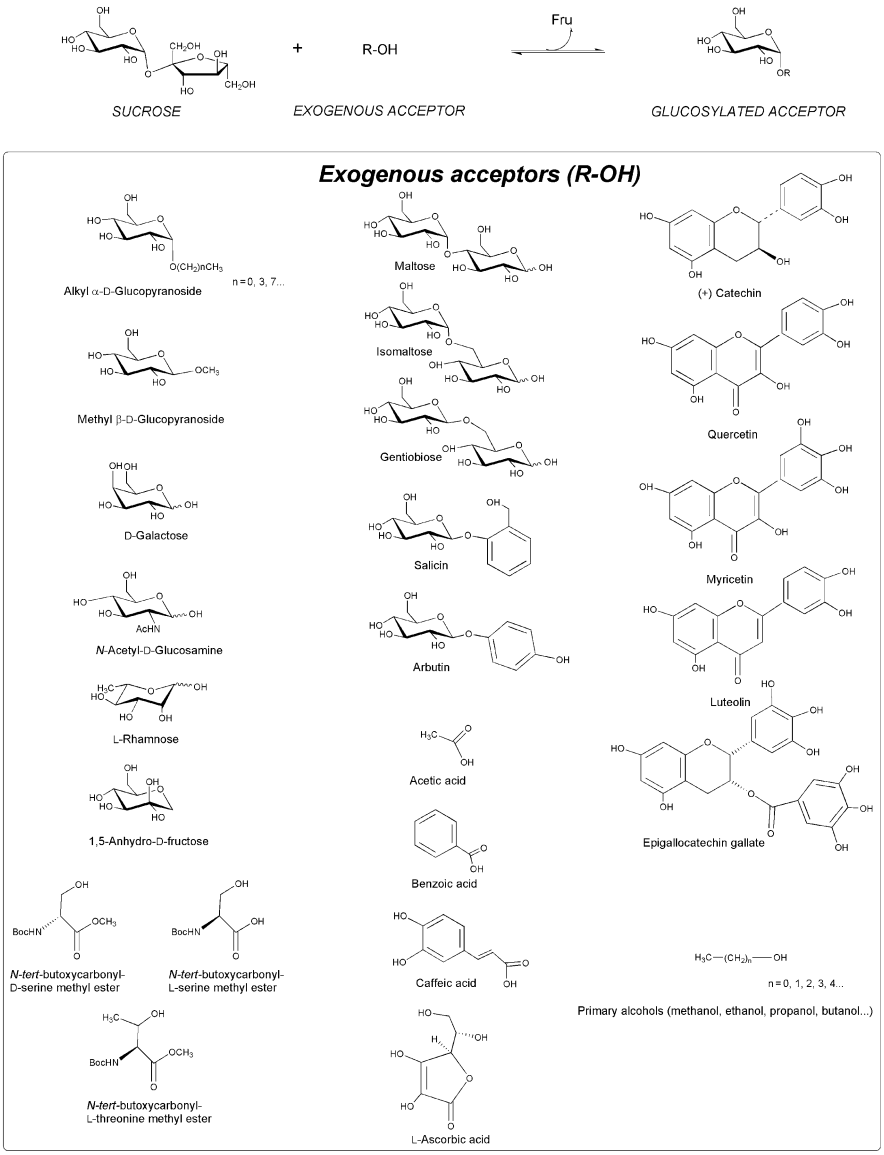

Fig. 7 Transglucosylation reaction of exogenous acceptors (R–OH) catalyzed by glucansucrases and/or sucrose phosphorylases. A representation of some exogenous acceptors recognized by the enzymes is shown

the production of oligoalternans [87–89] which have been demonstrated to control efficiently enteric bacterial pathogens [90, 91]. Some of these oligosaccharides are also being evaluated for the prevention of type II diabetes and as anti-cariogenic sucrose substitutes [75].

In addition to the synthesis of polysaccharides, the transglucosylation activity of GH family 13 amylosucrase from *N. polysaccharea* has been employed to modify amylo-polysaccharides, underlying the interest of this enzyme for the design of novel thickening agents, resistant starches, and original carbohydrate-based dendritic nanoparticles [73, 74].

A large portion of biologically active natural products are glycosylated. As the sugar moieties are often important for bioactivity, alteration of the glycosylation patterns of the parent structures has the potential to produce modified molecules with new activities. In more recent reports, the potential of native glucansucrases to glucosylate exogenous sugar and non-sugar acceptors has been exploited (Fig. 7). The resulting products were shown to depend on both the enzyme and the acceptor. Of interest, the synthesis of various glycosides and α-gluco-conjugates has been achieved using as acceptors: carbohydrates (e.g., galactose, xylose, D-tagatose, L-glucose, L-rhamnose, *N*-acetyl-D-glucosamine, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, butyl α-D-glucopyranoside, octyl α-D-glucopyranoside, raffinose and 1,5-anhydro-D-fructose derivatives; [92–98]), aromatic compounds (e.g., catechin, luteolin, quercetin, myricetin, epigallocatechin gallate, arbutin, salicin [23, 24, 99–104]), alcohols (e.g., salicyl alcohol, primary alcohols of different chain length [105, 106]), and amino acid derivatives [105] (Fig. 7). In many cases, the glucosylated products displayed improved biological activities and better pharmacological and physicochemical properties (e.g., sweetness, water solubility, stability, and bioavailability) [104, 107–111].

Although less investigated, the transglucosylation activity of sucrose phosphorylases to glucosylate phenolic and alcoholic hydroxyl groups of various compounds has also been described. Sucrose phosphorylase from *Pseudomonas saccharophila* was long ago reported to glucosylate D- and L-sugars such as D- and L-arabinose, L-rhamnose, L-fucose, D- and L-arabitol and D-xylitol to produce glucosylated disaccharides [112, 113]. More recently, recombinant sucrose phosphorylase from *Bifidobacterium longum* and *Leuconostoc mesenteroides* were used to produce stable glucoside derivatives of anti-oxidants such as L-ascorbic acid [114], caffeic acid [115], catechins [116, 117], and hydroquinones [118]. A novel transglycosylation reaction of sucrose phosphorylase to carboxylic acid compounds (benzoic acid and acetic acid) was recently reported [119, 120]. The glucosylation of these compounds permitted the significant reduction of the sour taste of these acidic compounds used in the beverage industry.

3 Engineering Novel Sucrose-Utilizing Transglucosidases

The use of α-transglucosidases in the large-scale manufacture of novel bioderivatives is still limited by several factors such as enzyme selectivity, stability, and, in some cases, efficiency. To overcome these limitations and further enlarge the applications of these enzymes, the latest protein engineering technologies have been used to tailor biocatalysts with specific properties for novel oligosaccharide,

polysaccharide, and glucoconjugate production. Nonetheless, these examples still remain scarce.

3.1 Enzyme Engineering Strategies

Over the last decade, strategies for creating variant tailored enzymes have been developed. Combinatorial methods, also known as directed evolution, are based on the generation of random libraries of modified genes coding for the enzyme using random mutagenesis and/or recombination [121, 122], and selection and/or screening for the property of interest of the resulting proteins. The advantage of such approaches is that three-dimensional structure elucidation is not required. These methods have resulted in remarkable improvements in enzyme activity for specific substrates, and global properties such as solvent and heat tolerance. The determination of high resolution crystal structures of enzymes, protein engineering based on rational design, and site-directed mutagenesis have enabled enzyme properties to be re-designed and improved. In particular, these rational strategies have proven to be extremely efficient in generating biocatalysts, including glycoenzymes with novel substrate specificities.

3.2 Improving and Controlling Natural Enzyme Reactions

3.2.1 Altering Biocatalyst Properties

The potential use of *N. polysaccharea* amylosucrase as an industrial tool for the synthesis, modification, or degradation of starch and related α-glucan polymers is hampered by its low catalytic efficiency on sucrose ($k_{cat} = 1\ s^{-1}$), its low stability ($t_{1/2} = 21$ h at 30 °C), and the synthesis of byproducts such as sucrose isomers. In an effort to improve the properties of this enzyme, a combinatorial engineering strategy (directed evolution) involving error-prone PCR and gene shuffling followed by selective screening was applied to generate more efficient variants of the amylosucrase from *N. polysaccharea*. The large libraries of variants were screened using a selection method that allowed only the growth of amylosucrase active clones on solid mineral medium containing sucrose as the sole carbon source [123]. The catalytic efficiency of the best isolated enzyme variant (N387D) was improved by about threefold. Another improved variant (E227G) was found capable of synthesizing longer amylose chains than the wild-type enzyme [123, 124].

From a random library of 60,000 clones, three improved variants of amylosucrase (two double mutants and one single mutant) were isolated that showed an improvement of their thermostability at 50 °C by 3.5- to 10-fold compared to the wild-type. The activity half-life of the best variant was 32 min at 50 °C, while the wild-type was completely inactivated at this temperature in less than 3 min.

At 50°C, amylose synthesis using the improved mutant and high sucrose concentration (600 mM) allowed the production of amylose chains twice as long as those obtained by the parental enzyme at 30°C [125, 126].

The transglucosylation activity of glucansucrases from the GH70 family was also enhanced by directed evolution [127, 128]. Using ultrasoft X-rays to irradiate the dextransucrase gene of *Leuconostoc mesenteroides* B-742CB, a novel dextransucrase with a catalytic activity increased by 2.3-fold was isolated that synthesized polymers with a higher degree of α-1, 3 branching than the parent enzyme (2.7 times) [127].

3.2.2 Controlling Biopolymer Product Size and Structure

Most of the applications of α-glucans concern derived products of lower molecular weights, ranging from 1 to 70 kDa, which are currently obtained by acidic hydrolysis followed by solvent fractionation. Recently, engineered *L. mesenteroides* NRRL B-512F dextransucrases (DSR-S), truncated at the C-terminal domain, have made possible the direct synthesis of IMOs and dextrans of controlled molecular weight (10 and 40 kDa) in one pot (directly from sucrose) that are of interest for food, health, and fine chemical applications [129].

DSR-E glucansucrase from *L. mesenteroides* NRRL B-1299, which naturally synthesizes both α-1, 6 and α-1, 2 glucosidic linkages, has also been engineered to generate an enzyme that catalyzes the formation of only α-1, 2 branches [130]. This new enzyme offers great potential as the α-1, 2 linkage, when present in oligosaccharides, has been shown to confer prebiotic properties. Experimental conditions have recently been established that enable the production of new polymers with controlled size and α-1, 2 linkage content [131].

Using a semi-rational engineering approach, modifications were introduced near the transition state-stabilizing amino acids (R624 and V630) of the glucansucrase GTF-R from *S. oralis*, leading to a drastic alteration of the enzyme specificity from α-1, 6 to α-1, 3 linkage while the transglycosylation activity and efficiency of the enzyme were conserved. Unlike the wild-type GTF-R that produces mainly α-1, 6 glucosidic linkages (62%), mutants synthesized glucan polymers with predominantly α-1, 3 linkages (up to 46%; mutans) [132]. Glucansucrase enzymes were switched from polymer to oligosaccharide synthesis by the substitution of one single amino acid. A GTF-R variant [132] was found to re-direct its transglucosylating activity toward the formation of oligosaccharides rather than polymers, for which the amounts synthesized were considered to be negligible compared to the wild-type GTF-R.

To reduce the extent of hydrolysis, several approaches can be attempted: (1) continuous removal of the transglucosylation product by crystallization or selective adsorption onto different carriers; (2) the presence of a suitable glycosyl acceptor that reacts as a nucleophile faster than water; (3) the use of high concentration of acceptor; (4) engineering the active site of the enzyme.

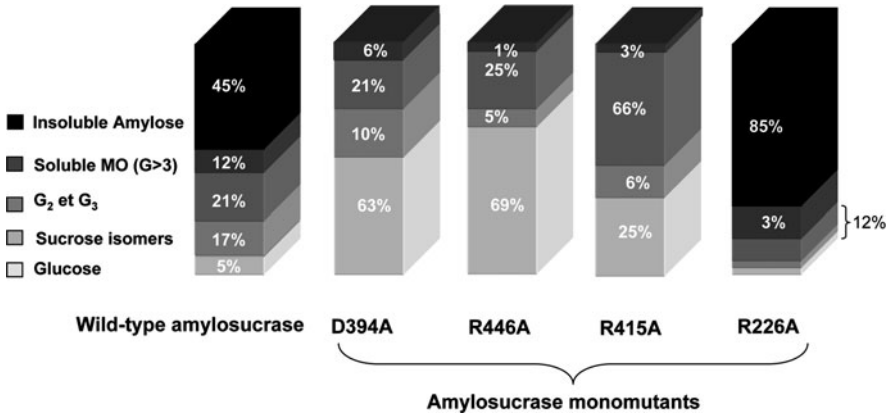

Fig. 8 Relative reaction yields of the products obtained at the end of reaction from 100 mM sucrose concentration using the wild-type amylosucrase from *N. polysaccharea*, D394, R446A, R415A, and R226 monomutants [133]. Analysis of the soluble and insoluble products was performed by HPAEC and HPLC. The yields of the soluble compounds were expressed as a percentage of the sucrose consumed. *MO* maltooligosaccharides, *G* α-D-glucose, G_2 maltose, G_3 maltotriose

In this regard, remarkable results were obtained for amylosucrase from *N. polysaccharea*, for which rational engineering led to the isolation of an amylosucrase variant with reduced or abolished side reactions, such as sucrose isomer formation and sucrose hydrolysis. The introduction of one single mutation (R226A) at the acceptor binding site of amylosucrase from *N. polysaccharea* fully diverted the transglucosylation activity of the enzyme toward the exclusive formation of an amylose-like polymer and almost no formation of shorter oligosaccharides. The amylose-like polymer yield was increased from 45% in wild-type to 85% in R226A mutant (Fig. 8). Another mono-mutation at the acceptor binding site (R415A) of amylosucrase allowed the controlled synthesis of short maltooligosaccharides of degree of ploymerization no longer than three. Conversely, introduction of alanine single mutations at the acceptor binding site (D394A and R446A) strongly limited the transglucosylation activity and thus led to creation of variants with higher hydrolysis activity than the wild-type [133].

3.3 Opportunities for Applications Through the Use of Engineered Enzymes

3.3.1 Synthesis of Novel Biopolymer Structures

The discovery of new types of sucrase enzymes provides exciting opportunities for the synthesis of tailor-made glucans and oligosaccharides for a range of diverse applications.

Guided by sequence comparisons, engineering of GH70 reuteransucrase (GTF-A) from *Lactobacillus reuteri* strain 121 has succeeded in altering α-1, 4 glucosidic linkage specificity into mainly α-1, 6 bond specificity, thus leading to the synthesis of dextran rather than a reuteran polymer [134]. The same research group later showed that reuteransucrase enzymes from *Lactobacillus reuteri* strain 121 (GTF-A) and *Lactobacillus reuteri* strain ATCC 55730 (GTF-O) could be hybridized to increase the transglucosylation/hydrolysis activity ratio and produce novel polymer products of variable and controlled structure and size distribution [135]. More recently, mutagenesis of the glucansucrase enzyme from *Lactobacillus reuteri* strain 180 (GTF-180) allowed the synthesis of modified exopolysaccharides (mEPSs) with altered physical properties, offering new opportunities for their use in the food industry [136].

Construction of chimeric glucansucrases from *L. mesenteroides* NRRL B-512F DSR-S and DSR-T5 led to the isolation of GS variants that produce glucans of different structures than those produced by the parental enzymes [137]. By exchanging the substrate-binding regions of two recombinant GS, chimeric glucansucrases produced water-soluble glucans containing different amounts of α-1, 6, α-1, 3, and α-1, 4 linkages.

In combination with chemical synthesis, glucansucrases (GTF-R from S*treptococcus oralis* and GTF-A from *Lactobacillus reuteri*) were used to produce thioglucosides, which are tolerated by most biological systems but are less sensitive to acid/base or enzyme-mediated hydrolysis than *O*-glucosides [138].

3.3.2 Chemo-Enzymatic Synthesis of Antigenic Carbohydrates

Combined with chemical synthesis, the use of biocatalysts has great potential to open the way to novel molecular diversity. Recently, an elegant *in vitro* chemoenzymatic route was reported that takes advantage of glucansucrase engineering to produce complex microbial cell-surface oligosaccharides and circumvents synthetic boundaries of glycochemistry [139]. The study was focused on the synthesis of oligosaccharides mimicking the O-antigen of *Shigella flexneri* serotypes 1b and 3a, which could be used for the development of multivalent carbohydrate-based vaccines. Most known *S. flexneri* O-antigen repeats share a linear tri-rhamnose-*N*-acetyl-glucosamine tetrasaccharide backbone and the serotype specificity is partly defined by the α-D-glucosyl branching onto the backbone. In the synthetic pathway foreseen for the production of the O-antigen repeats, glucosylation was considered using an α-transglucosidase in order to overcome the poor α/β stereoselectivity of the chemical glucosylation process. Given that no native enzyme is able to proceed to this reaction, a semi-rational engineering approach was followed to design novel α-transglucosidases able to act on non-natural and conveniently protected target substrates compatible with a subsequent chemical elongation. Recombinant amylosucrase from *Neisseria polysaccharea* was used as a scaffold to tailor the appropriate glucosylation tools. By targeting the randomization at the

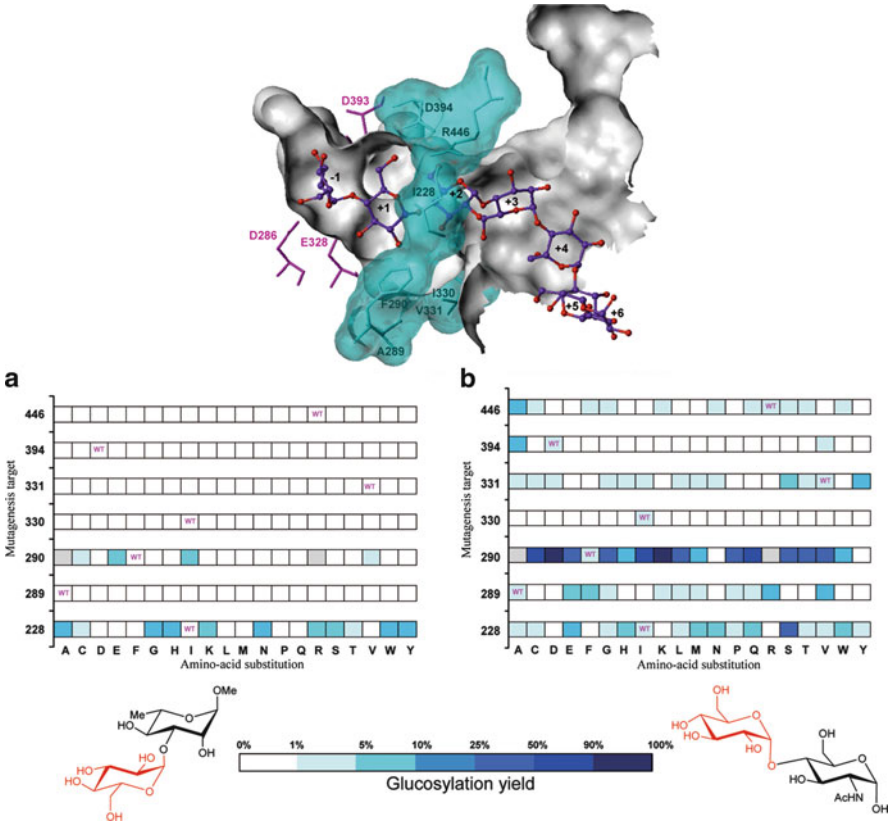

Fig. 9 Construction and screening of a single-mutant library of amylosucrase from *N. polysaccharea* for their ability to glucosylate methyl α-L-rhamnopyranoside (**a**) and allyl 2-*N*-acetyl-2-deoxy-α-D-glucopyranoside (**b**) WT: wild-type amylosucrase [139]

acceptor binding site (+1 subsite), a small and focused library of 133 single-mutants, corresponding to the systematic mutagenesis of seven positions by the 9 possible amino acids, was created and screened for variants with altered specificity (Fig. 9). The efficiency of the approach was demonstrated by the high frequency of positive hits (45 mutants of interest out of 133 screened). Amylosucrase variants with either completely new specificity toward methyl α-L-rhamnopyranoside or significantly enhanced toward allyl 2-*N*-acetyl-2-deoxy-α-D-glucopyranoside were isolated. Yet limited, the catalytic efficiency (k_{cat}/K_m) of the engineered enzymes toward the target acceptors was found considerably improved compared to the parental wild-type enzyme (Fig. 10). The best variants were then used to synthesize glucosylated building blocks that were converted into acceptors and donors compatible with chemical elongation toward oligosaccharide fragments of the O-antigens of the targeted serotypes.

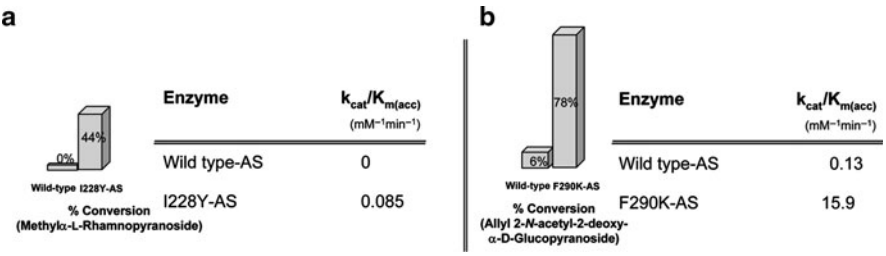

Fig. 10 Characterization of amylosucrase mutants improved for the glucosylation of (**a**) methyl α-L-rhamnopyranoside and (**b**) allyl 2-*N*-acetyl-2-deoxy-α-D-glucopyranoside [139]

The chemo-enzymatic process illustrated herein for the synthesis to *S. flexneri* O-antigenic oligosaccharides may be seen as a powerful alternative to the chemical route. More generally, this concept validation offers new opportunities in the synthesis of complex carbohydrates.

4 Conclusion: Future Directions

The examples presented above impressively show that naturally occurring sucrose-utilizing α-transglucosidases, in particular glucansucrases, can produce a broad range of glucans, gluco-oligosaccharides, and gluco-derivatives from renewable material. However, the development of novel synthetic pathways has been limited by the catalytic efficiency, stability, selectivity, and specificity of these enzymes. When considering manufacturing, enzyme fitness becomes even more critical and optimization is absolutely required. We have seen that recent advances in glucansucrase structure-function relationship understanding combined with progress in protein engineering technologies make it possible to envision the generation of improved glucansucrases with novel and well-defined properties. Neo-glucansucrases with totally new specificities able to catalyze reactions for which no equivalence exists in nature have been successfully generated. Such progress will undoubtedly enrich the repertoire of glucansucrases and lead to the emergence of even more useful biosynthetic tools. In particular, sucrose-utilizing biocatalysts could offer novel opportunities to optimize properties or access to many bioactive glucosides (e.g., antibiotics, hormones, sweeteners, alkaloids, flavonoids, etc.) reviewed by V. Křen et al. in the current series.

What progress will have to be made to enlarge further the use of glucansucrases in synthetic biology? Which directions should be followed to fulfill this objective?

First of all, a deeper comprehension of glucansucrase specificities at the molecular level will require the acquisition of a larger number of three-dimensional structures that have been a real challenge for crystallographers. Efforts will need to be focused on this goal which will provide essential information to guide the rational design of novel enzymes. However, the success of enzyme engineering strategies will also be

highly dependent on the design of efficient screening protocols, carefully established to isolate the desired variants from large enzyme collections. It means that new assays enabling automated and sensitive screening of the desired function have to be proposed. The combination of these approaches is anticipated to generate a large amount of data that will hopefully open the way to statistical analyses permitting new rules relating enzyme structure and sequence to activity to be established.

Finally, another critical aspect for rapid and predictive design is the lack of information available about the dynamics of these enzymatic systems. Prediction of the effect of a mutation on the enzyme structure and dynamics remains a difficult task. Molecular dynamics and simulations of protein and substrate motions combined with the use of biophysical techniques enabling conformational changes to be trapped have to be developed. Multidisciplinary approaches will undoubtedly lead to major advances in this field and help to promote the key role that computational methods must play for efficient re-engineering of enzymes. These new ways to accelerate the evolution process and identify mutation sites important for optimizing enzyme characteristics will help to provide rapidly a well-expressed, efficient, stable, and specific enzyme, in other words the ideal glucansucrase for biocatalysis.

References

1. Monsan P, Paul F (1995) FEMS Microbiol Rev 16:187
2. Lee JC, Greenberg WA, Wong CH (2006) Nat Protoc 1(6):3143
3. Persidis A (1997) Nat Biotechnol 15(5):479
4. Plante OJ, Palmacci ER, Seeberger PH (2001) Science 291:1523
5. Wang CC, Lee JC, Luo SY, Kulkarni SS, Huang YW, Lee CC, Chang KL, Hung SC (2007) Nature 446:896
6. Crout DH, Vic G (1998) Curr Opin Chem Biol 2:98
7. Hanson S, Best M, Bryan MC, Wong CH (2004) Trends Biochem Sci 29:656
8. Koeller KM, Wong CH (2001) Nature 409:232
9. Wong CH (1989) Science 244:1145
10. Wong CH, Shen GJ, Pederson RL, Wang YF, Hennen WJ (1991) Methods Enzymol 202:591
11. Yan F, Wakarchuk WW, Gilbert M, Richards JC, Whitfield DM (2000) Carbohydr Res 328:3
12. Zaks A, Klibanov AM (1985) Proc Natl Acad Sci USA 82:3192
13. Zaks A, Klibanov AM (1988) J Biol Chem 263:3194
14. Samain E, Drouillard S, Heyraud A, Driguez H, Geremia RA (1997) Carbohydr Res 302:35
15. Faijes M, Planas A (2007) Carbohydr Res 342:1581
16. Honda Y, Kitaoka M (2006) J Biol Chem 281:1426
17. Kim YW, Lee SS, Warren RA, Withers SG (2004) J Biol Chem 279:42787
18. Mackenzie LF, Wang Q, Warren RAJ, Withers SG (1998) J Am Chem Soc 120:5583
19. Okuyama M, Mori H, Watanabe K, Kimura A, Chiba S (2002) Biosci Biotechnol Biochem 66:928
20. Monsan P, Ouarné F (2009) In: Chalarampopoulos D, Rastall RA (eds) Prebiotics and probiotics science and technology. Springer, Berlin, p 293
21. Coutinho PM, Henrissat B (1999) In: Gilbert HJ, Davies G, Henrissat B, Svensson B (eds) Recent advances in carbohydrate bioengineering. The Royal Society of Chemistry, Cambridge, p 3
22. Jensen MH, Mirza O, Albenne C, Remaud-Siméon M, Monsan P, Gajhede M, Skov LK (2004) Biochemistry 43:3104

23. Bertrand A, Morel S, Lefoulon F, Rolland Y, Monsan P, Remaud-Siméon M (2006) Carbohydr Res 341:855
24. Meulenbeld GH, Hartmans S (2000) Biotechnol Bioeng 70:363
25. Davies G, Henrissat B (1995) Structure 3:853
26. Henrissat B, Davies G (1997) Curr Opin Struct Biol 7:637
27. Mirza O, Skov LK, Remaud-Siméon M, de Montalk GP, Albenne C, Monsan P, Gajhede M (2001) Biochemistry 40:9032
28. Skov LK, Mirza O, Henriksen A, De Montalk GP, Remaud-Siméon M, Sarcabal P, Willemot RM, Monsan P, Gajhede M (2001) J Biol Chem 276:25273
29. Skov LK, Mirza O, Sprogoe D, Van der Veen BA, Remaud-Siméon M, Albenne C, Monsan P, Gajhede M (2006) Biocat Biotrans 24:99
30. Mirza O, Skov LK, Sprogoe D, van den Broek LAM, Beldman G, Kastrup JS, Gajhede M (2006) J Biol Chem 281:35576
31. Sprogoe D, van den Broek LAM, Mirza O, Kastrup JS, Voragen AGJ, Gajhede M, Skov LK (2004) Biochemistry 43:1156
32. Watanabe K, Hata Y, Kizaki H, Katsube Y, Suzuki Y (1997) J Mol Biol 269:142
33. Brzozowski AM, Davies GJ (1997) Biochemistry 36:10837
34. MacGregor EA (1996) FEBS Lett 378:263
35. Pijning T, Vujicic-Zagar A, Kralj S, Eeuwema W, Dijkhuizen L, Dijkstra BW (2008) Biocat Biotrans 26:12
36. Seo ES, Christiansen C, Hachem MA, Nielsen MM, Fukuda K, Bozonnet S, Blennow A, Aghajari N, Haser R, Svensson B (2008) Biologia 63:967
37. Binder TP, Robyt JF (1983) Carbohydr Res 124:287
38. Gold AM, Osber MP (1971) Biochem Biophys Res Commun 42:469
39. de Montalk GP, Remaud-Siméon M, Willemot RM, Planchot V, Monsan P (1999) J Bacteriol 181:375
40. Pizzut-Serin S, Potocki-Veronese G, van der Veen BA, Albenne C, Monsan P, Remaud-Siméon M (2005) FEBS Lett 579:1405
41. Emond S, Mondeil S, Jaziri K, André I, Monsan P, Remaud-Siméon M, Potocki-Veronese G (2008) FEMS Microbiol Lett 285:25
42. Ha SJ, Seo DH, Jung JH, Cha J, Kim TJ, Kim T-J, Kim Y-W, Park CS (2009) Biosci Biotechnol Biochem 73:1505
43. Kim MJ, Kwon T, Lee HJ, Kim KH, Chung DK, Ji GE, Byeon ES, Lee JH (2003) Biotechnol Lett 25:1211
44. Kitao S, Nakano E (1992) J Ferment Bioeng 73:179
45. Lee JH, Yoon SH, Nam SH, Moon YH, Moon YY, Kim D (2006) Enzyme Microbial Technol 39:612
46. Lee JH, Moon YH, Kim N, Kim YM, Kang HK, Jung JY, Abada E, Kang SS, Kim D (2008) Biotechnol Lett 30:749
47. Nomura K, Sugimoto K, Nishiura H, Ohdan K, Nishimura T, Hayashi H, Kuriki T (2008) Biosci Biotechnol Biochem 72:82
48. Monchois V, Remaud-Siméon M, Russell RR, Monsan P, Willemot RM (1997) Appl Microbiol Biotechnol 48:465
49. Dols M, Remaud-Siméon M, Vignon M, Monsan P (1997) Appl Microbiol Biotechnol 62:47
50. Monchois V, Willemot RM, Remaud-Siméon M, Croux C, Monsan P (1996) Gene 182:23
51. Monchois V, Willemot RM, Remaud-Siméon M, Croux C, Soucaille P, Monsan P (1996) Ann NY Acad Sci 799:151
52. Arguello-Morales MA, Remaud-Siméon M, Pizzut S, Sarcabal P, Willemot R, Monsan P (2000) FEMS Microbiol Lett 182:81
53. Neubauer H, Bauche A, Mollet B (2003) Microbiol 149:973
54. Shimamura A, Nakano YJ, Mukasa H, Kuramitsu HK (1994) J Bacteriol 176:4845
55. Fujiwara T, Hoshino T, Ooshima T, Sobue S, Hamada S (2000) Infect Immun 68:2475
56. Gilmore KS, Russell RR, Ferretti JJ (1990) Infect Immun 58:2452

57. Hanada N, Isobe Y, Aizawa Y, Katayama T, Sato S, Inoue M (1993) Infect Immun 61:2096
58. Hanada N, Fukushima K, Nomura Y, Senpuku H, Hayakawa M, Mukasa H, Shiroza T, Abiko Y (2002) Biochim Biophys Acta 1570:75
59. Simpson CL, Cheetham NW, Giffard PM, Jacques NA (1995) Microbiology 141(Pt 6):1451
60. Vickerman MM, Sulavik MC, Nowak JD, Gardner NM, Jones GW, Clewell DB (1997) DNA Seq 7:83
61. Kralj S, van Geel-Schutten GH, Dondorff MMG, Kirsanovs S, van der Maarel M, Dijkhuizen L (2004) Microbiol -Sgm 150:3681
62. Shiroza T, Ueda S, Kuramitsu HK (1987) J Bacteriol 169:4263
63. Ueda S, Shiroza T, Kuramitsu HK (1988) Gene 69:101
64. Russell RR, Gilpin ML, Mukasa H, Dougan G (1987) J Gen Microbiol 133:935
65. Konishi N, Torii Y, Yamamoto T, Miyagi A, Ohta H, Fukui K, Hanamoto S, Matsuno H, Komatsu H, Kodama T, Katayama E (1999) J Biochem 126:287
66. Kralj S, van Geel-Schutten GH, Rahaoui H, Leer RJ, Faber EJ, van der Maarel MJ, Dijkhuizen L (2002) Appl Environ Microbiol 68:4283
67. Kralj S, Stripling E, Sanders P, van Geel-Schutten GH, Dijkhuizen L (2005) Appl Environ Microbiol 71:3942
68. Kang HK, Oh JS, Kim D (2009) FEMS Microbiol Lett 292:33
69. Albenne C, Skov LK, Mirza O, Gajhede M, Potocki-Veronese G, Monsan P, Remaud-Siméon M (2002) FEBS Lett 527:67
70. Moulis C, Joucla G, Harrison D, Fabre E, Potocki-Veronese G, Monsan P, Remaud-Siméon M (2006) J Biol Chem 281:31254
71. Kim D, Kang HK, Seo ES, Kim GJ, Kimura A (2006) J Biomed Nanotech 2:120
72. Potocki-Veronese G, Putaux JL, Dupeyre D, Albenne C, Remaud-Siméon M, Monsan P, Buleon A (2005) Biomacromol 6:1000
73. Putaux JL, Potocki-Veronese G, Remaud-Siméon M, Buleon A (2006) Biomacromol 7:1720
74. Rolland-Sabate A, Colonna P, Potocki-Veronese G, Monsan P, Planchot V (2004) J Cereal Sci 40:17
75. Seo ES, Nam SH, Kang HK, Cho JY, Lee HS, Ryu HW, Kim D (2007) Enzyme Microbial Technol 40:1117
76. Hamada S, Slade HD (1980) Microbiol Rev 44:331
77. Koepsell HJ, Tsuchiya HM, Hellman NN, Kazenko A, Hoffman CA, Sharpe ES, Jackson RW (1953) J Biol Chem 200:793
78. Buchholz K, Seibel J (2003) In: Eggleston G, Cote GL (eds) Oligosaccharides in food and agriculture. Oxford University Press, Washington, DC, p 63
79. Monsan P, Auriol D (2004) In: Neeser JR, German JB (eds) Bioprocesses and biotechnology for functional foods and nutraceuticals. Marcel Dekker Inc., New York, p 135
80. Remaud-Siméon M, López-Munguía A, Pelenc V, Paul F, Monsan P (1994) Appl Biochem Biotech 44:101
81. Djouzi Z, Andrieux C, Pelenc V, Somarriba S, Popot F, Paul F, Monsan P, Szylit O (1995) J Appl Bacteriol 79:117
82. Valette P, Pelenc V, Djouzi Z, Andrieux C, Paul F, Monsan P, Szylit O (1993) J Sci Food Agric 62:121
83. Djouzi Z, Andrieux C (1997) Br J Nutr 78:313
84. Sanz ML, Gibson GR, Rastall RA (2005) J Agric Food Chem 53:5192
85. Lamothe JP, Marchenay Y, Monsan P, Paul F, Pelenc V (1992) French patent 2678166
86. Cote GL (2009) Carbohydr Res 344:187
87. Arguello Morales MA, Remaud-Siméon M, Willemot RM, Vignon MR, Monsan P (2001) Carbohydr Res 331:403
88. Cote GL, Dunlap CA (2003) Carbohydr Res 338:1961
89. Cote GL, Robyt JF (1982) Carbohydr Res 111:127
90. Holt SM, Miller-Fosmore CM, Cote GL (2005) Lett Appl Microbiol 40:385
91. Holt SM, Teresi JM, Cote GL (2008) Lett Appl Microbiol 46:73

92. Champion E, André I, Mulard LA, Monsan P, Remaud-Siméon M, Morel S (2009) J Carbohydr Chem 28:142
93. Cote GL, Dunlap CA, Appell M, Momany FA (2005) Carbohydr Res 340:257
94. Cote GL, Dunlap CA, Vermillion KE (2009) Carbohydr Res 344:1951
95. Demuth K, Jordening HJ, Buchholz K (2002) Carbohydr Res 337:1811
96. Richard G, Morel S, Willemot RM, Monsan P, Remaud-Siméon M (2003) Carbohydr Res 338:855
97. Richard G, Yu S, Monsan P, Remaud-Siméon M, Morel S (2005) Carbohydr Res 340:395
98. Seo DH, Jung JH, Ha SJ, Yoo SH, Kim TJ, Cha J, Park CS (2008) In: Park KH (ed) Carbohydrate-active enzyme structure function and application. CRC Press, Boca Raton, p 125
99. Jung J-H, Seo D-H, Ha S-J, Song M-C, Cha J, Yoo S-H, Kim T-J, Baek N-I, Moo-Yeol B, Park C-S (2009) Carbohydr Res 344:1612
100. Meulenbeld GH, Zuilhof H, van Veldhuizen A, van den Heuvel RHH, Hartmans S (1999) Appl Environ Microbiol 65:4141
101. Moon YH, Lee JH, Ahn JS, Nam SH, Oh DK, Park DH, Chung HJ, Kang S, Day DF, Kim D (2006) J Agric Food Chem 54:1230
102. Moon YH, Lee JH, Jhon DY, Jun WJ, Kang SS, Sim J, Choi H, Moon JH, Kim D (2007) Enzyme Microbial Technol 40:1124
103. Moon YH, Nam SH, Kang J, Kim YM, Lee JH, Kang HK, Breton V, Jun WJ, Park KD, Kimura A, Kim D (2007) Appl Microbiol Biotechnol 77:559
104. Seo ES, Lee JH, Park JY, Kim D, Han HJ, Robyt JF (2005) J Biotechnol 117:31
105. Seibel J, Hellmuth H, Hofer B, Kicinska AM, Schmalbruch B (2006) Chembiochem 7:310
106. Yoon SH, Fulton DB, Robyt JF (2004) Carbohydr Res 339:1517
107. Chung MJ, Sung NJ, Park CS, Kweon DK, Mantovani A, Moon TW, Lee SJ, Park KH (2008) Eur J Pharmacol 578:159
108. Lee YS, Lee MH, Lee HS, Lee SJ, Kim YW, Zhang R, Withers SG, Kim KS, Lee SJ, Park KH (2008) J Agric Food Chem 56:5324
109. Lee CK, Le QT, Kim YH, Shhim JH, Lee SJ, Park KH (2008) J Agric Food Chem 56:126
110. Seo DH, Jung JH, Ha SJ, Song M-C, Cha J, Yoo S-H, Kim TJ, Baek N-I, Park CS (2009) J Mol Catal B 60:113
111. Tramice A, Andreotti G, Trincone A (2008) Biotechnol J 3:545
112. Doudoroff M, Hassid WZ, Barker HA (1947) J Biol Chem 168:733
113. van den Broek LAM, van Boxtel EL, Kievit RP, Verhoef R, Beldman G, Voragen AGJ (2004) Appl Microbiol Biotechnol 65:219
114. Kwon T, Kim CT, Lee JH (2007) Biotechnol Lett 29:611
115. Shin MH, Cheong NY, Lee JH, Kim KH (2009) Food Chem 115:1028
116. Kitao S, Ariga T, Matsudo T, Sekine H (1993) Biosci Biotechnol Biochem 57:2010
117. Kitao S, Matsudo T, Saitoh M, Horiuchi T, Sekine H (1995) Biosci Biotechnol Biochem 59:2167
118. Kwon TY, Lee JH (2004) Korean J Microbiol Biotechnol 32:286
119. Sugimoto K, Nomura K, Nishiura H, Ohdan K, Nishimura T, Hayashi H, Kuriki T (2007) J Biosci Bioeng 104:22
120. Sugimoto K, Nomura K, Nishiura H, Ohdan K, Nishimura T, Hayashi H, Kuriki T (2008) Biologia 63:1015
121. Stemmer WP (1994) Proc Natl Acad Sci USA 91:10747
122. Stemmer WP (1994) Nature 370:389
123. van der Veen BA, Potocki-Veronese G, Albenne C, Joucla G, Monsan P, Remaud-Siméon M (2004) FEBS Lett 560:91
124. van der Veen BA, Skov LK, Potocki-Veronese G, Gajhede M, Monsan P, Remaud-Siméon M (2006) FEBS J 273:673
125. Emond S, André I, Jaziri K, Potocki-Veronese G, Mondon P, Bouayadi K, Kharrat H, Monsan P, Remaud-Siméon M (2008) Protein Sci 17:967

126. Emond S, Mondon P, Pizzut-Serin S, Douchy L, Crozet F, Bouayadi K, Kharrat H, Potocki-Veronese G, Monsan P, Remaud-Siméon M (2008) Protein Eng Des Sel 21:267
127. Kang HK, Seo ES, Robyt JF, Kim D (2003) J Mol Catal B 26:167
128. Nam SH, Ko EA, Jang SS, do Kim W, Kim SY, Hwang DS, Kim D (2008) Biotechnol Lett 30:135
129. Moulis C, Vaca-Medina G, Suwannarangsee S, Monsan P, Potocki-Véronèse G, Remaud-Siméon M (2008) Biocat Biotransform 26:141
130. Fabre E, Bozonnet S, Arcache A, Willernot RM, Vignon M, Monsan P, Remaud-Siméon M (2005) J Bacteriol 187:296
131. Brison Y, Fabre E, Moulis C, Portais JC, Monsan P, Remaud-Siméon M (2010) Appl Microbiol Biotechnol 86(2):545–554
132. Hellmuth H, Wittrock S, Kralj S, Dijkhuizen L, Hofer B, Seibel J (2008) Biochemistry 47:6678
133. Albenne C, Skov LK, Mirza O, Gajhede M, Feller G, D'Amico S, André G, Potocki-Veronese G, van der Veen BA, Monsan P, Remaud-Siméon M (2004) J Biol Chem 279:726
134. Kralj S, van Geel-Schutten IGH, Faber EJ, van der Maarel M, Dijkhuizen L (2005) Biochemistry 44:9206
135. Kralj S, van Leeuwen SS, Valk V, Eeuwema W, Kamerling JP, Dijkhuizen L (2008) FEBS J 275:6002
136. van Leeuwen SS, Kralj S, Eeuwema W, Gerwig GJ, Dijkhuizen L, Kamerling JP (2009) Biomacromolecules 10:580
137. Funane K, Ishii T, Terasawa K, Yamamoto T, Kobayashi M (2004) Biosci Biotechnol Biochem 68:1912
138. Hellmuth H, Hillringhaus L, Hobbel S, Kralj S, Dijkhuizen L, Seibel J (2007) ChemBioChem 8:273
139. Champion E, André I, Moulis C, Boutet J, Descroix K, Morel S, Monsan P, Mulard LA, Remaud-Siméon M (2009) J Am Chem Soc 131:7379

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Difructose Dianhydrides (DFAs) and DFA-Enriched Products as Functional Foods

Carmen Ortiz Mellet and José M. García Fernández

Abstract This review provides an overview of the current status of the chemistry and biology of di-D-fructose dianhydrides (DFAs) with a focus on their potential as functional foods. The history of this family of cyclic ketodisaccharides has expanded for almost 100 years and offers a paradigmatic example of artificial synthetic molecules that were identified as natural products later on and finally encountered in our own table. Issued from fundamental investigations on the reactivity of carbohydrates in strongly acidic media, DFAs remained laboratory curiosities for decades. Early reports on their isolation from plants raised doubts, until the formation of some DFA representatives by the action of microorganisms on fructans was reported in the middle 1980s. Since then, research on DFAs has run in parallel in the areas of microbiology and carbohydrate chemistry. Evidence of the potential of these compounds as functional food was accumulated from both sides, with the development of biotechnological processes for mass production of selected candidates and of chemical methodologies to prepare DFA-enriched products from sucrose or inulin. In 1994 a decisive discovery in the field took place in the laboratory of Jacques Defaye in Grenoble, France: the presence of DFAs in a commercial sucrose caramel was evidenced in a quite significant 18% mass proportion! The development of an efficient analytical protocol for DFAs and the stereoselective synthesis of individual standards allowed one to demonstrate that DFAs and their glycosylated derivatives (glycosyl-DFAs) are universally formed during caramelization reactions. They are not *potential* food products; they have actually always been in our daily food. Most important, they seem to exert

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beneficial effects: they are acariogenic, low-caloric, and promote the growth of beneficial microflora in the gut.

Most recent evidence indicates that DFAs can even protect the intestinal tract against agressive agents favor the assimilation of antioxidants, and act as a drug-like food for the treatment of colon ailments such as inflammatory bowel disease (Crohn disease). The development of efficient methodologies for the preparation of DFA-enriched caramels, compatible with the food and agricultural industry regulations, may lead to new *natural* functional foods and nutraceuticals based on DFAs in the near future.

Keywords Caramel, Difructose dianhydrides, Fructose, Functional foods, Inulin, Prebiotics, Sucrose

Contents

1 Introduction

A food or food ingredient can be regarded as *functional* "if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects" [1]. Among functional foods, *prebiotics* have been defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, and/or activity of one or a limited number of beneficial bacteria in the colon and thus improve host health" [2, 3]. The term *nutraceuticals* was launched for the particular case of food ingredients that have a proven beneficial effect on human health [4]. The beneficial action of these compounds ranges from the supply of essential mineral or vitamins to protection against disease.

Research on prebiotics has intensified over the last 15 years or so, with a recent interest in those having additional nutraceutical potential [5, 6]. In principle, any foodstuff that reaches the colon (e.g., non-digestible carbohydrates, some peptides, and certain lipids) can be classified as potential prebiotics [7]. Most oligosaccharides are not sensitive to gastric acid, escape degradation and adsorption in the upper gastrointestinal tract, and reach the colon intact [8]; here they promote the proliferation of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, which constitute a significant proportion of the intestinal microflora and are claimed to have several beneficial effects in the host [9]. Potential prebiotic oligosaccharides can be classified according to their chemical constituents and degree of polymerization (DP), and include manno-oligosaccharides [10], pectic-oligosaccharides [11], isomalto-oligosaccharides [12], xylo-oligosaccharides [13], galacto-oligosaccharides/transgalactosylated-oligosaccharides (GOS/TOS) [14], and fructans such as fructo-oligosaccharides (FOS), inulin, or levan [14].

For the perceived benefits on human health, fructose-based oligo and polysaccharides are probably the most commonly used fibers in the production of functional foods. Difructose dianhydrides (DFAs; the alternative abbreviation DFDAs is encountered in several publications) and their glycosylated derivatives (glycosyl-DFAs) represent a recent addition to this list. From the structural point of view, DFAs are cyclic fructodisaccharides resulting, formally, from the dimerization reaction of D-fructose with the loss of two water molecules and generation of two reciprocal glycosidic linkages. Consequently, they can be considered as the first members of the cyclofructan family [15]. The formation of DFAs by thermal and mineral acid activation of D-fructose and inulin was already known at the beginning of the twentieth century [16], but they remained laboratory curiosities until recent times [17, 18]. Their isolation from several microorganisms and higher plants and the characterization of some representatives as non-caloric, acariogenic sweeteners with potential bifidogenic properties stimulated interest in the chemistry and nutritional properties of DFAs. The identification in 1994 of DFAs as major nonvolatile components in caramel (up to 18% in an industrial sucrose caramel) represented a benchmark that provided a definitive stimulus to the field [19]. The transformation of sucrose into DFAs following a culinary transformation perfectly symbolized the chemical questions that a discipline just born, namely *Molecular Gastronomy*, was intended to address [20, 21]. Nowadays, research on DFAs is enjoying a renaissance that embraces areas such as natural products, chemical synthesis, food analysis, nutrition, and health. This review presents an overview of the advances in the chemistry and biology of DFAs, emphasizing the inter- and multidisciplinary character of the current body of knowledge and the future perspectives, particularly in view of their potential applications as functional foods.

2 Natural Occurrence of Di-D-Fructose Dianhydrides

Publications on DFAs have frequently suffered from confusion regarding wrong structural assignment of some diastereomers until recent times. In addition, the old notation system based on the use of the terms *difructose dianhydride* (*DFA*;

di-D-fructofuranose dianhydrides) or *diheterolevulosan* (*DHL*; di-D-fructopyranose or D-fructofuranose D-fructopyranose dianhydrides), followed by a roman number that is reminiscent of the sequential order in which they were discovered, still coexists with the use of the systematic nomenclature proposed by the IUPAC–IUMB organization [22]. This is particularly true for manuscripts dealing with isolation of DFAs from natural sources. Figure 1 depicts the chemical formulae of the most relevant DFA diastereomers reported up to date with indication of the abbreviated IUPAC nomenclature and, when applicable, the corresponding traditional name.

2.1 DFAs in Higher Plants

The first report on DFA formation in higher plants dates back to 1933, when Schlubach and Knoop [23] isolated a compound tentatively identified as α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (**10**, also known as DFA I) from Jerusalem artichoke. Alliuminoside, a difructofuranose 2,6′:6,2′-dianhydride for which configuration at the glycosidic linkages was not determined, was isolated from tubers of *Allium sewertzowi* [24]. However, the fact that these results have not been further confirmed throws some doubt onto whether the DFAs were actually from plant origin or were formed by the presence of microorganisms. The enzymic formation of α-D-fructofuranose β-D-fructofuranose 1,2′:2,3′-dianhydride (**1**, DFA III) in sterilized homogenates of the roots of *Lycoris radiata*, a plant use in China as a traditional folk medicine, unequivocally demonstrated the capacity of this plant to produce this particular DFA [25]. The compound was further extracted from the intact bulbs by supercritical carbon dioxide and its structure unequivocally established by NMR [26].

2.2 DFA-Producing Microorganisms

The investigation of DFAs as natural products blossomed in the mid-1970s when Tanaka and Uchiyama reported the isolation of an extracellular inulinase from *Arthrobacter ureafaciens*, a soil bacteria that synthesizes α-fructofuranose β-fructofuranose 1,2′:2,3′-dianhydride (**1**, DFA III) from inulin [27, 28]. Since this seminal work, several microorganisms, many of which belong to the *Arthrobacter* genus, have been shown to produce enzymes that promote the transformation of fructans (inulin or levan) into DFAs. Up to now, four DFA isomers have been obtained by enzymatic degradation of fructans: **1** (DFA III), **10** (DFA I), di-β-fructofuranose 2,6′:6,2′-dianhydride (**15**, DFA IV), and α-D-fructofuranose β-D-fructofuranose 1,2′:2,6′-dianhydride (**16**, DFA V) [29]. As a unique feature, and in stark contrast to common fructan decomposing enzymes, DFA-producing enzymes catalyze an intramolecular transglycosylation reaction through which the second glycosidic bond from the non-reducing fructose is transferred to the terminal D-fructofuranose residue (see Scheme 1 for the case of inulin).

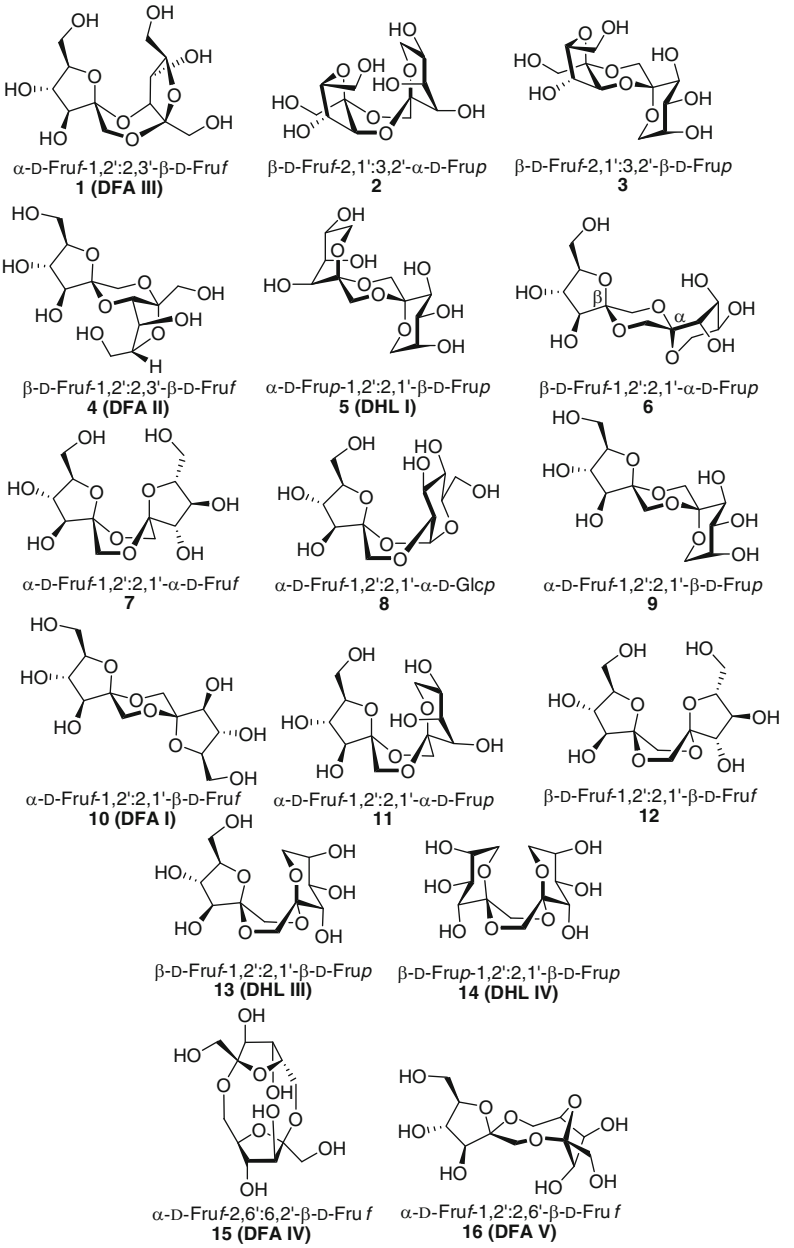


Fig. 1 Chemical structures of di-D-fructose dianhydrides (**1**–**7** and **9**–**14**) and of a D-fructose–D-glucose mixed dianhydride (**8**), with indication of the preferred conformation of six-membered rings

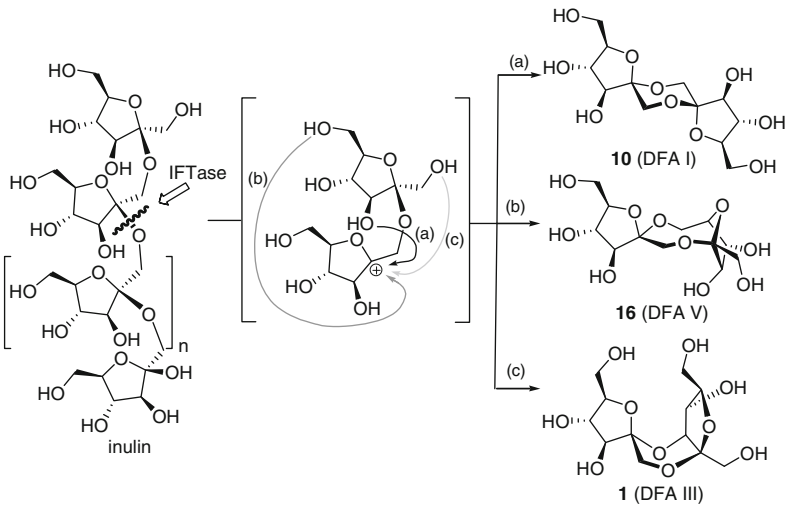
This mechanism operates for both inulin degrading (IFTase) [30] and levan degrading (LFTase) enzymes [31–33].

3 Synthetic Strategies to DFAs

The ensemble of structures collected in Fig. 1 reflects the broad structural diversity existing in the DFA family. The difficulty in accessing individual pure standards for analytical identification, quantification, or evaluation of DFAs has been a major hurdle for research on their nutritional properties. Enzymatic production of DFAs from inulin or levan proceeds with full control of the regio- and stereoselectivity of the glycosylation process, leading to single DFA isomers. However, the range of DFA structures available from the current set of inulin and levan fructotransferases is restricted to a few representatives. In contrast, chemically promoted DFA formation allows access to a much broader pool of derivatives. Purification of individual compounds from reaction mixtures represents a major complication in this case. The strategies developed for the chemical synthesis of DFAs, their scope, and their limitations are discussed in the following paragraphs.

3.1 DFA Formation by Treatment of Fructose and Fructose-Containing Oligo- and Polysaccharides with Mineral Acids

DFAs were first isolated from the syrups obtained by the treatment of fructose or inulin with mineral acids, such as hydrochloric or nitric acids [17], which



Scheme 1 Proposed mechanism for the formation of DFAs from inulin by the action of inulin fructotransferase (IFTase)

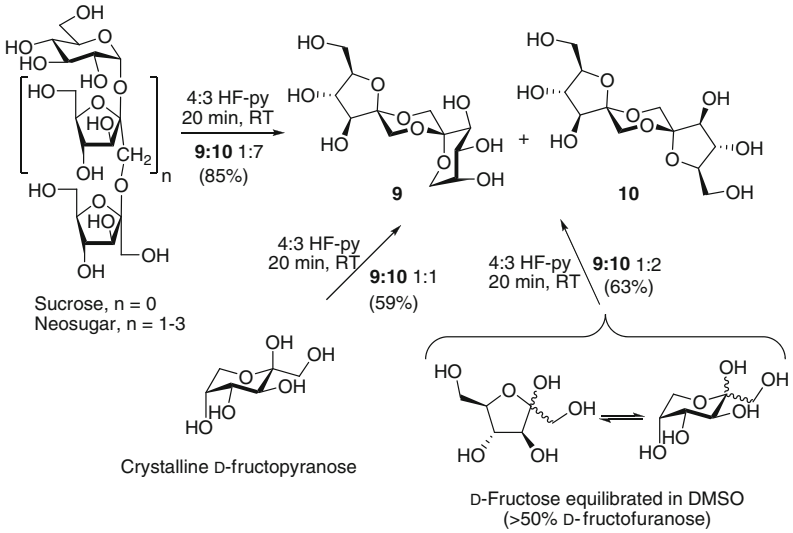
correspond in fact to Fischer glycosylation conditions. The proportions of DFAs formed by these methods remain rather low due to concomitant non-specific dehydration reactions, being of very limited synthetic utility. Slightly better yields have been reported by using methanolic sulfuric acid or trifluoroacetic acid [34]. Actually, formation of DFAs from D-fructose syrups under acidic pH was perceived as an unwanted side reaction in some instances, being detrimental for crystallization yields during industrial production of D-fructose [35, 36]. Hydrolytic methods used for the quantification of fructose equivalents in herbaceous biomass similarly leads to the formation of DFAs to a certain extent, which can result in underestimated values [37]. Traces of DFAs have also been detected in the residue resulting from the production of bioethanol by fermentation of molasses, which probably arise from D-fructose dimerization during sugar beet processing [38].

3.2 Activation of Fructose, Sucrose, Glycosylfructoses, and Inulin with Anhydrous Hydrogen Fluoride and HF-Reagents

Among chemical methods, protonic activation of D-fructose, sucrose, FOSs (neosugar), and inulin with anhydrous hydrogen fluoride (HF) and HF-reagents was found to be extremely efficient in promoting conversion to DFAs [34]. In contrast to mineral acids, HF selectively activates fructose units at the anomeric position, with formation of fructosyl fluorides which are in equilibrium with the corresponding fructosyl oxycarbenium ions. Further dimerization affords mixtures of DFAs whose composition depends on the starting D-fructose source and on reaction conditions. By using the complex pyridinium poly(hydrogen fluoride) and acting on the HF-pyridine ratio, Defaye and coworkers evidenced that the reaction outcome is dependent, to some extent, upon the initial cyclic form of the D-fructose moieties. Thus, under identical kinetic reaction conditions (HF-pyridine 4:3), sucrose or neosugar afforded significantly higher proportions of the difructofuranose dianhydride **10** as compared to crystalline D-fructopyranose, for which the furanose–pyranose derivative **9** was the major product. When commercial D-fructopyranose was equilibrated in dimethylsulfoxide, which increases the presence of the fructofuranose form up to 50%, prior to HF-pyridine activation, a parallel increase in the relative proportion of **10** over **9** was observed. Optimization of the reaction conditions allowed the production of both isomers on a preparative scale (Scheme 2) [39].

HF activation of sucrose provoked fast hydrolysis of the disaccharide into the corresponding monosaccharide substituents, followed by fructose dimerization to give a mixture of DFAs from which compounds **3**, **5**, **9**, and **14** were isolated. The formation of reversion glucooligosaccharides and glucosyl-DFAs, resulting from in situ HF-promoted glucosylation of DFAs, was also observed, but isolation of pure compounds from the mixture proved impractical [40].

Glycosyl-DFAs with precise glycosylation patterns could be obtained by using glycosylfructoses as starting materials. Thus, 6-*O*-α-D-glucopyranosyl-D-fructofuranose (isomaltulose, palatinose), 5-*O*-α-D-glucopyranosyl-D-fructopyranose (leucrose),



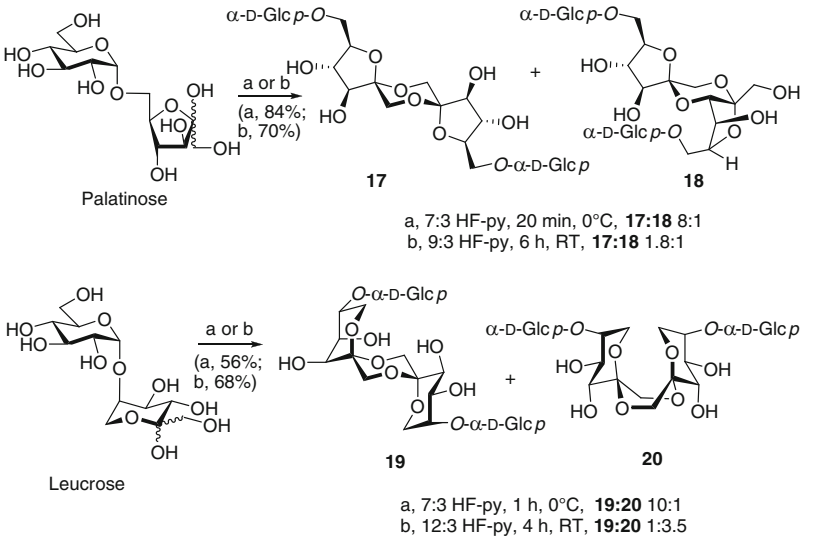
Scheme 2 Synthesis of α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**9**) and α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (**10**) from D-fructose, sucrose and D-fructose oligosaccharides by the action of pyridinium poly(hydrogenfluoride) complex

4-*O*-α-D-glucopyranosyl-D-fructose (maltulose), and 3-*O*-α-D-glucopyranosyl-D-fructose (turanose) underwent cyclodimerization when dissolved in pyridinium poly (hydrogen fluoride) to give mixtures of diglucosylated-DFAs [41]. In the cases of palatinose and leucrose the glucosyl substituent blocks the cyclic form of the D-fructose moiety in the disaccharide, restricting the production of DFA structures to 6,6′-di-*O*-α-D-glucopyranosyl difructofuranose (**17**, **18**) and 5,5′-di-*O*-α-D-glucopyranosyl difructopyranose (**19**, **20**) derivatives, respectively (Scheme 3). It was possible to modulate the relative proportion of diastereomers by acting on the HF-pyridine ratio. Maltulose and turanose afforded complex mixtures of 4,4′-di-*O*-α-D-glucopyranosyl and 3,3′-di-*O*-α-D-glucopyranosyl DFA pseudotetrasaccharides.

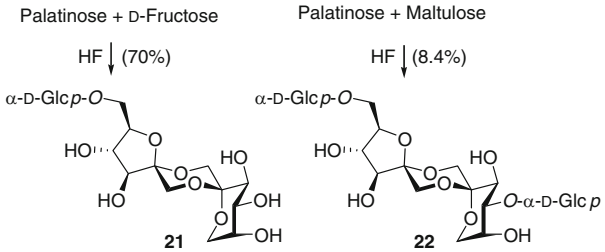
Interestingly, dimerization of glycosylfructoses could also be conducted in pure HF at high concentration of the substrate without affecting the pre-existing glycosic linkages [42]. Monoglucosyl-DFAs (e.g., **21**) and unsymmetrically glucosylated diglucosyl-DFAs (e.g., **22**) were prepared by mixing either a given glucosylfructose (e.g., palatinose) and D-fructose or two different glucosylfructoses (e.g., palatinose and maltulose; Scheme 4). The compounds thus prepared were used as standards for a more detailed characterization of the sucrose-HF mixture.

1-*O*-α-D-Glucopyranosyl-D-fructose (trehalulose) exhibited a completely different reactivity in HF. The activated furanosyl species was now intramolecularly trapped by *O*-2′-glycosidation to give β-D-fructofuranose and β-D-fructopyranose α-D-glucopyranose 1,1′:2,2′-dianhydrides (**23**, **24**, Scheme 5) [43, 44].

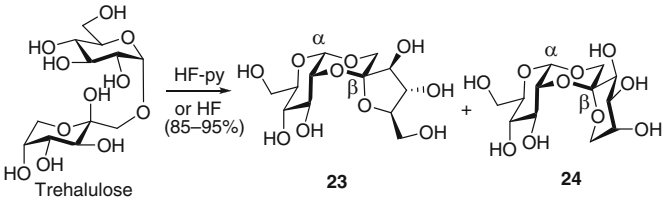
The ensemble of results accumulated on the protonic reactivity of D-fructose and oligosaccharides thereof by using HF-based reagents led to the proposal of a



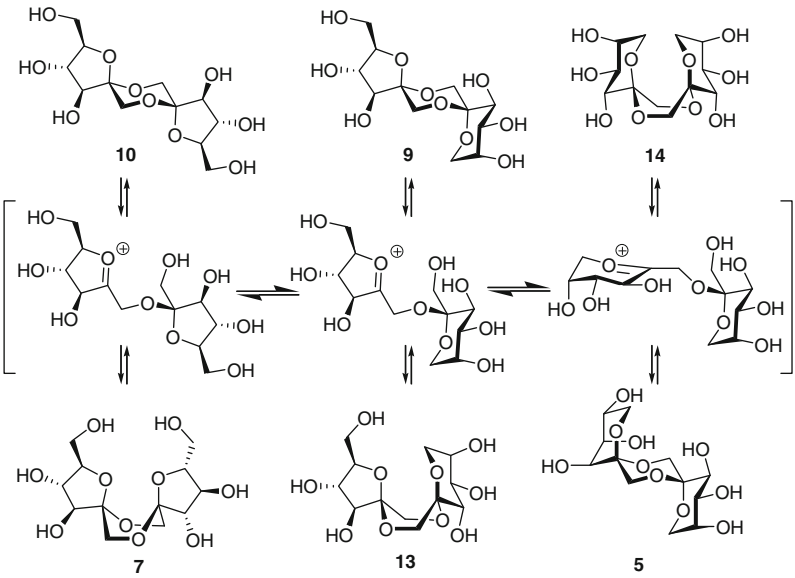
Scheme 3 Preparation of diglucosyl-DFAs from palatinose and leucrose by the action of pyridinium poly(hydrogenfluoride) complex



Scheme 4 Preparation of monoglucosyl- and diglucosyl-DFAs with different substitution patterns at the two D-fructose residues from mixtures of D-fructose and a glycosylfructose or of two-component mixtures of glycosylfructoses, by activation with HF



Scheme 5 Synthesis of D-fructose–D-glucose mixed dianhydrides from trehalulose by the action of HF-reagents

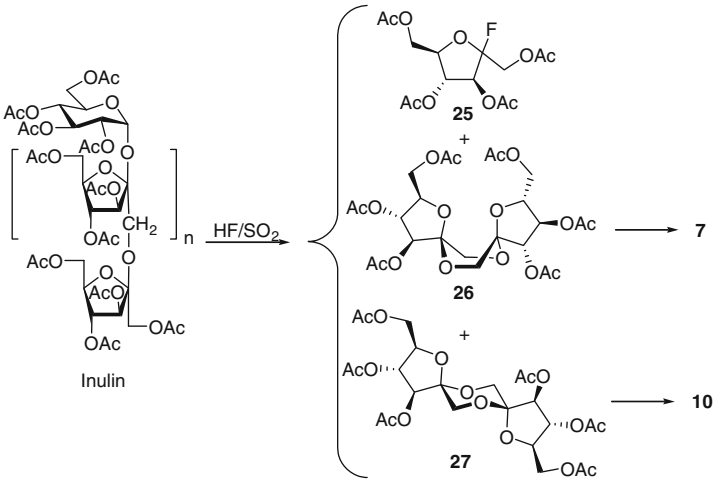


Scheme 6 DFA isomerization reactions

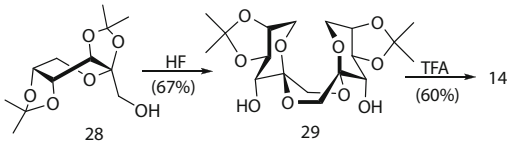
general mechanism for the formation of DFAs. Following activation, an interconverting mixture of reactive fructofuranosyl and fructopyranosyl oxycarbenium ions is formed. The five-membered fructofuranosyl species reacts faster to afford difructofuranose DFAs as the kinetic reaction products (e.g., compounds **1**, **7**, or **10**). Under thermodynamic conditions, the difuranose compounds isomerize, through a transient fructodisaccharide, to the more stable fructopyranose-containing DFAs (e.g., **9**, **13**, or **14**; Scheme 6). In the presence of glucose, the DFAs formed undergo glucosylation to give glucosyl-DFAs. The resemblance of this scenario with that resulting from thermal activation of sucrose turned to be decisive for elucidation of the processes taking place during caramelization.

3.3 Protecting Group Strategies: Intermolecular D-Fructose Dimerization

Protonic activation of D-fructose, sucrose, or inulin presents serious limitations when looking for pure individual DFA standards due to the reversibility of the dimerization and isomerization processes. Beyond a certain enrichment of the DFA mixtures in kinetic (e.g., **10**) or thermodynamic components (e.g., **9**), selectivity is rather poor and chromatographic purification is difficult. The use of protecting groups to block the cyclic form of the D-fructose precursor was first explored by Defaye and coworkers in 1988. Activation of inulin acetate with HF in sulfur dioxide afforded a three-component mixture of the tetra-*O*-acetyl-β-D-fructofuranosyl



Scheme 7 HF-catalyzed synthesis of DFAs from inulin acetate



Scheme 8 Stereoselective synthesis of di-β-D-fructopyranose 1,2′:2,1′ dianhydride (**14**) from 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose by HF activation

fluoride **25** and the difuranose DFAs **7** (erroneously assigned as **11** in the original reference) and **10** as the corresponding hexa-*O*-acetates **26** and **27** (Scheme 7). The relative proportions varied from 2:1 to 1:7, depending on reaction conditions. Interestingly, pure **27** could be isolated from the crude mixtures by direct crystallization from ether [45]. A second example from the same laboratory reported the synthesis of the dipyranose DFA **29** by HF activation of 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose **28** (Scheme 8) [46].

A proper analysis of the above results has to take into consideration the particular conformational properties of DFAs, which are governed by stereoelectronic effects. The relatively flexible 1,4-dioxane central ring adopts a chair or boat conformation for dispiro-DFAs having different or identical configuration at the anomeric centers, respectively, in order to place the oxygen substituents in pseudoaxial orientation, fitting the conformational anomeric effect [47], and the carbon substituents in pseudoequatorial position (Fig. 2).

The *chair* DFAs are thermodynamically favored, and can be obtained in good yield if furanose–pyranose interconversion is prevented by protecting groups. Thus, even though compound **10** is a kinetic isomer when considering the whole set of DFAs, it is thermodynamically favored in the difuranose DFA subset. The *boat* conformers are neither kinetically nor thermodynamically favored. The term

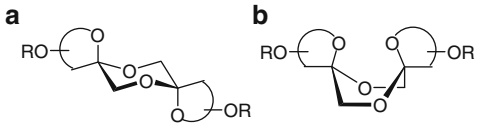


Fig. 2 Schematic representation of the *chair* and *boat* conformations of dispiro-DFAs for derivatives having different (**a**) or identical configuration at the anomeric centers (**b**)

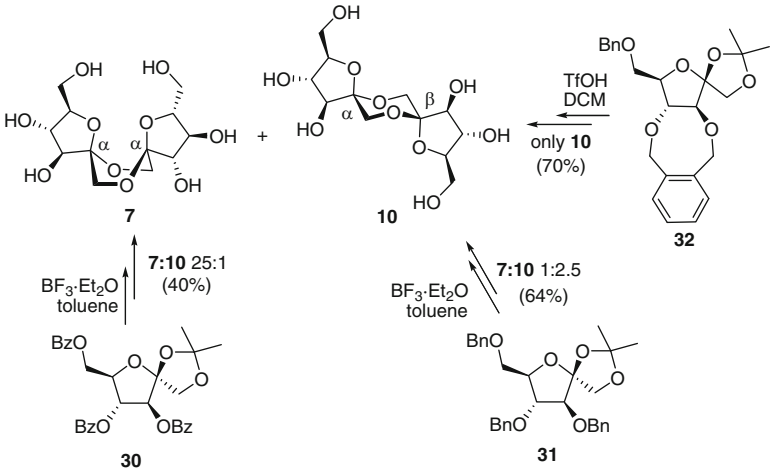
contra-thermodynamic has been coined for spiroketal derivatives featuring these characteristics (e.g., **7** or **14**) [48]. Their stereoselective synthesis requires stereodirecting strategies and irreversible or slow-reversible conditions. Thus, formation of **26** in significant proportions from inulin acetate (Scheme 7) can be explained by *O*-2 acetate participation during the dual glycosylation process, which directs the stereochemical course to the α-configuration. The reaction is not fully irreversible, however; it progress with time to the *chair* conformer **27**. In the case of the fructopyranose diacetonide **29**, the isopropylidene group at the 4,5-*O*-position prevents inversion of the $^{2}C_{5}$ conformation (characteristic of β-configured D-fructopyranose moieties) into the $^{5}C_{2}$ conformation (α-D-fructopyranose moieties) in the monosaccharide rings, acting as an element of stereocontrol towards the contra-thermodynamic di-D-fructopyranose dianhydride (see structures of **5** and **14** in Fig. 1).

The use of HF as promoter for the stereoselective synthesis of DFAs is handicapped by the occurrence of concomitant isomerization processes and the hazards associated with HF storing and handling. A more convenient approach has been recently reported based in the use of 1,2-*O*-isopropylydene-β-D-fructofuranose (**30**–**32**) or β-D-fructopyranose (**33**–**35**) derivatives as precursors and boron trifluoride or trifluoromethanesulfonic (triflic) acid as catalyst [49, 50]. The reaction is conducted in dichloromethane or toluene and proceeds under irreversible conditions, leading to two-component mixtures of *O*-protected difuranose (Scheme 9) or dipyranose DFAs (Scheme 10). The stereochemical outcome can be controlled, to some extent, by using participating (e.g., benzoyl; **30**, **34**) or nonparticipating protecting groups (e.g., benzyl; **31**, **33**). Alternatively, the cyclic 3,4-*O*-(*o*-xylylene) protecting group (**32**, **35**) has been incorporated as an element of remote stereochemical control during the dimerization reaction [51].

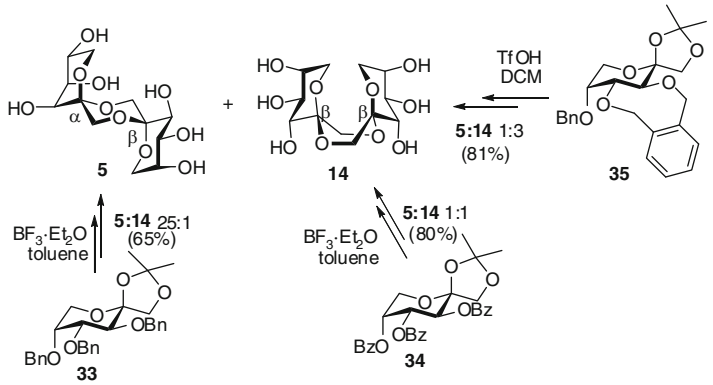
The above methodology proved very successful for accessing pure standards of DFAs **5**, **7**, **10**, and **14**, which are among the most abundant DFAs in kinetic mixtures of diastereomers as well as in caramel. It is, however, intrinsically limited to compounds having identical ring size at both monosaccharide moieties. Moreover, the di-β-D-fructofuranose 1,2:2,1′-dianhydride isomer **12** remained elusive.

3.4 Rigid Spacer-Mediated Strategies: Intramolecular Spiroketalization

To improve the approach and broaden the spectrum of DFA diastereomers available by stereoselective synthesis, the concept of *rigid spacer-mediated spirocyclization*

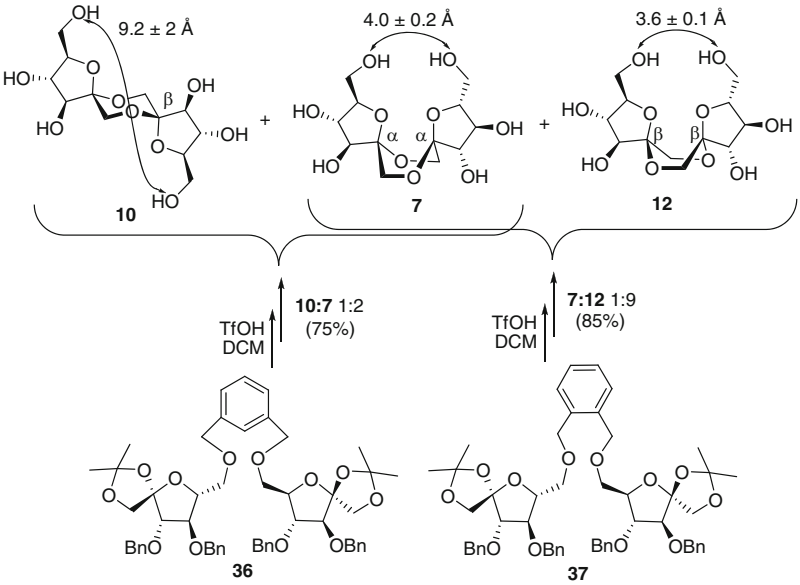


Scheme 9 Stereoselective synthesis of di-D-fructofuranose 1,2′:2,1′-dianhydrides from 1,2-isopropylidene-β-D-fructofuranose precursors by selective acidic activation in organic solvents



Scheme 10 Stereoselective synthesis of di-D-fructopyranose 1,2′:2,1′-dianhydrides from 1,2-isopropylidene-β-D-fructopyranose precursors by selective acidic activation in organic solvents

was implemented. The strategy consists in linking the two reacting monosaccharide subunits by an appropriate tether in order to transform the intermolecular dimerization into an intramolecular process. By judicious choice of the bridge, the conformational space available during DFA formation can be restricted to favor a particular cyclic disaccharide. The *o*-, *m*-, and *p*-xylylene segments proved very convenient as distance restriction elements. They were inserted between the appropriate hydroxyl positions in the D-fructose moieties by bridging reactions using the corresponding commercially available α,α′-dibromoxylenes. For instance, a conformational analysis of the dispiro difuranose DFAs **7**, **10**, and **12** revealed a much



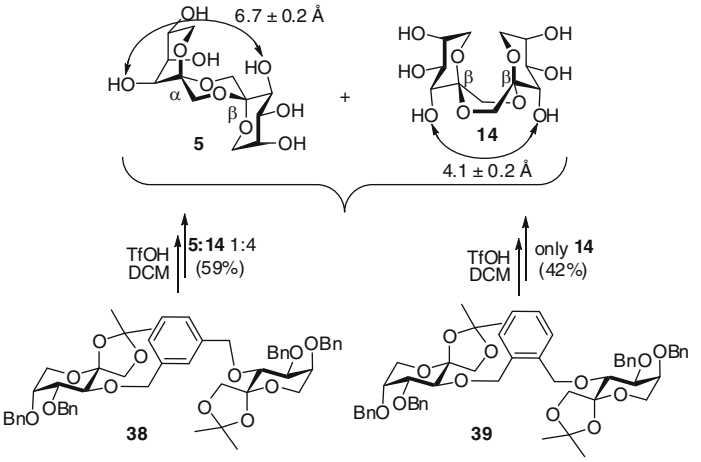
Scheme 11 Rigid spacer-mediated synthesis of di-D-fructofuranose 1,2′:2,1′-dianhydrides. The distance between the bridged oxygen atoms is indicated

shorter *O*-6–*O*-6′distance for the α,α C_2 symmetric isomer **7**, which is still shorter in the case of the β,β-isomer **12**. Consequently, insertion of 6,6′-di-*O*-*m*- and *o*-xylylene tethers (**36**, **37**) resulted in high yields of the di-α- and di β-D-fructofuranose 1,2:2,1′-dianhydride derivatives, respectively (Scheme 11) [52]. A similar analysis showed significant differences in the *O*-3–*O*-3′distance in the dipyranose DFAs **5** and **14**. A stereoselective synthesis of the later was conceived by preparing a D-fructopyranose precursor in which these secondary positions were connected through an *o*-xylylene bridge (**38**, **39**, Scheme 12) [53].

The stereoselective synthesis of DFAs having furanose and pyranose moieties represents a more difficult challenge. The four diastereomeric possibilities, namely the two *boat* compounds α,α (**11**) and β,β (**13**), and the two *chair* derivatives α,β (**9**) and β,α (**6**) are present in either kinetic or thermodynamic mixtures of DFAs. The inter-residue distances between selected oxygen atoms are indicated in Fig. 3.

The *O*-3–*O*-3′distance is notably shorter in the β-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride **13** than in the other three DFAs in this subset (Fig. 3). Its synthesis was accomplished by linking two D-fructopyranose moieties having different protecting group patterns with the *o*-xylylene bridge (**40**, Scheme 13). In one of those D-fructose residues pyranose → furanose interconversion became possible after activation with TfOH (→**41**). The higher reactivity of the five-membered ring towards spirocyclization led to the β,β-isomer with total selectivity in 41% yield [54].

The *chair* DFAs **6** and **9** can be accessed by introducing a distance restriction element between positions O-6 and O-3′. Compound **9** is thermodynamically much



Scheme 12 Rigid spacer-mediated synthesis of di-D-fructopyranose 1,2′:2,1′-dianhydrides. The distance between the bridged oxygen atoms is indicated

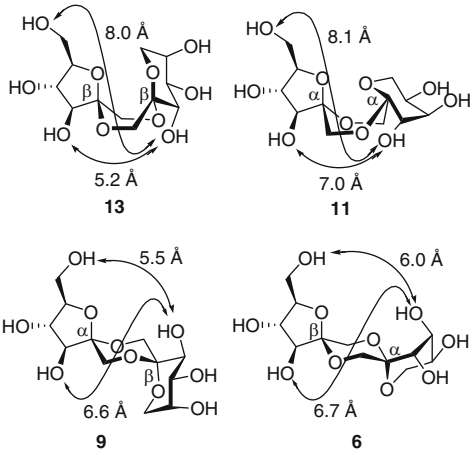
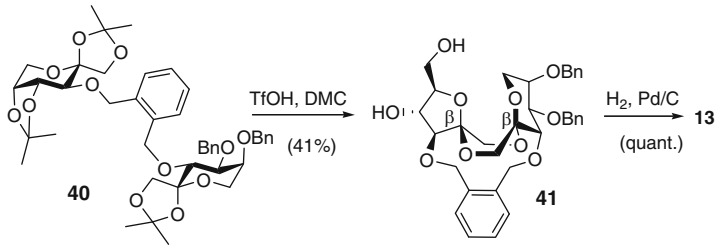
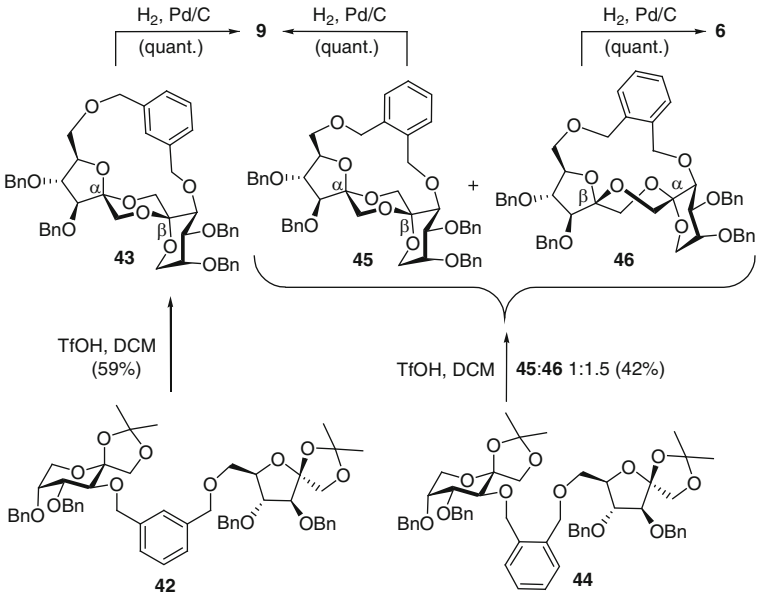


Fig. 3 Structures of the four D-fructofuranose D-fructopyranose 1,2:2, 1′-dianhydride diastereomers with indication of selected interatomic distances



Scheme 13 *o*-Xylylene-mediated synthesis of β-D-fructofuranose β-D-fructopyranose 1,2′:2, 1′-dianhydride (**13**)



Scheme 14 Xylylene-mediated synthesis of the D-fructofuranose D-fructopyranose 1,2′:2,1′-dianhydrides **9** and **6**

more stable than **6**; it is actually the more abundant DFA at the thermodynamic equilibrium. When a relatively flexible tether such as *m*-xylylene was used to connect a D-fructofuranose and a D-fructopyranose residue (**42**), the α,β derivative **43** was the only isolated compound. Interestingly, the less stable DFA **6** is conformationally more flexible. When the *o*-xylylene bridge, which imposes a shorter distance than that expected for a *chair* conformation, was inserted (**44**), the β,α derivative **46** became the major reaction product over the α,β derivative **45** (Scheme 14) [55].

3.5 Enzymatic Strategies Towards the Synthesis of DFAs

Stereoselective chemical synthesis of DFAs is instrumental in obtaining pure standards for the validation of analytical methods for the identification and quantification of DFAs in mixtures, particularly in food products. Yet it is inadequate for mass production of DFAs or nutritional applications. Biotechnological approaches using inulin or levan fructosyltransferases, while limited to certain diastereomers, show much promise in these respects. Most of the reports on enzymatic synthesis of DFAs are related to the monospiranic difuranose DFA III (**1**) and the non-spiranic DFA IV (**15**), while reports on the dispiranic DFA I (**10**) are much less frequent. Their synthesis has become an issue of industrial interest [56].

Isolation of DFA I-forming enzymes from *Arthrobacter* spp. was first reported by Kobayashi and coworkers [57]. The enzyme optimal operating conditions were pH 6 and 40°C and it was strongly inhibited by Hg^{2+}, Fe^{3+}, and other metal cations, a common feature for most of these enzymes [58]. *Streptomyces* spp. has also been shown to be a source of DFA I-forming enzyme [59]. More recently, Haraguchi and coworkers have reported new DFA I-forming enzymes from *Arthrobacter* spp. with slightly prolonged heat stability [60, 61].

Yokota and coworkers reported the isolation of an extracellular DFA III-producing inulin fructotransferase (IFTase) (EC 4.2.2.18; inulase II) from *Arthrobacter* sp. H65-7 featuring high productivity (up to 90 U mL^{-1}) and thermal tolerance (retention of 80% activity after treatment at 70°C) [62, 63]. Actually, DFA III has been industrially manufactured from purified inulin by using this enzyme at the Shimizu Factory (Nippon Beet Sugar Mfg. Co., Ltd.) since 2004. Recently, the use of crude inulin instead of purified inulin has been shown to be compatible with this production process [64]. Improved enzymes that remain active after prolonged heating have also been reported [65, 66].

DFA IV can be produced from levan (β-2,6-fructan) by the action of levan fructotransferase (EC 4.2.2.16; LFTase) from *Arthrobacter nicotinivorans* GS-9. Minor traces of fructose and short oligofructosides (1-kestose, nystose, and fructofuranosyl nystose) were removed by fermenting the reaction solution with baker's yeast. As a drawback, the productive enzyme could not be recycled. Although further improvements on LFTases performance in DFA IV formation have been achieved, only in a few of these cases are the DFA yield and purity as high as in the case of the inulin converting enzymes [67]. These enzymes are generally more easily inactivated by the presence of metal cations (Mn^{2+}, Cu^{2+}, Fe^{3+}, or Ag^{+}) [68] and are very sensitive to substrate origin (molecular weight and branching) [69, 70]. Genetic engineering, by combining the genes encoding for levansucrase (EC 2.4.1.10; levan-producing enzyme from sucrose), LFTase, and regulatory genes in recombinant *Bacillus subtilis*, allowed the production of DFA IV directly from sucrose in a single culture production system [71, 72].

4 DFAs in Nutrition

Based on the evidence accumulated over the years on the protonic reactivity of carbohydrates, Defaye and García Fernández hypothesized that dianhydride formation was a main driving force in the transformations involving ketoses and ketosides. This consideration, together with the longstanding observation of the acidic character of caramel, led to these researchers to apply a derivatization/chromatographical protocol, previously found successful for isolating pure samples of DFAs from the mixture arising from sucrose activation with HF, to a commercial sucrose caramel [19]. The similarities observed between both products were striking. This work traced the way from organic chemistry to molecular gastronomy and nutrition in the field of DFAs.

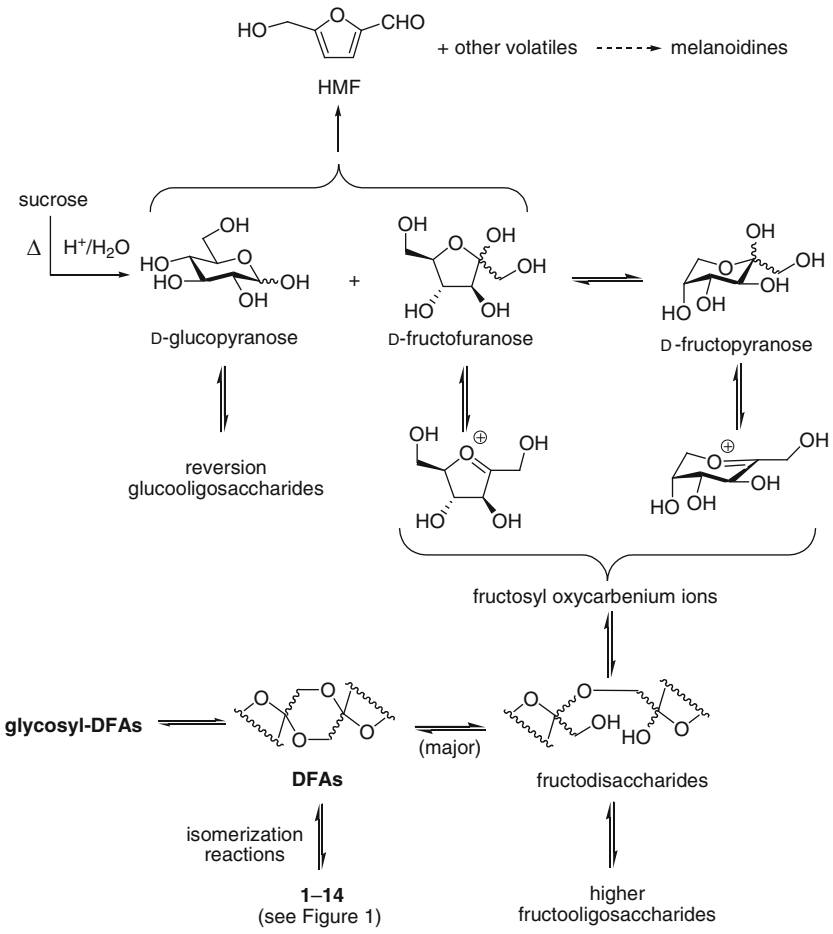
4.1 DFAs as Oligosaccharide Components of Caramel

Caramelization, together with the Maillard reaction, belongs to the group of nonenzymatic browning reactions taking place upon cooking of sugar-rich foods, and caramel itself has been used for millennia to impart color and flavor to food and beverages [73]. Caramelization commonly occurs when sugars, or products containing high proportion of sugars, are heated either dry or in concentrated aqueous solutions, alone or in the presence of certain additives. Upon thermal treatment of sugars, dehydration and self-condensation reactions occur, giving rise to volatiles (principally 5-hydroxymethylfurfural, HMF), pigments (melanoidines), and oligosaccharidic material, among which di-D-fructose dianhydrides (DFAs) and glycosylated-DFA derivatives of different DP have been identified. Following the isolation of DFAs **10** (DFA I), **9** (DHL II), **7** (erroneously assigned as **12** in the original report), **4** (DFA II), and **5** (DHL I) from an aromatic industrial sucrose caramel in 1994 [19, 74], the presence of DFAs in roasted chicory [75], heat dried fruits [76], natural and sugar-roasted torrefacto coffee [77, 78], and traditional tequila [79] has also been proven. Nowadays, it is well-established that the chemical transformations leading to DFAs take place universally during caramelization processes, even when glucose or glucose syrups are used as the starting sugar; D-glucose isomerizes to a certain extent to D-fructose through Lobry de Bruyn–Alberda van Eckenstein aldose → ketose rearrangement in the early stages of caramelization [80–82].

Comparison of HF- (which can be considered as a caramelization promoter privileging the DFA formation pathway) and heat-induced (classical) caramelization led to the proposal of the general mechanism depicted in Scheme 15 for the particular case of sucrose as the starting sugar. Dimerization of D-fructose to give DFAs and further DFA interconversion, via a transient fructodisaccharide, are reversible processes that compete with irreversible intramolecular dehydration leading to volatiles, especially to HMF [83, 84]. Although difructofuranose DFAs (e.g., compounds **7** or **10**) are kinetically favored, they isomerize to the thermodynamically more stable D-fructopyranose-containing derivatives (e.g., **5**, **9**, or **13**). Up to 14 different dianhydride structures have been identified in sucrose caramel, 13 of which (compounds **1**–**7** and **9**–**14**) are DFAs and one (compound **8**) is a mixed D-fructose D-glucose dianhydride. In a second step, DFAs can be glycosylated by monosaccharide (or oligosaccharide) units to give glycosyl-DFAs. Condensation and non-specific dehydration processes involving volatile and nonvolatile components are at the origin of the high molecular weight melanoidines responsible for color development.

4.2 Identification and Quantification of DFAs in Food Products by Analytical Methods

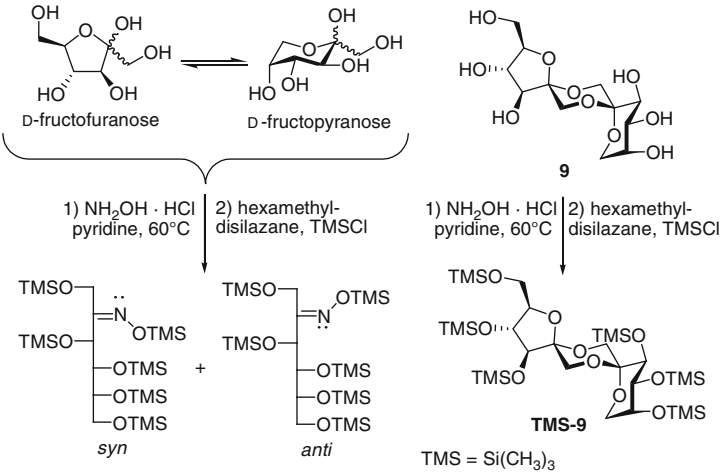
The identification of DFAs in caramel and the implication of this discovery in food processing and nutrition stressed the need for robust methods for the fast



Scheme 15 General transformations occurring during sucrose caramelization

identification and quantification of DFAs in reaction mixtures as well as in food and biological samples. In contrast to other food oligosaccharides, e.g., FOS, in which the individual constituents generally possess a regular structure and vary exclusively in the DP, DFAs form a complex family of isomeric compounds, with identical molecular weights, that differ in the cyclic form of the D-fructose moieties, the stereochemistry of the glycosidic linkages, and the positions involved in cyclodimerization, making their separation a much more difficult challenge.

High performance liquid chromatography (HPLC) has, to date, failed to provide satisfactory methods for the separation of the 14 DFAs present in sucrose caramel. In 1999 an analytical method based on GC for the identification and quantification of DFAs in commercial sucrose caramel was reported [85]. The protocol has proven extremely useful over the years, allowing not only the proving of caramel authenticity, but also the detection of adulteration by fraudulent addition of partially



Scheme 16 Oximation-trimethylsilylation derivatization reactions prior to GC analysis of caramel

caramelized products to foodstuffs, e.g., honey [86]. It involves prior derivatization of the dry sample through a reaction sequence that involves oximation followed by trimethylsilylation. Reducing sugars (e.g., D-fructose) then affords two peaks in the GC chromatogram, corresponding to the per-*O*-trimethylsilylated *syn*- and *anti*-oximes, whereas non-reducing derivatives, such as sucrose or DFAs, provide single peaks corresponding to the per-*O*-trimethylsilyl ethers (Scheme 16).

The use of phenyl β-D-glucopyranoside as internal standard and authentic samples of DFAs obtained by synthesis allowed determination of the corresponding response factors for quantitative analysis. The relative abundance of DFA diastereomers in a D-fructose caramel obtained by heating a concentrated solution of sucrose in the presence of 10% citric acid was found to correspond to a kinetic distribution, the α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride **10** being the major component in the mixture (Fig. 4).

4.3 Preparation of DFA-Enriched Products

The above findings imply that DFAs have been in the human diet since prehistoric times. Several attempts to develop caramelization procedures that favor DFA formation, in order to investigate their impact in nutrition, have been reported. Products containing DFAs and glycosyl-DFAs in proportions up to 50% have been previously prepared by pyrolysis of sucrose [87, 88] and inulin [89]. In the case of sucrose, a particular type of caramel is produced by heating at 145–147°C for a short time (7 min), which was designated as *sucrose thermal oligosaccharide caramel* (*STOC*). This product was found to contain about 50% of oligosaccharides with a high proportion of fructose. Gel permeation chromatography (GPC) of

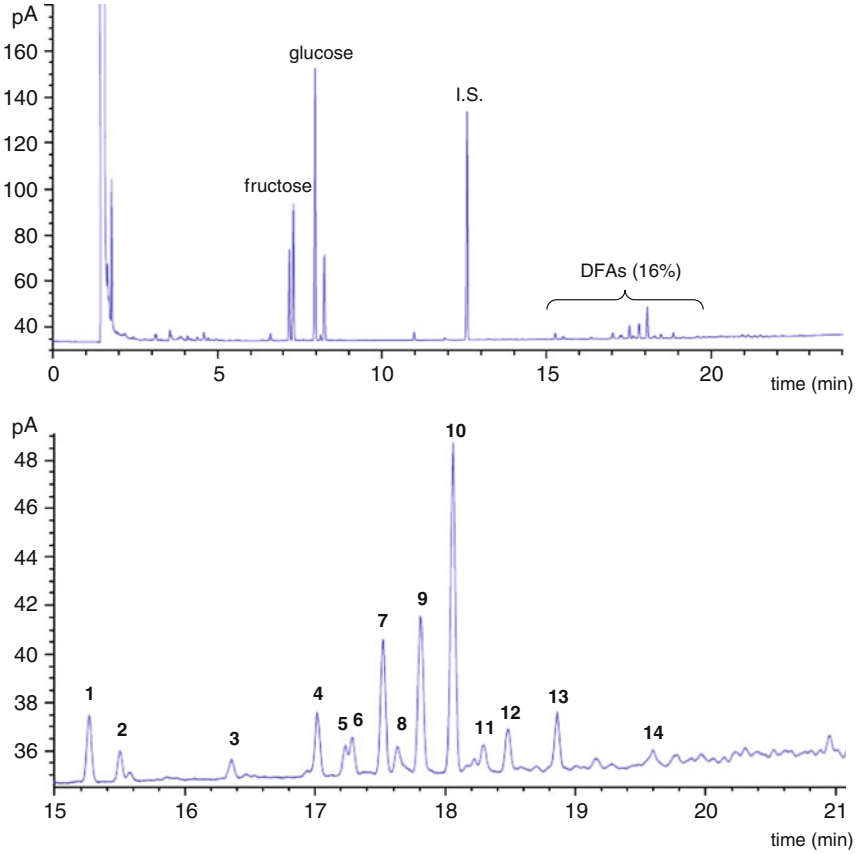


Fig. 4 GC chromatogram of a classical aromatic sucrose caramel after oximation-trimethylsilylation. Peak assignment corresponds to structures in Fig. 1. Internal standard (I.S.) is phenyl β-D-glucopyranoside. See [85] for chromatographical conditions

STOC followed by GC led to the identification of a fraction consisting of disaccharides of which the major component was α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (**10**, DFA I). The presence of glycosylated-DFA I was also proven by methylation analysis, glycosylation occurring preferentially at the primary *O*-6 and *O*-6′ positions. The general structure **45** was proposed for the higher oligomers in STOC (Fig. 5).

After further re-examination of STOC, DFAs **1**–**13** were identified in the mixture. The di β-D-fructofuranose 1,2′:2,1′-dianhydride (**14**) was also probably present although it was not identified at that time. The same DFAs, excepting the mixed fructose–glucose dianhydride **8**, were found in the product of thermolysis of inulin in the presence of citric acid at 160–180°C. The relative abundance of the individual DFA isomers was significantly different to that encountered in STOC, however, with an increased proportion of DFAs **1**, **7** and **10**. The D-fructose–D-glucose mixed

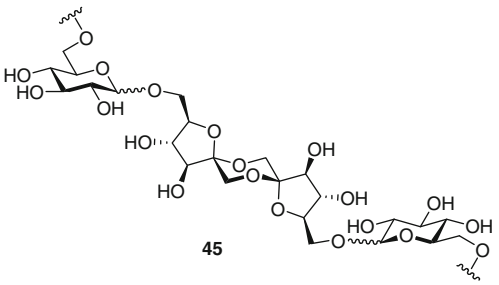


Fig. 5 General structure of the higher oligomers present in sucrose thermal oligosaccharide caramel (STOC)

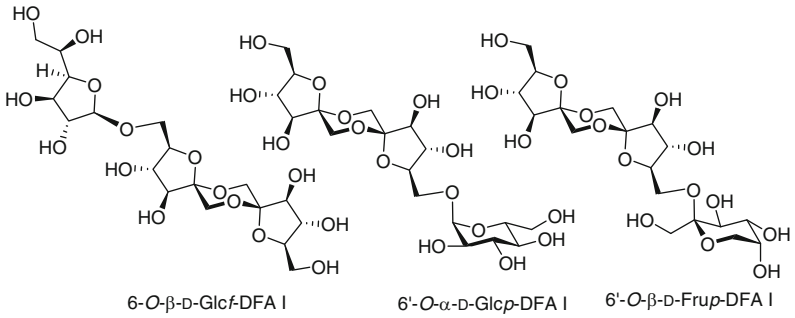


Fig. 6 Structures of the glycosyl-DFA trisaccharides isolated from the thermolysis products of sucrose (STOC) or inulin

dianhydride **8** was absent. Instead, the α-D-fructofuranose β-D-fructofuranose 1,2′:2,6′-dianhydride (**15**, DFA V) was encountered in the mixture after short pyrolysis times. A mechanism analogous to that depicted in Scheme 1 for the formation of DFAs from inulin by the action of inulin fructotransferase (IFT) was invoked to account for the experimental results [90].

The thermolysis products of sucrose and inulin both contained glycosyl-DFAs in the higher oligosaccharide fractions. Two trisaccharides having the DFA I core (**10**) were isolated from STOC and fully characterized by NMR, namely the 6-*O*-β-D-glucofuranosyl (6-*O*-β-D-Glc*f*-DFA I) and the 6′-*O*-α-D-glucopyranosyl (6′-*O*-α-D-Glc*p*-DFA I) derivatives. A trisaccharide incorporating a β-D-fructopyranosyl substituent at position *O*-6′ (6-*O*-β-D-Fru*p*-DFA I) was isolated by liquid chromatography (LC) from the pyrolysis product of inulin (Fig. 6) [89]. The presence of *O*-6,6′-diglucosylated derivatives of **10** in the product of citric acid condensation of isomaltulose (6-*O*-α-D-glucopyranosyl-D-fructofuranose, palatinose) has also been reported [91]. Their structures are identical to that of the tetrasaccharides obtained by the action of HF-based reagents on palatinose (see Scheme 3).

The high temperatures used in the pyrolysis methodology make difficult a strict control of the procedure and its reproducibility. A caramelization method allowing high conversions into DFAs with total control on the composition of the final product and technically appropriate for nutritional studies and food applications

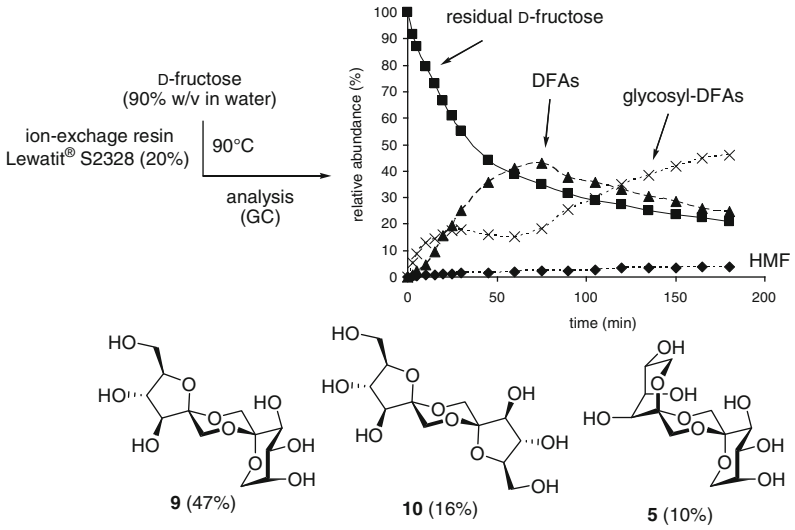


Fig. 7 Kinetics of D-fructose conversion after Lewatit S2328 catalyzed caramelization. The structures of the three major DFAs present in the final product after 2 h with indication of their relative proportions in the DFA fraction are depicted

was therefore highly desirable. Recently, Ortiz Mellet, García Fernández, and coworkers have developed a technology to produce caramels with a high content of DFAs and oligosaccharides thereof from commercial D-fructose based on the use of acid ion-exchange resins as caramelization promoters [92]. The kinetics of formation of these compounds as a function of D-fructose concentration, catalyst proportion, temperature, catalyst nature, and particle size has been investigated. The use of sulfonic acid resins, such as the strongly acidic, macroporous type resin Lewatit S2328, allows caramelization to be conducted at remarkable low temperatures (70–90°C). Dimerization to give DFAs, which can be further fructosylated to give glycosyl-DFAs, is a preferred pathway when highly concentrated D-fructose solutions in water (70–90%, w/v) are used, leading to conversions into DFA derivatives (ensemble of DFAs and glycosyl-DFAs) up to 70–80% in 1–2 h, with relatively low proportions of HMF (<2%).The relative abundance of individual DFA structures can be modulated by acting on the catalyst nature and reaction conditions, which offers a unique opportunity for nutritional studies of DFA-enriched caramels with well-defined compositions. In any case, the thermodynamic derivative **9** was the major DFA structure in the final caramel, accounting for about 50% of the total DFA fraction (Fig. 7), which is a remarkable difference as compared with the products obtained in the presence of citric acid. In this respect the DFA-enriched product obtained by ion-exchange resin-promoted caramelization of D-fructose resembles the DFA mixtures obtained by HF-based activation of D-fructose or inulin.

4.4 DFAs as Functional Foods

The available information on the nutritional properties of individual DFA isomers is limited to those representatives that can be produced by biotechnological processes, that is compounds **1** (DFA III), **10** (DFA I), and **15** (DFA IV). While the potential of DFAs as functional foods was soon realized, the initial reports of otherwise relevant results on their nutritional properties were included only in patents or published in national Japanese journals which are not always accessible. In all cases they are rated as low-caloric sweeteners; DAF III, for instance, has half the sweetness of sucrose and is chemically highly stable, with lower degradability and Maillard reactivity under acidic conditions than sucrose [93]. Anticariogenic and anti-tooth decaying effects have also been claimed. Moreover, these DFAs promote in vitro growth of bifidobacteria.

DFA III and DFA IV, for which technical processes of mass production are more advanced, have been shown to be non-digestible oligosaccharides that are utilized by intestinal microorganisms and are converted in organic acids such as acetate, butyrate, and lactate. These organic acids reduce the pH of cecal contents and increase the calcium solubility, which leads to enhanced calcium absorption [94]. More detailed analyses have been made of the effect of DFA III administration on the intestinal microbiota of humans and rats [95–98]. The DFA III-assimilating bacterium *Riminococcus productus* AHU1760 was isolated from subjects who ingested DFA III for 12 months. The results indicated that DFA ingestion tended to increase total organic acids in feces and to decrease fecal pH and the secondary bile acid (SBA) ratio in total bile acids. Since SBAs have been found to promote colon carcinogenesis and gallstone formation, it is concluded that DFA III may prevent colorectal cancer and be a new prebiotic candidate [97].

Both DFA III and DFA IV affect epithelial tissue junctions, thereby promoting calcium, magnesium, and zinc absorption in the small and large intestine of rats [99, 100]. α-D-Fructofuranose β-D-fructofuranose 1,2′:2,3′ dianhydride (**1**, DFA III) also prevents tannic acid-induced suppression of iron absorption and contributes to bone strength, thereby protecting against anemia [101] and preventing osteoporosis [102]. DFA III has been further proven to be an enhancer of the paracellular transport in the small intestine [103]. The increase in the tight junction permeability induced by this DFA results from alterations to F-actin and claudin-1 via Ca^{2+} independent mechanisms [104]. DFA III also promotes the availability of the quercetin glycoside αG-rutin (Fig. 8), a water soluble flavonoid glycoside with antioxidant activity with potential health benefits that is manufactured commercially [105, 106].

Reports on the nutritional properties of heterogeneous products with a high content in DFAs are still rather scarce. Acidic condensates of palatinose, which contain significant proportions of glucosyl-DFAs, were found to increase *Bifidobacteria* in the human intestine [107]. Some studies have revealed that addition of STOC at relatively low levels to the diet of chickens resulted in major increases in beneficial *Lactobacilli* and *Bifidobacteria* in the cecum. It also caused a major increase in growth rate and significant improvement in feed conversion [108].

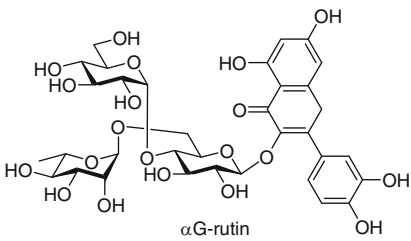


Fig. 8 Structure of the antioxidant flavonoid αG-rutin

More recently, it has been found that DFA-enriched products obtained by acid ion-exchange resin-promoted caramelization of fructose and containing about 70% of DFAs and glycosyl-DFAs promoted the development of a beneficial microflora in rats in vitro in a dose dependent manner, which is in agreement with the behavior of a classical prebiotic [109]. These caramels also exerted evident beneficial effects in protecting against colitis in a rat model for the human inflammatory bowel disease (Crohn disease), decreasing the macroscopic damage index in the colon and the levels of mieloperoxidase as compared with untreated animals. Notably, the effect was much higher than that obtained using pure DFA III, indicating that either the mixture contains more active components or they act in synergy to produce the observed beneficial effects. The protective effect against colitis by the DFA-enriched caramels is further reflected in a quite significant recovery of the populations of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria*. Similarly, this effect is higher for animals fed with DFA-enriched caramels as compared with animals fed with pure DFA III, especially for *Lactobacilli*. It was concluded that DFA-enriched caramels act by preventing colon damage and by recovering from colon damage, thereby behaving as a drug-like food, the definition of a nutraceutical.

5 Concluding Remarks

A much greater body of knowledge must be gained about the effects of ingesting DFAs, the possible synergies between different isomers, and their interactions with other food components. The lack of efficient production and purification methodologies and the limitations of the current analytical tools to investigate DFAs and glycosyl-DFAs in biological samples have been major limitations for nutritional and metabolomic approaches. During the last decade, major advances concerning the stereoselective synthesis of DFAs have been achieved that may significantly change the current status of these cyclodisaccharides in carbohydrate chemistry and biology. The increasing interest of the food industry in developing new functional products is motivating pure academic research in the field, which will certainly bring about new applications.

Expectations for commercial viability of DFAs are further sustained by the fact that the raw materials from which they can be obtained are readily available from

relatively inexpensive agricultural feedstocks. Sucrose, fructose, and oligosaccharides such as inulin or levan are abundant starting materials. Inulin and levan are storage carbohydrates in higher plants, such as Jerusalem artichoke or chicory and ryegrass or cocksfoot, respectively. Chicory, in particular, might eventually be considered to replace sugar beet in certain geographical areas where climate restrictions hampers the cultivation of the latter. Efficient large scale preparation of these oligosaccharides using sucrose industry technologies has been demonstrated. The possibility to produce DFA-enriched products by soft caramelization procedures compatible with food applications in the absence of additives opens further possibilities for commercial development.

References

1. Diplock AT, Aggett PJ, Ashwell M, Bornet F, Fern EB, Robertfroid MB (1999) Br J Nutr 81 (4 Suppl 1):S1
2. Gibson GR, Roberfroid MB (1995) Br J Nutr 125:1401–1412
3. Gibson GR, Probert HM, van Loo J, Rastall RA, Roberfroid M (2004) Nutr Res Rev 17:259–275
4. Pszczola DE (1992) Food Technol 46:77–79
5. Lomax AR, Calder PC (2009) Br J Nutr 101:633–658
6. Hugenholtz J, Smid EJ (2002) Curr Opin Biotechnol 13:497–507
7. Fooks LJ, Fuller R, Gibson GR (1999) Int Dairy J9:53–61
8. Delzene NM (2003) Proc Nutr Soc 62:177–182
9. Bounik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourie B, Brouns F, Bornet F (2004) J Nutr 80:1658–1664
10. Zentec J, Marquart B, Pietrzak T (2002) J Nutr 132:S1682–S1684
11. Olano-Martín E, Williams MR, Gibson GR, Rastall RA (2003) FEMS Microbiol Lett 218:101–105
12. Morgan AJ, Mul AJ, Beldman G, Voragen AGJ (1992) AGRO Food Industry High Technol Nov/Dec 3:35–38
13. Campbell JM, Fahy GC, Wolf BW (1997) J Nutr 127:130–136
14. Macfarlane GT, Steed H, Macfarlane S (2008) J Appl Microbiol 104:305–344
15. Gattuso G, Nepogodiev SA, Stoddart JF (1998) Chem Rev 98:1919–1958
16. McDonald EJ (1946) Adv Carbohydr Chem 2:53–277
17. Manley-Harris M, Richards GN (1997) Adv Carbohydr Chem Biochem 52:207–239
18. García-Moreno MI, Benito JM, Ortiz Mellet C, García Fernández JM (2008) Molecules 13:1640–1670
19. Defaye J, García Fernández JM (1994) Carbohydr Res 256:C1–C4
20. This H, Rutledge D (2009) Anal Bional Chem 394:659–661
21. This H (2009) Acc Chem Res 42:575–583
22. McNaught AD (1996) Pure Appl Chem 68:1919
23. Shlubach HH, Knoop H (1993) Justus Liebigs Ann Chem 504:19–30
24. Strepkov SM (1958) Zh Obshch Khim 28:3143–3147
25. Uchiyama T (1983) Agric Biol Chem 47(2):437–439
26. Li H-Y, Hagiwara H, Zhu W, Yokoyama C, Harada N (1997) Carbohydr Res 299:301–305
27. Uchiyama T, Niwa S, Tanaka K (1973) Biochim Biophys 315:412–420
28. Tanaka K, Uchiyama T, Ito A (1972) Biochim Biophys 284:248–256
29. Kawamura M, Uchiyama T (2007) Chemistry and production of di-D-fructose dianhydrides. In: Norio S, Noureddine B, Shuichi O (eds) Recent advances in fructooligosaccharides research. Research Signpost, Kerala, India, pp 273–296

30. Uchiyama T (1975) Biochim Biophys Acta 397:153–163
31. Tanaka K, Kawaguchi H, Ohno K, Shohji K (1981) J Biochem 90:1545–1548
32. Tanaka K, Uchiyama T, Yamamuchi K, Suzuki Y, Hashiguchi S (1982) Carbohydr Res 99:197–204
33. Tanaka K, Karigane T, Yamaguchi F, Nishikawa S, Yoshida N (1983) J Biochem 94: 1569–1578
34. Defaye J, Gadelle A, Pedersen C (1985) Carbohydr Res 136:53–65
35. Chu YD, Shiau LD, Berglund KA (1989) J Crystal Growth 97:689–696
36. Wolfrom ML, Blair MG (1948) J Am Chem Soc 70:2406–2409
37. Nguyen SK, Sophonputtanaphoca S, Kim E, Penner MH (2009) Appl Biochem Biotechnol 158:352–361
38. Benito JM, Ortiz Mellet C, García Fernández JM (2005) Identificación y cuantificacción de azúcares resultantes de la dimerización de la sacarosa y de la D-fructosa (dianhídridos de fructosa) formados en la raíz de la remolacha o durante su procesado industrial. In: Echevarría C, Morillo-Valverde R (eds) Aspectos fisiológicos de la remolacha azucarera de siembra otoñal, 1st edn. Junta de Andalucía, Seville, Spain
39. García Fernández JM, Gadelle A, Defaye J (1994) Carbohydr Res 265:249–269
40. Defaye J, García Fernández JM (1994) Carbohydr Res 251:17–31
41. García Fernández JM, Defaye J (1992) Carbohydr Res 237:223–247
42. Defaye J, García Fernández JM (1994) Carbohydr Res 251:1–15
43. García Fernández JM, Defaye J (1992) Tetrahedron Lett 33(51):7861–7864
44. García Fernández JM, Ortiz Mellet C, Defaye J (1998) J Org Chem 63:3572–3580
45. Defaye J, Gadelle A, Pedersen C (1988) Carbohydr Res 174:323–329
46. García Fernández JM, Schnelle R-R, Defaye J (1995) Tetrahedron Asymmetry 6:307–312
47. Gerber-Lemaire S, Vogel P (2009) Carbohydr Chem 35:13–32
48. Takaoka LR, Buckmelter AJ, LaCruz TE, Rychnovsky SD (2005) J Am Chem Soc 127:528
49. Benito JM, Gómez-García M, Ortiz Mellet C, García Fernández JM, Defaye J (2001) Org Lett 3:549–552
50. Benito JM, Rubio EM, Gómez-García M, Ortiz Mellet C, García Fernández JM (2004) Tetrahedron 60:5899–5906
51. Balbuena P, Rubio EM, Ortiz Mellet C, García Fernández JM (2006) Chem Commun 2610–2612
52. Rubio EM, Ortiz Mellet C, García Fernández JM (2003) Org Lett 5(6):873–876
53. Rubio EM, García Moreno MI, Balbuena P, Lahoz FJ, Alvarez E, Ortiz Mellet C, García Fernández JM (2006) J Org Chem 71:2257–2266
54. Rubio EM, García Moreno MI, Balbuena P, Ortiz Mellet C, García Fernández JM (2005) Org Lett 7(4):729–731
55. Louis F, García Moreno MI, Balbuena P, Ortiz Mellet C, García Fernández JM (2008) Tetrahedron 64:2792–2800
56. Saito K, Tomita F (2000) Biosci Biotechnol Biochem 64:1321–1327
57. Seki K, Haraguchi K, Kishimoto S, Kobayashi S, Kainuma K (1988) Starch-Stärke 40: 440–442
58. Seki K, Haraguchi K, Kishimoto S, Kobayashi S, Kainuma K (1989) Agric Biol Chem 53:2089–2090
59. Kushibe S, Sashida R, Morimoto Y, Ohkishi H (1993) Biosci Biotechnol Biochem 57: 2054–2058
60. Haraguchi K, Yoshida M, Ohtsubo K (2003) Biotechnol Lett 25:1049–1053
61. Haraguchi K, Yoshida M, Yamanaka T, Ohtsubo K (2003) Carbohydr Polym 53:501–505
62. Yokota A, Hirayama S, Enemoto K, Miura Y, Takao S, Tomita F (1991) J Ferment Bioeng 72:258–261
63. Yokota A, Enemoto K, Tomita F (1991) J Ferment Bioeng 72:262–265
64. Kikuchi H, Inoue M, Saito H, Sakurai H, Aritsuka T, Tomita F, Yokota A (2009) J Biosci Bioeng 107(3):262–265

65. Haraguchi K, Yoshida M, Ohtsubo K (2005) Carbohydr Polym 59:411–416
66. Haraguchi K, Yoshida M, Ohtsubo K (2006) Carbohydr Polym 66:75–80
67. Cha J, Park NH, Yang SJ, Lee TH (2001) J Biotechnol 91:49–61
68. Jang K-H, Ryu E-J, Park B-S, Song K-B, Kang SA, Kim CH, Uhm T-B, Park Y-I, Rhee S-K (2003) J Agric Food Chem 51:2635–2636
69. Jang K-H, Jang E-K, Kim SH, Kim I-H, Kang SA, Koh I, Park Y-I, Kim Y-J, Ha -D, Kim CH (2006) J Microbiol Biotechnol 16:102–108
70. Jang E-K, Jang K-H, Koh I, Kim I-H, Kim SH, Kang SA, Kim CH, Ha S-D, Rhee S-K (2002) J Microbiol Biotechnol 12:603–609
71. Takesue N, Sone T, Tanaka M, Tomita F, Asano K (2007) Enzyme Microb Technol 41: 673–676
72. Takesue N, Sone T, Tanak M, Tomita F, Asano K (2009) J Biosci Bioeng 107(6):623–629
73. Tomasik P, Palasinski M, Wiejak S (1989) Adv Carbohydr Chem Biochem 47:203–278
74. Queneau Y, Jarosz S, Lewandowski B, Fitremann J (2007) Adv Carbohydr Chem Biochem 61:217–292
75. Defaye J, García Fernández JM (1995) Zuckerindustrie 120:700–704
76. Defaye J, García Fernández JM (2000) Actual Chim Novembre:24–27
77. Oosterveld A, Voragen AGJ, Schols HA (2003) Carbohydr Polym 54:183–192
78. Montilla A, Ruíz-Matute AI, Sanz ML, Martínez-Castro I, del Castillo MD (2006) Food Res Int 39:801–806
79. Waleckx E, Gschaedler A, Colonna-Ceccaldi B, Monsan P (2008) Food Chem 108:40–48
80. Speck JC Jr (1958) Adv Carbohydr Chem 13:63–103
81. Kroh LW, Jalyschko W, Häseler J (1996) Starch/Stärke 48:426–433
82. Jiang B, Liu Y, Bhandari B, Zhou W (2008) J Agric Food Chem 56:5138–5147
83. Simkovis I, Surina I, Vrican M (2003) J Anal Appl Pyrolysis 70:493–504
84. Antal MJ, Mok WSL, Richard GN (1990) Carbohydr Res 199:91–109
85. Ratsimba V, García Fernández JM, Defaye J, Nigay H, Voilley A (1999) J Chromatogr A 884:283–293
86. Ruíz-Matute AI, Soria AC, Martínez-Castro I, Sanz ML (2007) J Agric Food Chem 55: 7264–7269
87. Manley-Harris M, Richards GN (1994) Zuckerindustrie 114:924–928
88. Manley-Harris M, Richards GN (1994) Carbohydr Res 254:195–202
89. Manley-Harris M, Richards GN (1996) Carbohydr Res 287:183–202
90. Christian TJ, Manley-Harris M, Field RJ, Parker BA (2000) J Agric Food Chem 48: 1823–1837
91. Tanaka M, Nakajima Y, Nishio K, Hashimoto H (1993) J Carbohydr Chem 12:49–61
92. Suárez-Pereira E, Rubio EM, Pilard S, Ortiz Mellet C, García Fernández JM (2010) J Agric Food Chem 58:1777–1787
93. Rechner AR, Wagner E, Van Buren L, Van De Put F, Wiseman S, Rice-Evans CA (2002) Free Radical Res 36:1127–1135
94. Saito K, Hira T, Suzuki T, Hara H, Yokota A, Tomita F (1999) Biosci Biotechnol Biochem 63:655–661
95. Minamida K, Sujaya IN, Tamura A, Shigematsu N, Sone T, Yokota A, Asano K, Benno Y, Tomita F (2004) J Biosci Bioeng 98:244–250
96. Minamida K, Shiga K, Sujaya IN, Sone T, Yokota A, Hara H, Asano K, Tomita F (2005) J Biosci Bioeng 99:230–236
97. Minamida K, Asakawa C, Sujaya IN, Kaneko M, Abe A, Sone T, Hara H, Asano K, Tomita F (2006) J Biosci Bioeng 101:149–156
98. Minamida K, Kaneko M, Ohashi M, Sujaya IN, Sone T, Wada M, Yokota A, Hara H, Asano K, Tomita F (2005) J Biosci Bioeng 99:548–554
99. Hara H, Kondo K (2005) Biosci Biotechnol Biochem 69:839–841
100. Mineo H, Amano M, Chiji H, Shigematsu N, Tomita F, Hara H (2004) Dig Dis Sci 49: 122–132

101. Afsana K, Shiga K, Ishizuka S, Hara H (2003) J Nutr 133:553–3560
102. Shiga K, Hara H, Okano G, Ito M, Minami A, Tomita F (2003) J Nutr 133:4207–4211
103. Mineo H, Hara H, Shigematsu N, Okuhara Y, Tomita F (2002) J Nutr 132:3394–3399
104. Suzuki T, Hara H (2006) Life Sci 79:401–410
105. Matsumoto M, Matsukawa N, Chiji H, Hara H (2007) J Agric Food Chem 55:4202–4208
106. Matsuwaka N, Matsumoto M, Chiji H, Hara H (2009) J Agric Food Chem 57:1498–1505
107. Kashimura J, Nakajima Y, Benno Y, Endo K, Mitsuoka T (1989) Bifidobacteria Microflora 8:45–50
108. Orban JI, Patterson JA, Surron AL, Richards GN (1997) Poultry Sci 76:482–490
109. Rubio Castillo EM, Gómez García M, Ortiz Mellet C, García Fernández JM, Zarzuelo Zurita A, Gálvez Peralta JJ, Duval R (2007) Novel caramels with a high prebiotic oligosaccharide content, ES Patent WO2008107506

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Development of Agriculture Left-Overs: Fine Organic Chemicals from Wheat Hemicellulose-Derived Pentoses

Frédéric Martel, Boris Estrine, Richard Plantier-Royon, Norbert Hoffmann, and Charles Portella

Abstract This review is dedicated to wheat hemicelluloses and its main components D-xylose and L-arabinose as raw materials for fine organic chemistry. The context of the wheat agro-industry, its by-products, and extraction and hydrolysis of hemicelluloses to produce the pentoses are considered. The straightforward preparation of pentose-based surfactants, their properties, and their situation in the field of carbohydrate-based surfactants are addressed. Multistep transformations of pentoses are also described, first from a methodology point of view, with the aim of producing multifunctional enantiopure building-blocks, then considering targeted natural and/or bioactive products. Selected reactions of furfural, an important dehydration product of pentoses, are also presented.

Keywords D-Xylose, Enantiopure building-blocks, Furfural, L-Arabinose, Surfactants

Contents

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Abbreviations

δO/W	Oil/water interfacial tension
APG	Alkyl polyglucosides
APP	Alkyl polypentosides
CMC	Critical micelle concentration
DP	Degree of polymerization
D-Xyl	D-Xylose
GAG	Glycosaminoglycan
HLB	Hydrophilic–lipophilic balance
L-Ara	L-Arabinose
MOE	Modulus of elasticity
MOR	Modulus of rupture
MTHF	Methyltetrahydrofuran
RCM	Ring-closing metathesis
GAG	Glycosaminoglycan

1 Introduction

Wheat farming produces two abundant by-products, straw and bran, the dry matter of which contains more than 25% of hemicellulose, a heteropolysaccharide made up mainly of two aldopentoses: D-xylose, and L-arabinose (Fig. 1). These two 4-epimeric sugars are obtained by hydrolysis of hemicellulose. They constitute polar and enantiopure synthons used in the synthesis of surfactants and fine organic chemicals. A third important synthon, furfural, results from dehydration of pentoses. Hemicelluloses are the second most abundant natural organic chemicals next to cellulose.

We present here an overview of production of these three synthons and their transformations into a wide range of products, from surfactants to multifunctional building-blocks, natural and bioactive products.

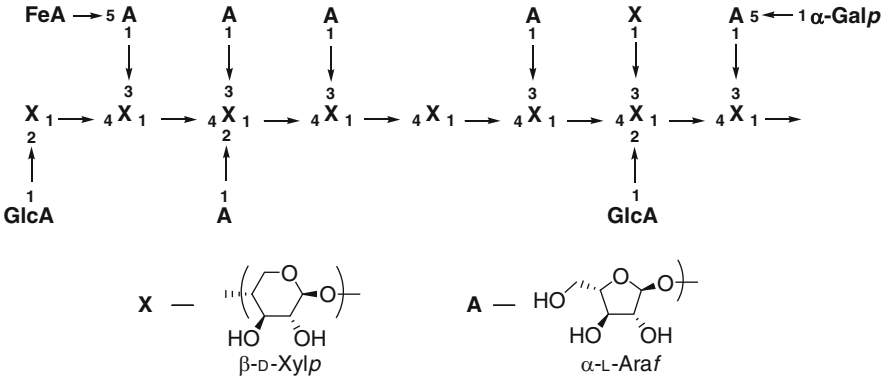


Fig. 1 Simplified structure of wheat hemicelluloses (see Fig. 2 for FeA, GlcA*p*, α-Gal*p*)

2 Hemicelluloses: From Wheat Bran and Straw to Pentoses

2.1 Wheat: Composition, Transformation and By-Products

Wheat is the second most produced cereal in the world, after corn [1]. It represents 28% of cereal grains (Table 1). Wheat is the most produced grain in the European Union, with 151 million tons being produced in 2008, well ahead of the production of 63 million tons of maize. The soft wheat *Triticum aestivum* represents 95% of wheat production. Its lower protein content (8–10 wt%) distinguishes it from hard wheat (*Triticum durum*), which contains more gluten (over 12% by weight), and is responsible for its technological qualities (production of pasta and semolina). In the European Union, wheat is exported (27% of the production), used for animal feed (44%), for seed (3%), for bio-ethanol (1%), and in the milling, starch, stationery, chemical, and pharmaceutical industries (25%). These developments require the separation of the different components of wheat.

All cereal grains contain three parts: the germ, the kernel or endosperm or floury endosperm, and envelopes and integuments forming the bran. The germ is the body of the future development of seedlings, and accounts for 2–3% of the grain. It is rich in oil and protein. The endosperm consists mainly of starch (80%) and proteins (12%). It is the reserve of the seedling and constitutes 78–80% of the grain. Finally, the bran is the protective covering of the grain, and represent between 15 and 20% of the mass of wheat grain. These three parts are separated in the mill. According to the efficiency of the mill, the bran may contain more or less flour, starch, and gluten. Thus, the composition of wheat bran is variable (Table 2).

Wheat bran is mostly used today in feed, and rarely for nonfood applications. The quantity of bran produced in the European Union represents more than 7 million tons per year. Straw, another coproduct of wheat processing, also has strong potential tonnages [2]; it contains a similar amount of hemicellulose as wheat

Table 1 World's production of cereals (2008–2009) in millions of tons

Corn	789	32.2%
Wheat (soft and hard)	687	28.2%
Rice (paddy)	650	26.7%
Barley	133	5.5%
Sorghum	55	2.3%
Millet	30	1.2%
Oats	30	1.2%
Rye	25	1.0%
Other (triticale, millet, spelt, etc.)	38	1.7%
Total	2,432	100.0%

Table 2 Composition of wheat bran

Hemicelluloses	27–31%
Starch	18–20%
Proteins	15–17%
Cellulose	11–13%
Minerals	6–7%
Fat	4.5–5.5%
Lignin	4–5%

bran [3]. Each hectare of cultivated wheat produces between 2 and 4 tons of straw. Straw is used in Europe as an organic soil (≥45%), as litter or fodder (≥50%), and as a source of bio-energy.

2.2 Hemicelluloses: Composition, Extraction, Hydrolysis

Hemicelluloses are macromolecules that are parietal components, characteristics of higher plants. They are short heteropolysaccharides (50–300 glycoside units). In the case of wheat bran, hemicelluloses are composed of linear chains of D-xylose linked by a β(1–4) glycosidic bond, and substituted by 33% of L-arabinose linked by α(1–3) and sometimes α(1–2) bonds. Other minor substituents are present: glucuronic, 4-*O*-methyl glucuronic, and galacturonic acids (α(1–2) ester linkage with D-xylose); D-galactose (α(1–3) linkage). Acetyl groups can also be attached to the hydroxyl group on C-3 of D-xylose [4]. Ferulic acid is esterified by the primary hydroxyl group of L-arabinose. An ether linkage between two ferulic moieties may constitute a bridge between two arabinoxylan chains. Similarly, ferulic acid can be etherified with lignin grafting covalently hemicelluloses to lignin (Fig. 2).

In Europe, the potential annual production of D-xylose and L-arabinose is 1.3 and 0.7 million tons, respectively. The wheat straw hemicelluloses have structures similar to those of wheat bran with only 5% of L-arabinose [5].

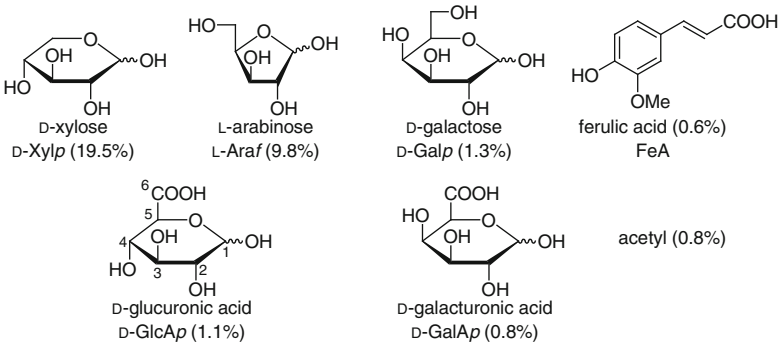


Fig. 2 Composition of hemicelluloses of wheat bran

To extract these pentoses there are different processes based on the nature of chemical bonds targeted: acid-catalyzed hydrolysis of the glycosidic bond, hot alkaline hydrolysis of ester bonds between chains, or hydrolysis with biological catalysts.

An acid hydrolysis process using sulfuric, hydrochloric, nitric, or phosphoric acids cleaves the glycosidic bonds [6]. The α(1–3) and α(1–2) bonds being more labile, hydrolysis releases first L-arabinose residues. Then the bonds in the xylan chains and with other substituents are hydrolyzed. The reaction products are mainly monomeric sugars. Hydrolysis is performed between 80 and 150°C for reaction times between 300 min and 20 min, respectively, and with concentrations in wheat bran of up to 150 g/L. The amount of acid used is 0.1–0.5 mol/L. Yields of hydrolysis range from 70 to 95% in sugars released.

Saponification in the presence of sodium, potassium, ammonium, or calcium hydroxides is performed between 40 and 100°C for 1–4 h. The amount of alkali used to treat 100 g/L wheat bran ranges from 0.5 to 2.0 mol/L. Saponification breaks mainly ester bonds with ferulic acid, which dissolves the chains of arabinoxylans. The yield of soluble hemicellulose is 20% [7] to 70% [8] if completed by hydrogen peroxide action. Complementary acid hydrolysis is required when monomeric sugars are targeted.

Biological processes involve the use of hydrolytic enzymes breaking β(1–4) linkages between two residues of D-xylose (endoxylanase, xylose oxidase), links α(1–3) and α(1–2) between D-xylose and L-arabinose (arabinofuranosidases), and ester bonds between ferulic acid and L-arabinose (feruloylesterases). Enzymes such as endoxylanase are now commercially available, but xylosidase, arabinofuranosidase, and feruloylesterases still are very marginal. The endoxylanase can hydrolyze xylans chains if steric hindrance is low [9]. It follows that the hydrolysis products are few because the xylans of wheat bran are highly substituted (one L-arabinose residue on average every two D-xylose residues). Without other enzymatic activities, hydrolysis leads only to very low solubility: 15–20% of hemicelluloses with endoxylanase (pH 5–6, 40–60°C, 100 g/L wheat bran for 24 h under gentle stirring). An amount of 50–200 mg of enzyme proteins is required for 100 g of

wheat bran. Biological processes may allow hemicelluloses separation from lignin, and help to separate and dissolve the isolated chains of arabinoxylans [10]. As for the alkaline saponification, biocatalytic processes should be supplemented by acid hydrolysis to obtain monomeric sugars.

The implementation of these reactions on an industrial scale requires a complex process of impregnation, contact, heating, separation, purification, and conditioning. An important aspect of the recovery of pentoses wheat bran is the need for very pure L-arabinose and D-xylose. The process begins by removing the starch from wheat bran to avoid releasing D-glucose on acid hydrolysis and its difficult separation from pentoses. To extract the starch, it is possible to perform multistep washing with cold water. The amount of water can be reduced by using hot water (60–80°C) or by enzymatic treatment with α-amylase. Washed bran is then subjected to acid, alkaline, or enzymatic hydrolysis, at concentration levels between 50 and 150 g/L substrate. The sugars released in the liquid phase can be separated from solid waste by centrifugal techniques or pressing, and then purified on ionic exchange resins in order to remove anions or cations coming from the hydrolysis step [11]. Colored compounds such as polyphenols are removed with activated carbon [12].

3 Pentoses-Derived Surfactants

Today, new surfactants have to be toxicologically safe and readily biodegradable [13]. In this context, carbohydrates represent a valuable feedstock [14].

Alkyl polyglucosides (APG) are nonionic surfactants produced from renewable raw materials such as fatty alcohols from vegetable oils and D-glucose from starch. They have become important as complements to ethoxylates [15]. Among these chemicals, butanol-based and decanol-based APG are used as hydrotropes in detergent formulation or as foam booster in toiletries and personal care products [16–20].

Pentose-based surfactants (APP for Alkyl PolyPentosides) are also gaining interest as efficient ingredients based on agriculture waste valorization (L-arabinose and D-xylose) [21–23].

3.1 Alkyl Polypentosides Based on Fischer-Type Glycosylation

Bertho et al. [23] have applied Fischer-type glycosylation and used several sources of hemicelluloses (Fig. 3).

The glycosidation of the reducing sugars obtained from hemicelluloses leads to the alkyl polypentosides surfactants. Glycosylation needs fatty alcohols as raw materials. Natural fatty alcohols derived, for example, from coconut or palm kernel oil are used in the alkyl polyglycoside synthesis to build up the hydrophobic part of the surfactants. These alcohols are used as blends obtained after transesterification and fractionation of oils leading to corresponding fatty acid methyl esters, and

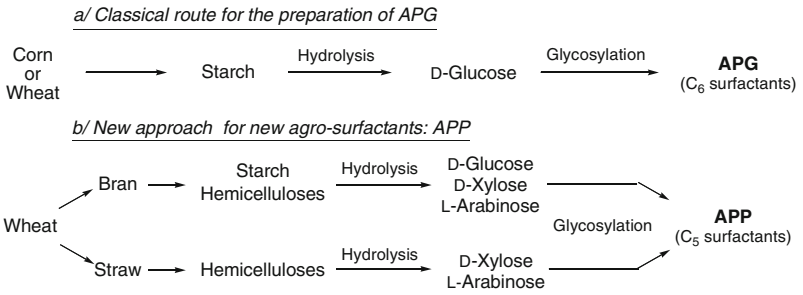
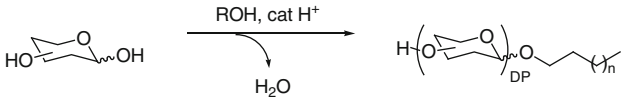


Fig. 3 Production of alkyl polyglucosides (APG) and alkyl polypentosides (APP)



Scheme 1 Fischer's glycosylation

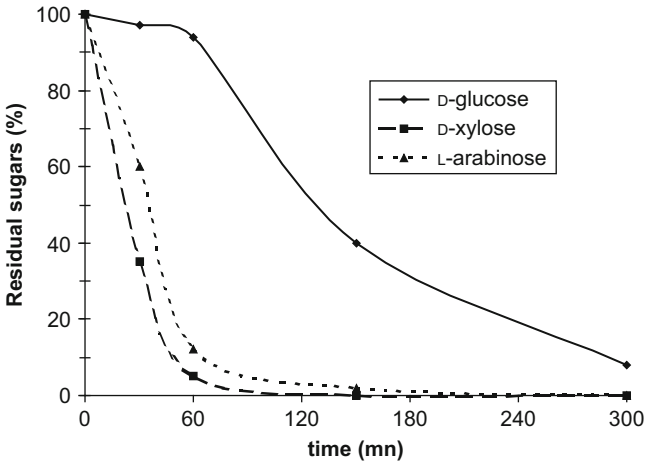
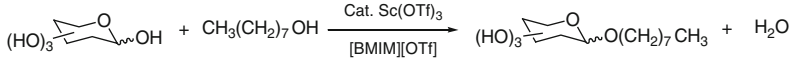


Fig. 4 Comparative glycosylation of pentoses and D-glucose at 80°C

subsequent hydrogenation. In contrast to esterification or telomerization, glycosylation is regioselective, and an oligomer mixture is formed due to coupling of more than one aldose unit to the alcohol (Scheme 1).

Fischer's glycosylation is now applied on an industrial scale for glucose-based and pentose-based alkyl polyglycosides [24]. It needs to be thermodynamically oriented by using aglycone excess at temperatures avoiding sugar degradation.

Pentoses are more reactive than glucose regarding the glycosylation reaction (Fig. 4). Preparation of APG usually requires operating over 120°C.



Scheme 2 Glycosylation of octanol under Lewis acid catalysis

Table 3 Glycosylation of octanol at 80°C under $Sc(OTf)_3$ catalysis

Carbohydrate	Time (h)	Isolated yield (%)	α/β
D-Glucose	24	44	79/21
D-Glucose[a]	24	74	75/25
D-Xylose	3	72	59/41
D-Xylose[a]	3	78	66/34

[a]Reaction performed in 1-butyl-3-methylimidazolium trifluoromethanesulfonate

In comparison, the grafting of "C5" sugars can be done under softer conditions (80°C), which reduce degradation of the starting materials and end products. This results in a cost effective process giving high quality products [25].

Augé and Sizun showed that octanol can be glycosylated in ionic liquids and in the presence of $Sc(OTf)_3$ as catalyst. In the case of D-xylose, the yield of glycoconjugation is good without the necessity to use ionic liquids (Scheme 2, Table 3) [26].

3.2 *Pentose-Based Surfactants from Palladium-Catalyzed Butadiene Oligomerization*

Pentose-based surfactants have also been obtained through Pd-catalyzed oligomerization of butadiene [27–29]. This aspect is developed in "Palladium-catalyzed telomerization of butadiene with polyols: from mono to polysaccharides" devoted to this type of products.

3.3 *Pentose-Based Surfactants: Properties, Applications, and Environmental Profile*

Another advantage of the pentoses glycosylation approach is the possibility to control polymerization degree in a broader range than with glucose derivatives. The average number of glycosidic units linked to an alcohol group (the degree of polymerization (DP)) is easily controlled for tailor made surfactant when pentoses are used as starting materials (Fig. 5) [28]. This is a very important characteristic with regard to physical and chemical properties and applications of alkyl polyglycosides (electrolytes tolerability, dynamic surface activity, foaming behavior, etc.).

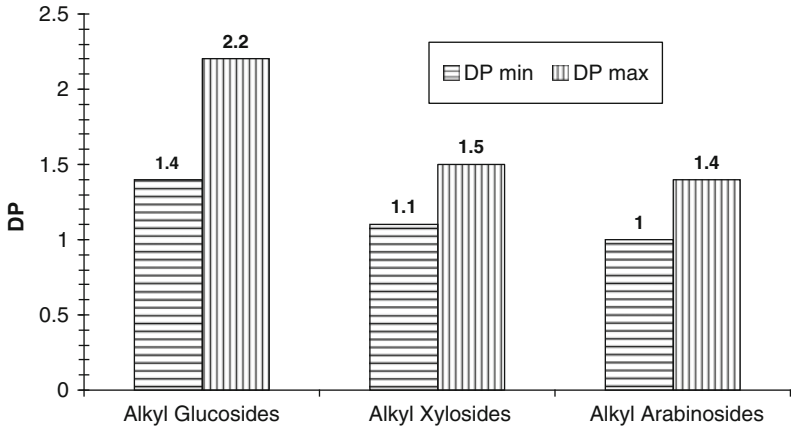


Fig. 5 Polymerization degree of alkyl polyglycosides

Table 4 Critical micelle concentration (CMC) and static surface (γ_{cm}) tension of alkyl glycosides with different head group and alkyl chain lengths

Surfactant	CMC (mg/L)	γ_{cmc} (mN/m)
2-(*E*)-7-Octadienyl β-D-xylopyranoside [30]	–	36
2-(*E*)-7-Octadienyl α-D-xylopyranoside [30]	500	31
2-(*E*)-7-Octadienyl α-L-arabinopyranoside [30]	180	34
Octyl α/β-D-glucoside [31]	7,632	26
Octyl β-D-glucopyranoside [31]	10,600	26
Decyl β-D-glucopyranoside [31]	994	26

The higher lipophilic nature of pentose-based surfactants mainly contributes to their lower aggregation concentration compared to their hexose equivalents when the lipophilic tail is unsaturated (Table 4). Lower CMC (Critical Micelle Concentration) gives a strong indication of the efficiency and pentose-based molecules behavior is a key advantage in the surfactant sector. Due to their unique physical and chemical behavior, pentose-based surfactants, and particularly APP have been exploited in concrete industrial applications where conventional alkyl polyglucosides are less successful [32]. The high wetting power of APP is also a key element in the formulation of highly effective cleaners [33]. Emulsifying properties of pentosides are better than conventional nonionic surfactants in terms of aggregation kinetics and unexpected synergies with major surfactants, even with cationics (when sanitizing properties are to be reached) [34].

Pentose-based surfactants are not considered as toxic or harmful in acute toxicity tests. APP also have uses in dishwashing and laundry detergents, cosmetics, and cleaning products which are discharged into domestic wastewater after use and thus enter the aquatic environment. It has been shown that pentose derivatives have good kinetics of biodegradation compared with conventional surfactants [35]. The production of pentose-based surfactants requires 37–41% less fertilizers and 36–57%

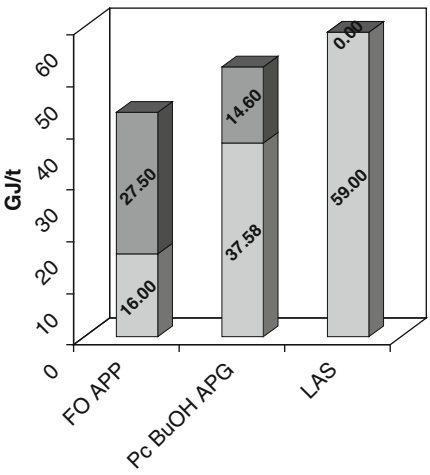


Fig. 6 Energy summary for the production of surfactants: short tail pentosides (FO APP: fusel oil alcohols APP) and glucosides (Pc BuOH APG: petrochemical Butanol APG) and linear alkyl benzene sulfonate (LAS)

less nonrenewable energy compared with the production of glucose-based surfactants (Fig. 6) [36].

4 D-Xylose and L-Arabinose as Starting Materials for Fine Organic Chemistry

D-Xylose, the second most abundant carbohydrate after D-glucose, is used to produce xylitol, a health-friendly food sweetener, which has also been recognized as one of the "Top value added chemicals from biomass" [37]. It is a source of rare sugars applying bioconversion methodologies [38].

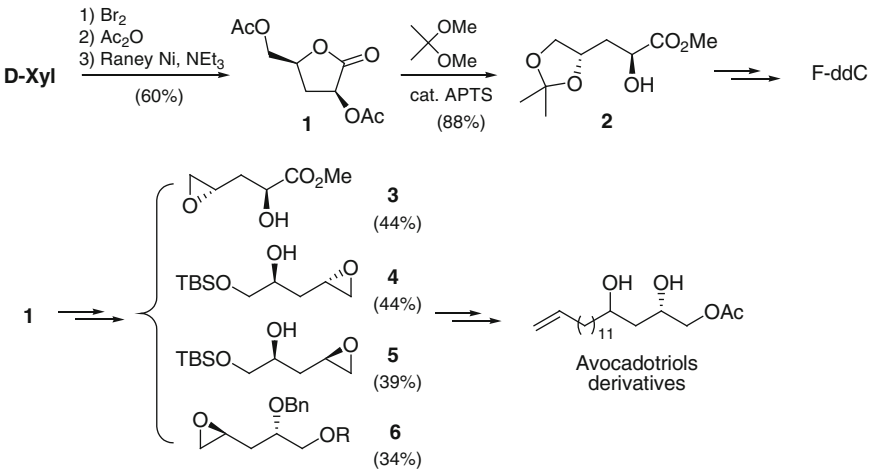
D-Xylose and L-arabinose are 4-epimers. This structural feature induces significant differences in their reactivity as for acid-catalyzed acetonation [39, 40].

Preparation of enantiopure multifunctional building-blocks and applications of D-xylose and L-arabinose in the synthesis of natural products and bioactive compounds are presented.

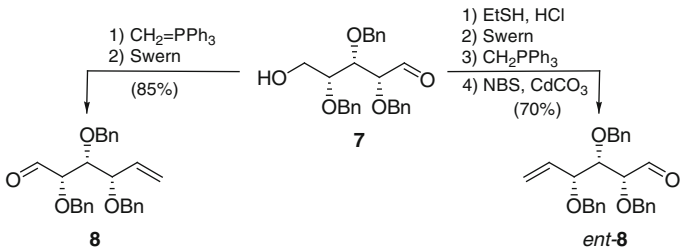
4.1 Enantiopure Building-Blocks

4.1.1 Masked 1,2,4-Triols

D-Xylose is converted in three steps into the 2,5-*O*-diacetyl 3-deoxy-D-erythropentono-1,4-lactone **1**, a versatile intermediate towards a series of 1,2,4-triol derivatives **2**–**6** [41–44]. These compounds are precursors in the synthesis of a fluoro analog of nucleoside (F-ddC from **2**) [41] and of avocadotriol derivatives (from **4** and **5**) [43] (Scheme 3).



Scheme 3 Conversion of D-xylose into D-*threo*-3-deoxy pentaldonic acid and 3-deoxypentitol derivatives



Scheme 4 D-Xylose: a compound with a latent plane of symmetry

4.1.2 D-Xylose: A Synthon with a Latent Plane of Symmetry

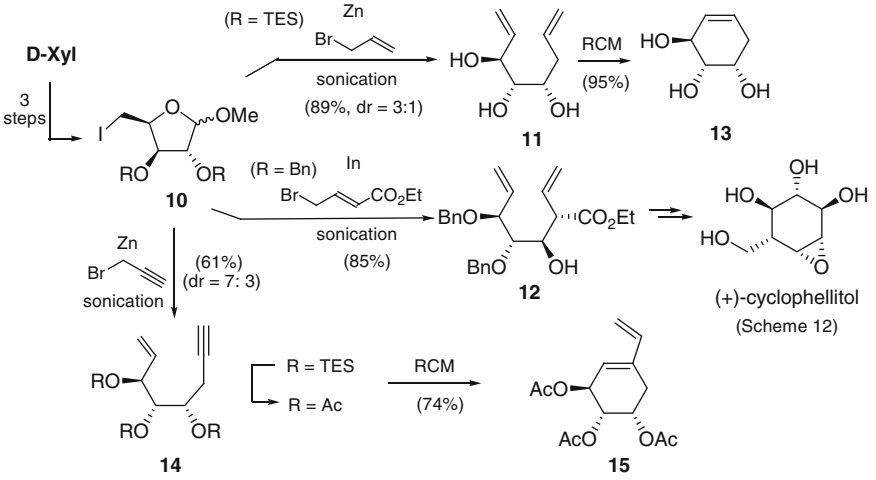
D-Xylose derivative **7** has a latent plane of symmetry, the terminal hydroxyl and carbonyl groups being virtually enantiotopic. With adapted reactions and a judicious order of performing them (Scheme 4), the two enantiomeric intermediates **8** and *ent*-**8** were prepared [45]. They are interesting starting materials for further diastereodivergent synthesis of conduritol and inositol derivatives [46].

4.1.3 Transformations of D-Xylose via Reductive Opening of a 5-Iodo-D-Xylofuranoside

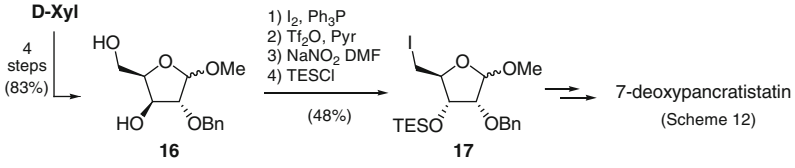
Carbohydrates have been starting materials for the synthesis of functionalized carbocycles for a long time [47–49]. More recently, ring-closing metathesis

(RCM) proved to be a tool of choice for such a purpose [50]. In three steps, D-xylose is converted into the 5-deoxy-5 iodo derivative **10**. Reductive ring opening using zinc (Bernet–Vasella type reaction [51–53]) leads to an enal which can be derivatized into the dienes **11** and **12** or an enyne **14** [54, 55] (Scheme 5). Interestingly, the diastereoselectivity of the reaction was reversed when indium was used in place of zinc, behavior which seems to be specific to the D-xylose derivatives. A high yielding RCM was performed using Grubbs 1 catalyst affording the trihydroxy cyclohexene **13** [54]. This strategy was applied to a short synthesis of (+)-cyclophellitol via the diene **12** [56]. Enyne metathesis of **14** (Grubbs 2 catalyst) produced diene **15** [57].

It may be necessary to differentiate the hydroxyl groups in positions C(2) and C(3) of pentoses for further synthetic purposes. According to Madsen, D-xylose is a better starting material than D-ribose for the synthesis of a 5-iodoribofuranoside having different protecting groups (Scheme 6) [58]. D-Xylose was converted in a high yielded four-step procedure into the intermediate **16**. Iodination and inversion of the alcohol at C(3) afforded the iodoribofuranoside **17** (Scheme 6), a precursor of 7-deoxypancratistatin (Scheme 11).



Scheme 5 Conversion of D-xylose into trihydroxycyclohexene derivatives

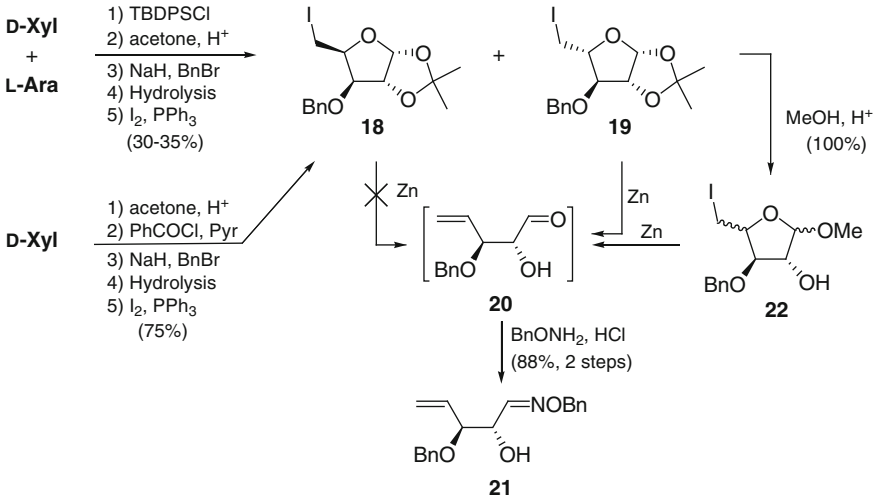


Scheme 6 Conversion of D-xylose to a 5-iodo-D-ribofuranoside

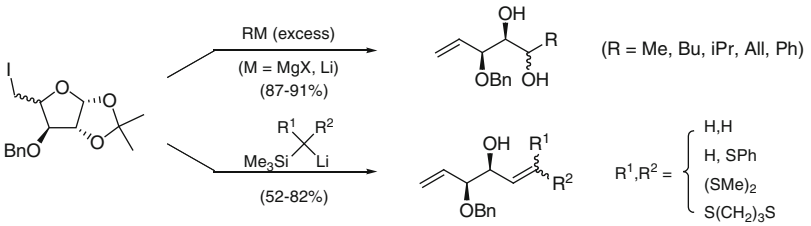
4.1.4 Use of Mixture of D-Xylose and L-Arabinose as Starting Materials

As D-xylose and L-arabinose are 4-epimers, both, as well as mixtures of them (as provided by hemicellulose), can be used to prepare enantiomerically pure synthetic intermediates the center C(4) of which is deoxy or sp^2-hybridized (alkenes, ketones). For instance, treatment of pure D-xylose with acetone under acidic conditions, followed by selective C(5) benzoylation, benzylation of the 3-alcoholic moiety, hydrolysis of the benzoate, and iodination furnishes the 5-iodo derivative **18**. L-Arabinose reacts differently than D-xylose with acetone and gives a pyranoside instead of a furanoside. Thus an alternative route to **18** was sought that can be applied both to D-xylose and L-arabinose [59]. It starts with the selective silylation of HO–C(5) [60], then acetonide formation protects alcohols moieties at C(1) and C(2). Subsequent benzylation of HO–C(3), hydrolysis of the silyl ether, and iodination provides **18** from D-xylose and **19** from L-arabinose (Scheme 7). Zinc reduction of **19** generates enal **20**, but not the reduction of **18** [61]. Thus **18** and **19** are converted first into their methyl furanosides **22**. The latter are reduced with Zn into **20**. Enal **20** is converted into oxime **21** [59].

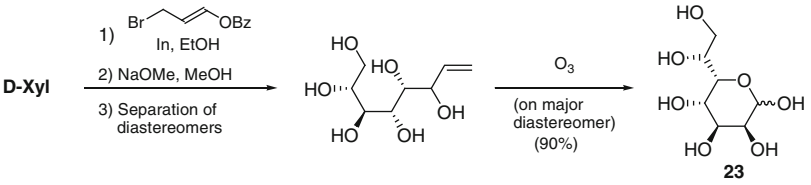
D-Xylose and L-arabinose can also be transformed via their iodofuranoside using halogen–metal exchange with an organomagnesium or organolithium reactant. A three-step domino process ending with a nucleophilic addition converts **18** into a 1-alkyl(aryl)pent-4-ene-1,2,3-triol with a moderate stereoselectivity (Scheme 8). Reactions of **18** with α-silylated organolithium reagents lead, via a four-step domino process ending with a Peterson type olefination, to a variety of multifunctional enantiopure 1,5-dienic building-blocks (Scheme 8) [62].



Scheme 7 Joint transformation of D-xylose and L-arabinose into (2*R*,3*R*)-3-benzyloxy-2-hydroxypent-4-enal



Scheme 8 Domino reactions of 5-iodofuranosides with organometallic reagents



Scheme 9 Indium mediated chain elongation of D-xylose

4.1.5 Chain Elongation

Based on a methodology (indium mediated allylation in protic media) adapted by Whitesides to the three carbon elongation of unprotected carbohydrate [63], including D-arabinose, Madsen has extended the methodology to elongation of D-xylose using 3-bromoprenyl esters. Ozonolysis of the terminal double bond finally affords D-*glycero*-L-*gulo*-heptose **23** [64]. The same strategy applied to L-arabinose would give L-*glycero*-L-*gulo*-heptose, according to the results reported for the D-analog (Scheme 9).

4.1.6 D-Xylose-Derived Glycodendrimers

Two series of large glycodendrimers containing terminal modified xylose residues were reported, recently. The first one, based on an organophosphorus-derived core [65], was obtained by condensation reaction from 4-hydroxyphenyl D-xylopyranoside. The third generation contains 48 xylose units (Fig. 7). The second glycodendrimer series was prepared by "click" cycloaddition coupling from 2′-azidoethyl 2,3,4-tri-*O*-acetyl-D-xylopyranoside [66] and alkynyl-terminated dendrimers [67]. This strategy allowed one to reach a large third generation dendrimer containing 243 terminal D-xylopyranoside residues (Fig. 7).

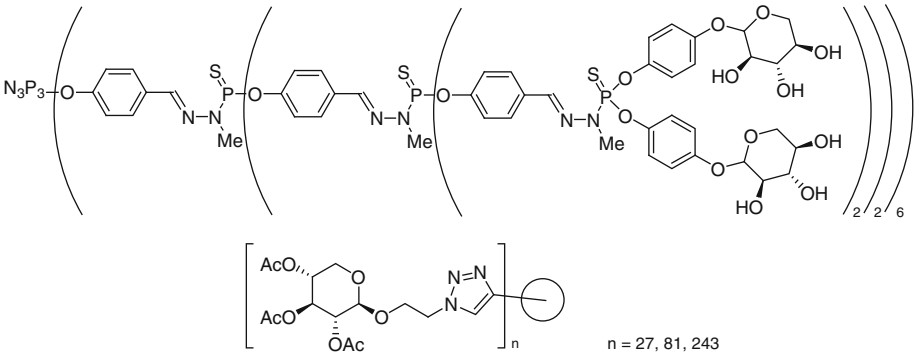


Fig. 7 D-Xylopyranoside-derived glycodendrimers

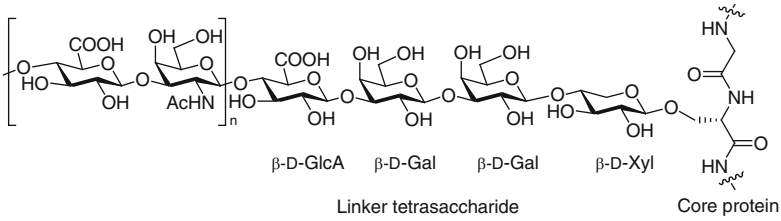


Fig. 8 Structure of proteoglycans

4.2 Synthesis of Compounds of Biological Interest

4.2.1 D-Xylosides as Primers of the Biosynthesis of Glycosaminoglycan Chains

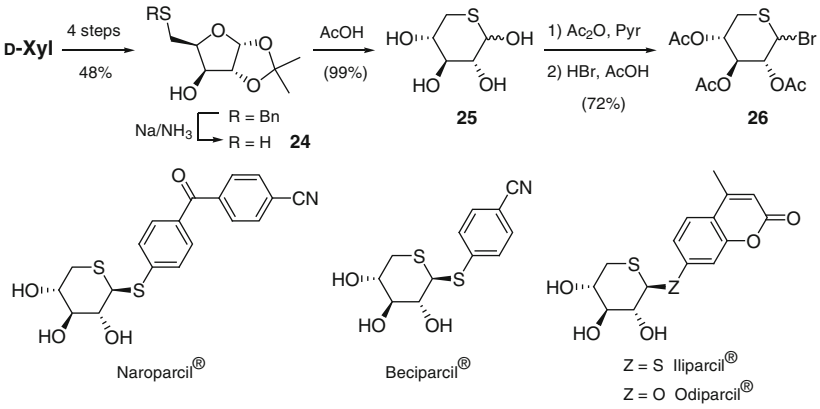
D-Xylose is unusual as a structural component in mammalian cells. It is only present as the linker between protein and carbohydrate in proteoglycans, a class of extracellular macromolecules composed of glycosaminoglycan (GAG) chains attached to a core protein. Biosynthesis of GAG chains starts with the formation of a glycosidic bond between a serine residue of the protein and the unique D-xylose of the GAG chain. Then a specific linker tetrasaccharide is synthesized and is used as the acceptor for the elongation of GAG chains (Fig. 8).

Interactions between GAG chains and various molecules or pathogens play many important biological functions. β-D-Xylopyranosides with a hydrophobic aglycon can penetrate the plasma membrane, initiate the first galactosylation step, and act as an exogenous primer for GAG biosynthesis. Hence, these xylosides have demonstrated interesting biological properties such as anti-thrombotic activity, preservation of human skin properties, and antiproliferative activity. Due to major

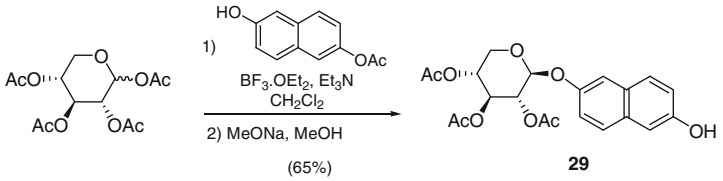
drawbacks of heparin therapy (intravenous mode of administration, hemorrhagic risk, etc.), an intensive search for new orally active antithrombotic drugs was developed by Laboratoires Fournier (now a Solvay Pharmaceuticals Company) in the last 20 years (for recent publications, see [68; 69–71]). A wide range of β-D-xylopyranosides with various hydrophobic aglycons were synthesized (Scheme 10). In vitro and in vivo studies have established that 5-thio β-D-xylopyranoside derivatives exhibited the best properties for the initiation of GAG biosynthesis and constituted a potential antithrombotic drug [72, 73]. Finally, 5-thio D-xylopyranosides with a substituted coumarin derivative as aglycon were found to be the most potent compounds. Within this series, Odiparcil® was evaluated in clinical trials in patients for the prevention of venous thromboembolism [74]. Recently, synthesis of new compounds with substituted pyridine derivatives as aglycons was investigated and preliminary bioassays showed a promising pharmacological profile [75, 76].

Recent studies have shown that GAG are essential components in the dermal matrix structure and that human aged skin contains less GAG than young skin. L'OREAL Recherche has recently developed Pro-Xylane™, a *C*-D-xylopyranoside which is an excellent inducer for the biosynthesis of sulfated GAG chains, currently developed in cosmetic skincare products [77–79] (see "Knoevenagel reaction of unprotected sugars; biological activity of Proxylan").

Previous studies have reported that β-D-xylopyranosides with 6-hydroxynaphthyl-based aglycons inhibit growth of tumor-derived cells due to the induction of heparane sulfate chains biosynthesis [80, 81]. Aromatic *O*-glycosylation is a difficult task due to several specific problems such as the weaker nucleophilicity and ambident reactivity of phenols [82]. The standard procedure was first achieved with a classical glycosylation reaction using 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose under $BF_3.OEt_2$ mediated conditions and a monoprotected 2,6-dihydroxynaphtalene in CH_2Cl_2 (Scheme 11) [83].



Scheme 10 Synthesis of 5-thio-D-xylose and some representative bioactive thioxylopyranosides



Scheme 11 General procedure for the preparation of 6-hydroxynaphthyl-β-D-xylopyranosides

A new synthetic route towards naphthyl xylosides was performed on solid support. Pure xylosides were obtained in 6–42% overall yield from the amino-methylated resin [84, 85]. Similarly, β-D-xylopyranosides bearing a fluorescent moiety as a probe for proteoglycan biosynthesis [83], bis-xylosylated dihydroxynaphthalenes [86] and 2-naphthyl-1-thio-β-D-xylopyranosides were also prepared and these compounds have exhibited a strong priming of GAG chains biosynthesis [87].

4.2.2 Synthesis of Carbocyclic Compounds from D-Xylose

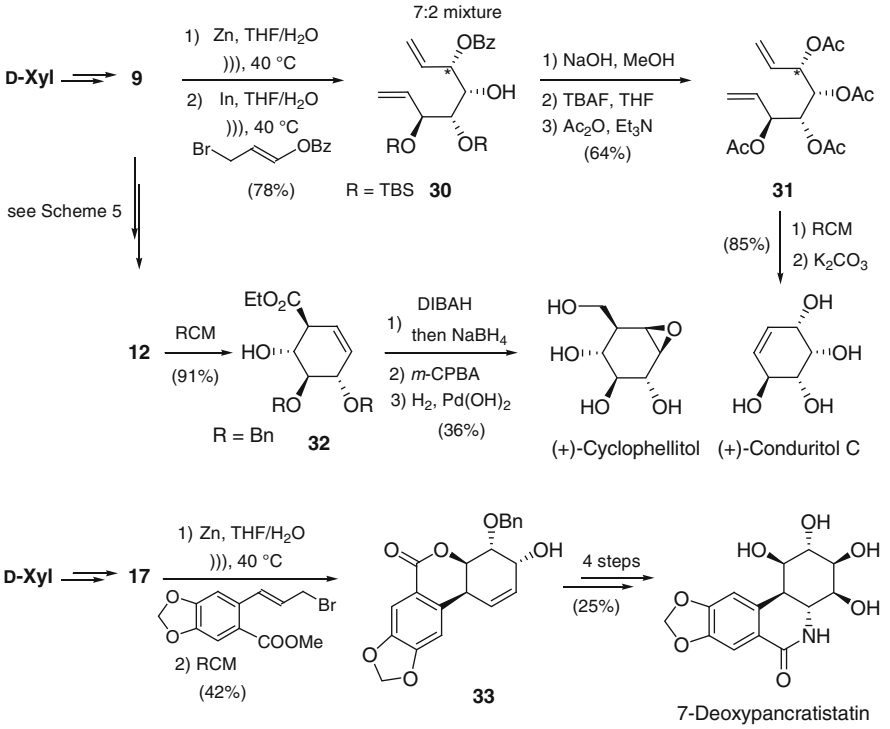
D-Xylose has been used as starting material in the synthesis of (+)-Conduritol C [88] and cyclophellitol [56] (Scheme 12). The combination of the zinc-mediated tandem reaction with RCM was also used in the synthesis of 7-deoxypancratistatin, a naturally occurring compound with activities against different cancer types. In the latter synthesis, the longest reaction sequence involves 13 steps from D-xylose with a 4% overall yield [58] (Scheme 12).

Transformation of aldehydes **8** and *ent*-**8** into conduritols and inositols was achieved in a few steps via dienes **34** and **35**. Conduritols B and F were easily obtained, after separation, by a RCM with the first-generation Grubbs' ruthenium catalyst [46]. Syntheses of *myo*-inositol and *chiro*-inositol were achieved in a few further steps in good yields. Similarly, both enantiomers of cyclophellitol were synthesized from 2,3,4-tri-*O*-benzyl-D-xylopyranose according to the same strategy [45] (Scheme 13).

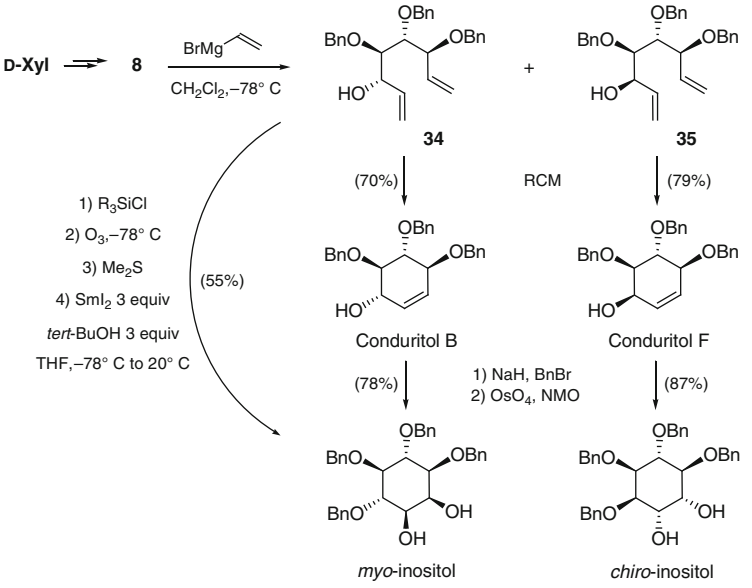
Another cyclization method towards synthesis of *myo*- inositols and *chiro*-inositols resulted from a SmI_2-promoted intramolecular pinacol reaction. Treatment of dienes **34** and **35** with ozone produced a dialdehyde after reduction of the ozonide with dimethyl sulfide. Subsequent SmI_2-mediated pinacol coupling gave the corresponding protected inositols as a mixture of diastereomers [89].

4.2.3 Synthesis of Sugar Analogs: Iminosugars and Thiosugars

Since the discovery of naturally occurring iminosugars in the 1960s, the interest in these sugar analogs has dramatically increased. These compounds have exhibited



Scheme 12 Syntheses of (+)-conduritol C, cyclophellitol and 7-deoxypancratistatin using the two-step strategy: reductive ring opening of 5-iodo-D-xylofuranoside and ring-closing metathesis

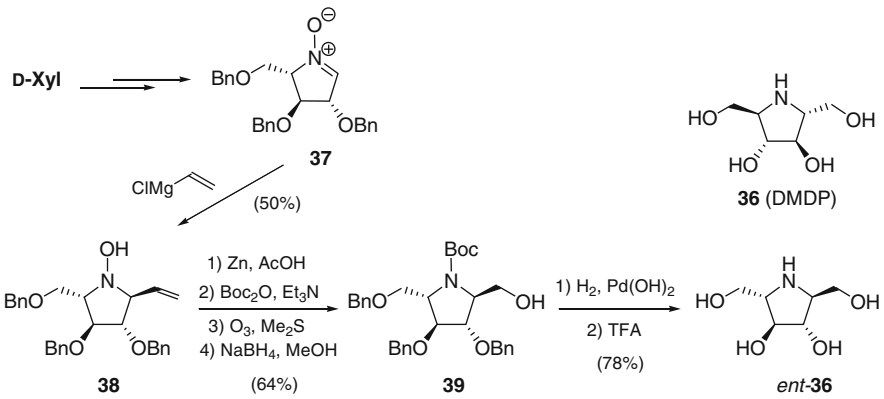


Scheme 13 Syntheses of benzylated derivatives of conduritol B, conduritol F, *myo*-inositol and *chiro*-inositol

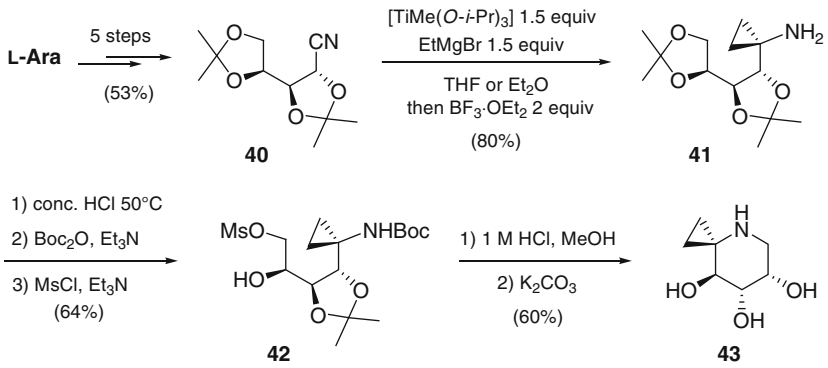
inhibitory activities against many enzymes of medicinal interest including glycosidases, glycosyl transferases, and metalloproteinases. These compounds are potential drugs in the treatment of diseases such as diabetes, cancers, or viral infections [90, 91].

An efficient and straightforward synthesis of isomers of DMDP **36** (2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine), a known naturally occurring glycosidase inhibitor, has been recently reported. D-Xylose is converted into nitrone **37** in a few steps. Vinylation of **37** gives hydroxyamine **38** which is reduced into the corresponding pyrrolidine with zinc. N-Protection, alkene ozonolysis, and reduction with $NaBH_4$ lead to compound **39**. Final deprotection gives 2,5-dideoxy-2,5-imino-L-mannitol (*ent*-**36**) (Scheme 14) [92].

Introduction of a spirocyclopropyl unit was investigated and a series of spirocyclopropyl-modified pyrrolidines and piperidines have been derived from L-arabinose (Scheme 15) [93–95]. Thus L-arabinose was converted into **40**,



Scheme 14 General synthetic approach toward the synthesis of the enantiomer of DMDP



Scheme 15 Synthetic route to a 5-spirocyclopropyl deoxyrhamnojirimycin from L-arabinose

precursor of cyclopropylamine **41**. Standard operations converted **41** into mesylate **42**. Intramolecular displacement of the mesylate by the deprotected cycloamine group afforded the 5-spirocyclopropyl 1-deoxyrhamnojirimycin **43**, a moderate inhibitor of L-fucosidase [96].

Recently, a new class of glucosidase inhibitors with an inner-salt sulfonium-sulfate was isolated from *Salacia reticulata*, a plant traditionally used in India and Sri Lanka for the treatment of diabetes (Fig. 9) [97–100].

Many syntheses of salacinol, its stereoisomers, and various analogs have been reported [101]. Total synthesis of salacinol and analogs was generally accomplished by the nucleophilic ring opening of a cyclic sulfate (L-*erythro* configuration) with a thiosugar (D-*arabino* configuration). For instance, thiosugar **46** was obtained in nine steps from D-xylose in 53% overall yield [102] (Scheme 16). An anomeric mixture (1:1) of methyl α- and β-D-xylopyranoside **44** was first prepared from D-xylose in five steps. After mesylation of the unprotected hydroxyl groups, treatment with sodium sulfide in DMF gave an anomeric mixture of compound **45**. Hydrolysis of the mixture with 4 N HCl followed by reduction of the resulting aldehyde and deprotection of the benzyl group led to the final thiosugar **46**.

For the preparation of new glycosidase inhibitors, modifications of the acyclic chain at the sulfur atom were investigated. Thus, cyclic sulfate **49** was synthesized from D-xylose in six steps [103, 104]. Using a judicious strategy for the protection–deprotection of the hydroxyl groups, D-xylose was easily transformed into the PMB-protected D-xylofuranose derivative **48**. After reduction to the corresponding xylitol by $NaBH_4$, treatment of the diol with thionyl chloride gave the cyclic sulfite, which was subsequently oxidized with sodium periodate and Ru(III) chloride as a catalyst to afford the cyclic sulfate **49**. Coupling with the thio-**50** and seleno-sugar

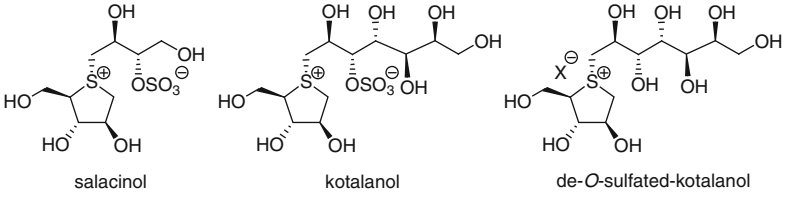
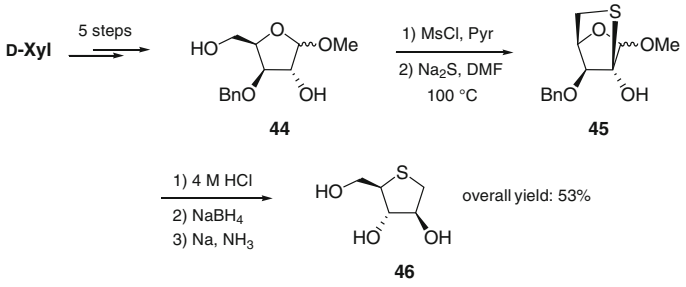


Fig. 9 Structures of bioactive thiosugar-derived sulfonium salts from *Salacia reticulata*



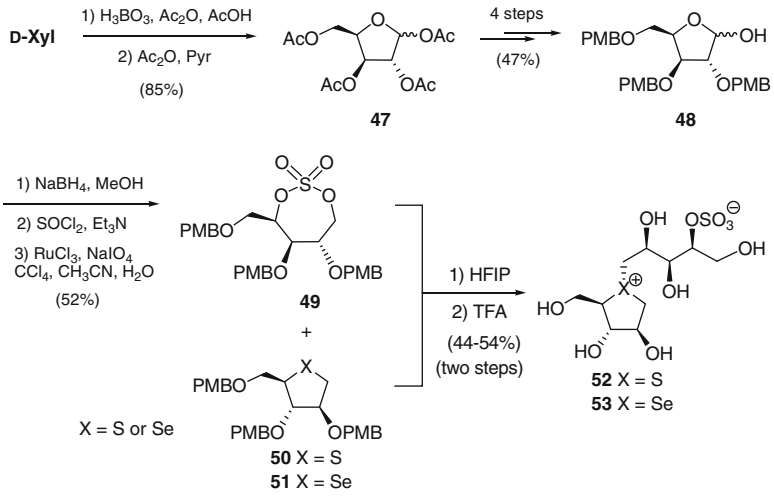
Scheme 16 Synthetic preparation of the thiosugar 1-deoxy-4-thio-D-arabinofuranose

51 (70°C, 1,1,1,3,3,3-hexafluoroisopropanol), followed by removal of the PMB groups with trifluoroacetic acid, provided the sulfonium **52** and selenonium derivatives **53** (Scheme 17).

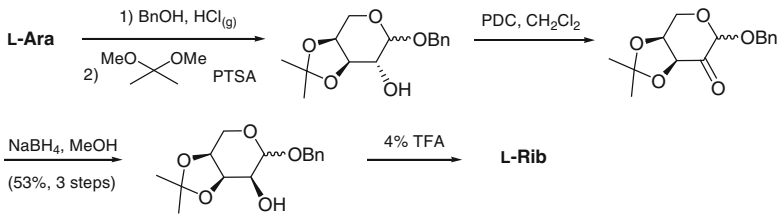
4.2.4 Synthesis of L-Nucleosides

L-Nucleosides represent a class of nucleoside analogs with an excellent profile for antiviral activity combined with minimal host toxicity [105]. Synthesis of new L-nucleosides can use L-ribose and 2-deoxy-L-ribose as starting materials. Synthetic preparations of L-ribose from natural epimeric L-aldopentoses such as L-arabinose or L-xylose has recently been reviewed [106]. The simpler approach used an oxidation–reduction at C-2 from L-arabinose as depicted in Scheme 18 [107, 108].

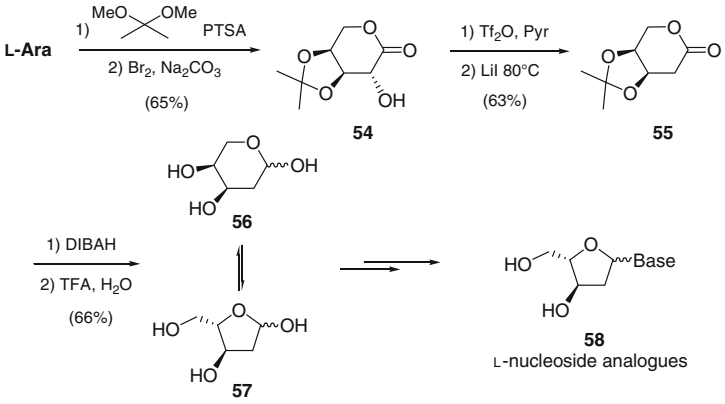
Most of the syntheses for an efficient preparation of 2-deoxy-L-ribose used L-arabinose as the starting material. Fleet and co-workers have developed a practical procedure for the synthesis of 2-deoxy-L-ribose from a readily available



Scheme 17 Synthesis of chain-modified analogs of glycosidase inhibitors salacinol and blintol



Scheme 18 Conversion of L-arabinose into L-ribose



Scheme 19 Conversion of L-arabinose into 2-deoxy-L-ribose

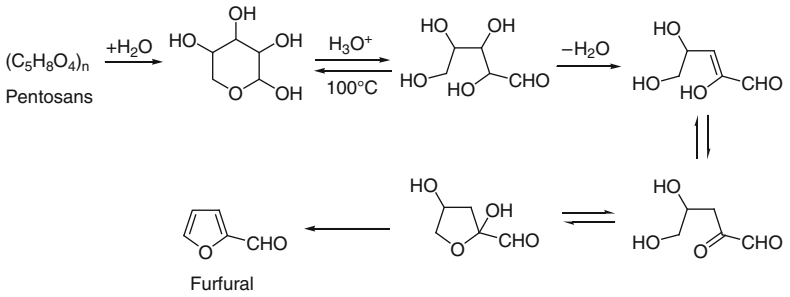
L-arabinopyranose derivative with the removal of the hydroxyl group at C-2 [109]. The monoacetonide of L-arabinopyranose obtained under kinetic control was oxidized into the corresponding 1,5-lactone **54** with bromine in dichloromethane. Deoxygenation at C-2 was achieved by treatment of the intermediate triflate with lithium iodide to give the lactone **55**. Reduction of **55** with DIBAH and subsequent deprotection of the isopropylidene group by acidic treatment gave 2-deoxy-L-ribose (**56** ⇌ **57**) in a good overall yield (Scheme 19), precursor of L-nucleosides and analogs **58**.

5 Chemistry of Furfural

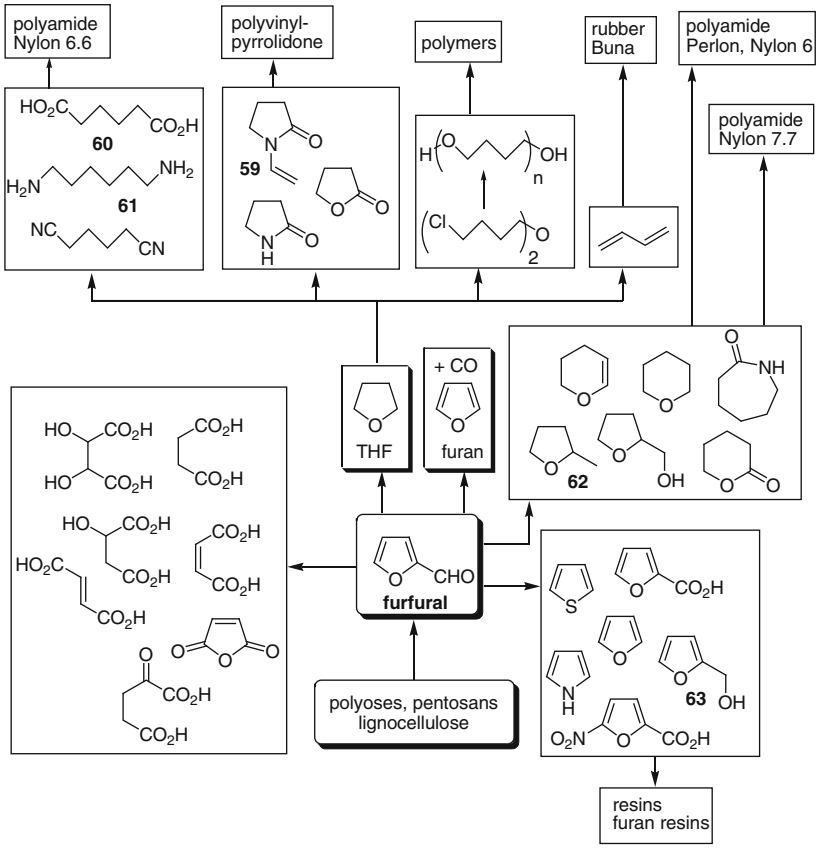
Furfural is easily produced from pentoses rich biomass by cyclodehydration. Various acidic reaction conditions have been applied [110, 111]. The precise order of the different dehydration steps is not known for certain. A plausible mechanism is depicted in Scheme 20 [112]. The worldwide production is currently about 300,000 tons per year. Thus, furfural is a low cost, polyfunctional substrate for the production of numerous bulk and fine chemicals.

5.1 Furfural as Starting Material for the Synthesis of Bulk Chemicals

Numerous applications of furfural as starting material for the production of bulk products have been reported (Scheme 21) [113]. Generally, two approaches can be distinguished. Products from biomass can be used to produce already existing



Scheme 20 Plausible mechanism of the acid-catalyzed formation of furfural



Scheme 21 Bulk products obtained from furfural

compounds obtained from fossil resources, mainly oil. Limited resources are thus replaced by renewable feedstock. The advantage of this approach for the chemical industry is that common installations can be used. An example is tetrahydrofuran

(THF) which can be transformed itself, for instance, into vinyl pyrrolidone **59**, a monomer for radical polymerization. Adipic acid **60** and 1,6-diaminohexane **61** Nylon 6,6 are also obtained from THF.

Methyltetrahydrofuran (MTHF) **62** possesses interesting solvent properties [114]. Many organometallic transformations work better in **62** than in Et_2O or THF. This solvent is also a good substitute for dichloromethane, for instance, in biphasic reactions.

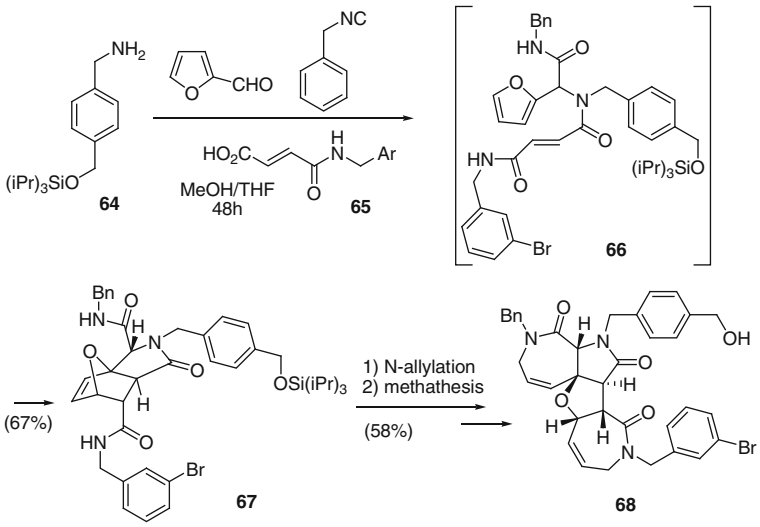
Reduction of furfural leads to furfuryl alcohol **63** which can be easily transformed into resins, thus replacing phenol formaldehyde resins (Scheme 21) [115]. A recent application concerns impregnation of wood (furfurylated wood). For instance, high retention of grafted or polymerized furfuryl alcohol improves a variety of properties such as an increased hardness, resistance to microbial decay and insect attack, as well as resistance to chemical degradation. Modulus of rupture (MOR), elasticity (MOE), and the dimensional stability are also significantly increased.

5.2 Furfural as Starting Material for Fine Chemicals

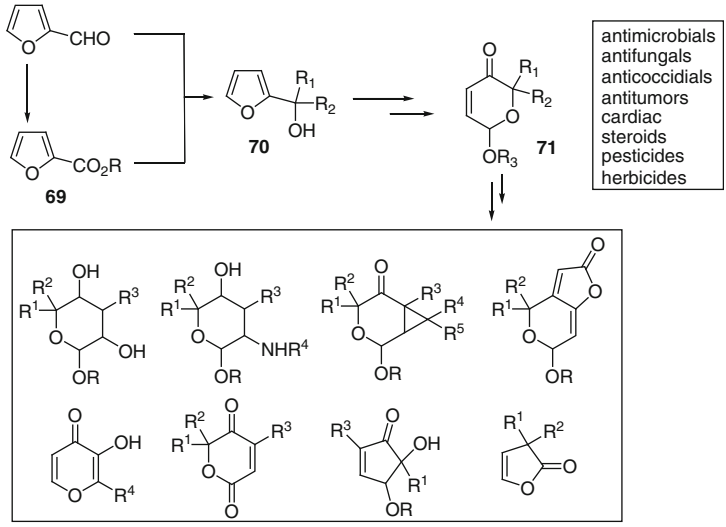
The chemistry of furfural is mainly determined by the aldehyde function and the heteroaromatic furan ring. Many chemical transformations are therefore similar to those of aromatic aldehydes. However, the aromatic character of the heterocycle is low when compared to isocyclic compounds such as benzene derivatives or other related heterocycles such as pyrroles and pyridines. Therefore, the furan ring also resembles to a diene which significantly enriches the chemistry of furfural [112]. Many applications of furfural to organic synthesis are reported in the literature [116]. Selected examples are described below.

The synergism of the two functional groups in chemical synthesis can be well demonstrated with multicomponent reactions [117]. For instance, the benzylamine derivative **64** reacts with furfural, benzylisonitrile [118] and fumaric derivative **65** (Ugi reaction) giving intermediate **66** which undergoes intramolecular Diels–Alder reaction producing stable adduct **67**. Two allyl functions are then introduced. Ring opening–closing metathesis using a ruthenium-based catalyst leads to the polycyclic product **68** (Scheme 22). The reaction sequence is an impressive example of diversity oriented synthesis [119, 120] in which the two functional groups of furfural (aldehyde, diene) are exploited.

Furfural can be oxidized into furfuroic acid that is esterified into esters **69**. Furfural and **69** react with organometallic reagents to generate substituted furfurols **70** (Scheme 23) [121–124] that can be transformed into hydroxypyranone derivatives **71** via an Achmatowicz rearrangement [121, 125–128]. Many of them can be transformed into other compounds of biological interest [129]. When the alcohol function in **70** is replaced by a protected amine the so-called aza–Achmatowicz reaction converts the latter into six-membered nitrogen-containing heterocycles [126, 130]. Due to a high density of functions in these products, they have been



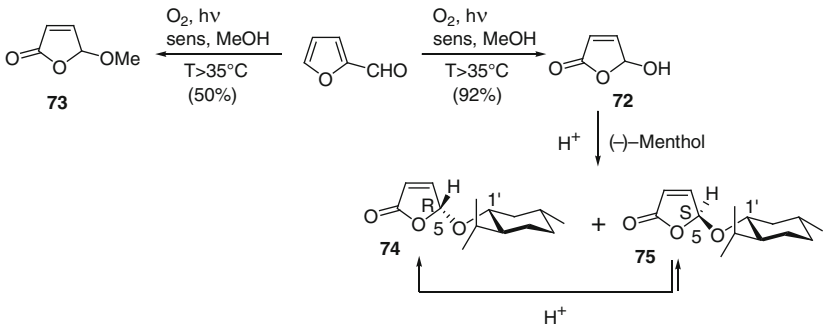
Scheme 22 Ugi reaction with furfural and subsequent intramolecular Diels–Alder reaction



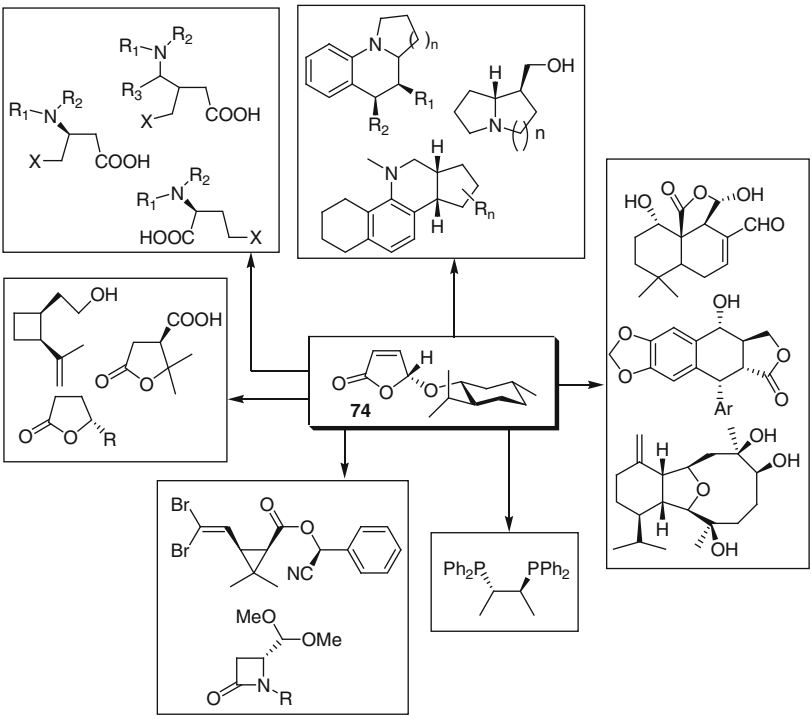
Scheme 23 Transformation of furfuryl alcohol derivatives into pyranones

used for the synthesis of a large variety of further nitrogen-containing heterocycles including alkaloids.

Photooxygenation of furfural [110, 131–137] gives either hydroxyfuranone **72** or corresponding alkoxyfuranone **73** depending on the reaction conditions (Scheme 24). Under acidic conditions (−)-menthol reacts with **72** giving a 1:1 mixture of diastereomeric acetals **74** and **75**. Acetal **74** crystallizes preferentially

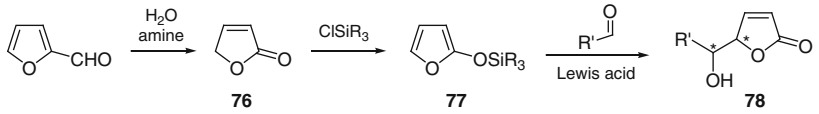


Scheme 24 Photooxygenation of furfural and synthesis of menthyloxyfuranone



Scheme 25 Synthetic applications of menthyloxyfuranone

and **75** can be equilibrated with **74** under acidic conditions [138–141]. Using (+)-menthol allows the preparation of *ent*-**74**. The latter acetal can also be prepared applying the Haarmann and Reimer (Symrise) process [142, 143]. Natural products, nitrogen-containing heterocycles, various amino acid derivatives, aroma, pheromones, chiral ligands for asymmetric catalysis, and products possessing a biological activity of pharmaceutical or agrochemical interest have been derived from **74** and *ent*-**74** (Scheme 25) [143–146].

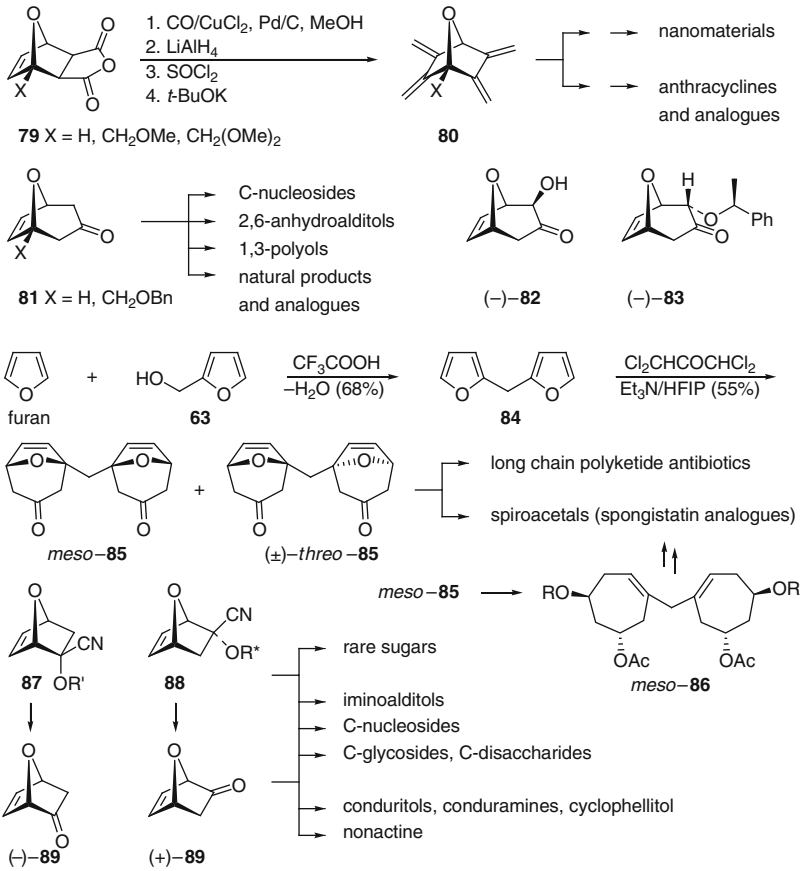


Scheme 26 Oxidation of furfural and synthesis of substituted furanones

Furfural is oxidized by H_2O_2 into furanone **76** (Scheme 26) [147–150]. Silyloxy furans **77** can be produced and transformed in a vinylogous Mukaiyama reaction into furanones **78** substituted at C(5) [150–153]. The reaction is carried out under Lewis acid catalysis, mainly with aldehydes. The relative configuration is controlled by the nature of the Lewis acid catalyst. The absolute configuration can be controlled by using different methods of asymmetric catalysis. These transformations have been intensively studied since the resulting lactones **78** possess various biological activities. Furthermore, they are partial structures of numerous natural products and also useful synthetic intermediates. Furfuraldehyde is readily converted into furfurylalcohol **63** by catalytic hydrogenation [154–161] or reaction with $NaBH_4$ [162, 163] or sodium dithionite [164, 165].

Like furfuraldehyde, **63** has been used to generate fine chemicals such as fragrant compounds [166] and enantiomerically pure synthetic intermediates for the preparation of products of biological interest (Scheme 27) [167–173]. Decarbonylation of furfural generates furan and carbon monoxide, two important industrial starting materials. The decarbonylation can be catalyzed by all kinds of catalysts [174–183]. Furan can be oxidized with air into maleic anhydride [184–187], an extremely valuable starting material for the industry. The Diels–Alder adducts **79** of maleic anhydride with furan (R=H) [188], methyl ether of furfuryl alcohol ($R=CH_2OMe$), and the dimethyl acetal of furfuraldehyde ($R=CH(OMe)_2$) [189, 190] have been converted into corresponding tetraenes **80** with high overall yields (64% in the case of R=H). The latter are useful synthons for the combinatorial preparation of linearly condensed polycyclic systems [191–201] (material sciences Molecular Lego [202]) including anthracyclines [203–209] as they react with dienophiles with a large reactivity difference between the addition of the first dienophile equivalent and the second. 8-Oxabicyclo[3.2.1] hept-6-en-3-ones **81** are obtained by [4+3]-cycloaddition of furans to 1,3-dibromo-2-oxyallyl cation generates followed by dehalogenation with Zn/Cu couple [210–219].

They are precursors in the synthesis of *C*-nucleosides [220–223] including enantiomerically enriched derivatives [224], 2,6-anhydroheptitols [225], 1,3-polyols [226–228], *C*-glycosides [229], thromboxane A2 analogs [230], muscarine and α-multistriatin analogs [231], β-alkoxy-δ-valerolactones relevant to natural products and drugs [232], hinokitiol [233], and GABA analogs [234]. Asymmetric deprotonation of **81** (XH) with (+)-bis[(*R*)-1-phenylethyl]amine, *n*-BuLi, and Et_3SiCl/Et_3N in THF gives the corresponding silyl enol ether that is oxidized with mCPBA in THF/H_2O into (−)-**82**, enantiomerically enriched precursors in



Scheme 27 Important synthetic intermediates derived from furan, furfuryl alcohol or/and furfuraldehyde

the synthesis of rare alditols and analogs [225]. Enantiomerically pure 3-oxo-8-oxabicyclo[3.2.1]oct-2-yl derivative (−)-**83** has been obtained by [4+3]-cycloaddition of furan with a chiral 1,2-dioxyallyl cation engendered in situ by acid-catalyzed heterolysis of enantiomerically pure mixed acetals derived from 1,1-dimethoxyacetone and enantiomerically pure 1-phenylethanol [235]. Dropwise addition of CF_3COOH to a mixture of furan and furfuryl alcohol **62** gives 2,2′-methylenebis (furan) **84** [236]. Double [3+4]-cycloaddition between 1,1,3-trichloro-2-oxyallyl cation and subsequent reductive work-up gives a 45:55 mixture of *meso*-**85** and (±)-*threo*-**85** (55% yield) that are separated readily by fractional crystallization. The *meso* compound was converted into *meso*-**86**, which was desymmetrized into a diol by Sharpless asymmetric dihydroxylation [237]. Further standard transformations permit the generation of all possible semi-protected 15-carbon polyketides

[238, 239] and of the polyol subunit of the polyene macrolide antibiotic RK-397 [240]. The same method allows the noniterative asymmetric synthesis of C_{15} polyketide spiroketals [241, 242], including advances precursor of the AB spiroketal of spongistatins [243].

Enantiomerically pure Diels–Alder adducts **87** and **88** of furan to 1-cyanovinyl esters and other products of saponification enones (−)-**89** and (+)-**89** [244–246] (with recovery of chiral auxiliaries ROH and R′OH) have been coined "naked sugars" as they contain six carbon centers and are enantiomerically pure like natural hexoses [247, 248]. They possess three unsubstituted carbon centers that can be substituted by all kinds of groups and/or functions with high regioselectivity and stereoselectivity [249]. This has permitted the de novo asymmetric synthesis of rare and valuable aldoses [250] and alditols [251–254], iminoaldoses and iminoalditols [255–258], thiosugars [259], *C*-nucleosides [260, 261], conduritols [262, 263] and conduramines [264–267], long-chain sugars [268, 269], branched sugars [270, 271], polyhydroxyindolizidines [272, 273] and analogs such as castanospermine [274], *C*-disaccharides [275, 276], *C*-glycosides of carbasugars [277–279], imino-*C*-disaccharides [280–284], and natural products, e.g., nonactine [285] and cyclophellitol [286]. Several other enantiomerically enriched 7-oxabicyclo[2.2.1] hept-2-ene derivatives have been described [287–293] and applied in the total, asymmetric synthesis of important products of biological interest [294–299]. What precedes, and what can be found in a recent review [300] on the application of 7-oxabicyclo[2.2.1]hept-2-ene derivatives, demonstrate that furfural, furfurol, furan, and other furans derived therefrom allow one to generate a very large molecular diversity and complexity.

6 Conclusion

In summary, hemicelluloses constitute a plentiful supply of D-xylose and L-arabinose pentoses that are renewable raw materials for a highly diversified chemistry. Their polar and hydrophilic character is exploited in surfactants that are less hydrophilic than other carbohydrate-derived surfactants. Their chiral and multifunctional features are of great interest for the synthesis of enantiopure complex structures, often in both their enantiomeric forms. This permits the generation of a large variety of bioactive compounds. Dehydration of pentoses gives furfural a highly valuable synthetic intermediate for materials (commodities) and compounds of biological interest (drugs, crop protection, etc.).

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References

1. Allen E, Vocke G (2006) Wheat situation and outlook yearbook. In: Knauth C (ed) USDA WHS-2006 publications
2. Klass DL (2004) Biomass for renewable energy and fuels. In: Encyclopedia of energy, Elsevier Inc.
3. Theander O, Westerlund E, Aman P (1993) Cereal Food World 33:135–141
4. Brillouet JM, Joseleau JP (1987) Carbohydr Res 159:109–126
5. Sun R, Mark Lawther J, Banks WB (1996) Carbohydr Polymer 29:325–331
6. Torget R, Hatzis C, Hayward TK, Hsu TA, Philippidis GP (1996) Appl Biochem Biotechnol 57/58:85–101
7. Bataillon M, Mathaly P, Nunes Cardinali AP, Duchiron F (1998) Ind Crop Prod 8:37–43
8. Maes C, Delcour A (2001) J Cereal Sci 34:29–35
9. Lequart C, Nuzillard JM, Kurek B, Debeire P (1999) Carbohydr Res 319:102–111
10. Ternrud IE, Lindberg JE, Magnusson AT, Theander O (1988) J Sci Food Agric 42:9–18
11. San Miguel Bento L (1990) Int Sugar J 92:116–125
12. Schenk FW (1992) Refining: carbon treatment and reactive precoats. In: Schenk W (ed) Starch hydrolysis products. VCH, USA
13. Focus on surfactants (2008) Special Chem Mag 6:5
14. Hirsinger F (1997) Life-cycle inventory. In: Rybinski W, Hill K (eds) Alkyl polyglycosides: technology properties and applications. Wiley-VCH, Weinheim
15. Biermann M, Schmid K, Shulz P (1994) Alkyl polyglycosides: technology and properties. Henkel Referate 30:7–15
16. Rybinski W, Hill K (1998) Angew Chem Int Ed 37:1328–1345
17. Van der Burgh LF, Sommer SJ (1983) Eur Patent EP 92355
18. Lüders H (1987) Technical processes for manufacturing alkyl polyglucosides. In: Balzer D, Harald L (eds) Nonionic surfactants – alkyl polyglucosides. Marcel Dekker, New York and Basel
19. Lüders H (1987) Eur Patent EP 253996
20. Eskuchen R, Nitsche M (1997) Technology and production of alkyl polyglycosides. In: Rybinski W, Hill K (eds) Alkyl polyglycosides: technology properties and applications. Wiley-VCH, Weinheim
21. http://www.icis.com/Articles/2007/08/15/4501288/wheatoleo-venture-looks-to-sugar-based-surfactants.html
22. Kamm B, Kamm M, Schmidt M, Hirth T, Schulze M (2006) Biorefineries – industrial processes and products. In: Kamm B, Gruber PR, Kamm M (eds) Biorefineries industrial processes and products. Wiley-VCH, Weinheim
23. Bertho JN, Mathaly P, Dubois V, De Baynast R (1997) US 5688930
24. Augé J (2008) Green Chem 10:225–231
25. Wust W, Eskuchen R (1990) EP 0357969
26. Augé J, Sizun G (2009) Green Chem 11:1179–1183
27. Estrine B, Bouquillon S, Hénin F, Muzart J (2004) Eur J Org Chem 2914–2922
28. Estrine B, Bouquillon S, Hénin F, Muzart J (2005) Green Chem 7:219–223
29. Estrine B, Bouquillon S, Hénin F, Muzart J (2003) WO 03 053987. Chem Abstr 139:54601
30. Hadad C, Damez C, Bouquillon S, Estrine B, Hénin F, Muzart J, Pezron I, Komunjer L (2006) Carbohydr Res 341:1938–1944
31. Böcker T, Thiem J (1989) Tenside Surf Det 26:318–324
32. Ernenwein C, Estrine B (2004) FR 2869242
33. Estrine B, Ernenwein C, Marinkovic S (2008) FR 2913896
34. Estrine B, Ernenwein C, Brésin A, Wintrebert A, Benvegnu T, Plusquellec D (2004) FR 2869912

35. Estrine B (2005) Development of new environmentally friendly surfactants derived from wheat byproducts. First international conference on renewable resources and biorefineries, Ghent, Belgium
36. Estrine B, (2008) Valorization of pentoses from biomass. Seventh World Surfactant Congress, Paris, France
37. Werpy T, Petersen G, Aden A, Bozel J, Holladay J, White J, Manheim A, Elliot D, Lasure L, Jones S, Gerber M, Ibsen K, Lumberg L, Kelley S (2004) Top value added chemicals from biomass. In: Results of screening for potential candidates from sugars and synthesis gas, vol 1. US Department of Energy. http://www1.eere.energy.gov/biomass/pdfs/35523.pdf
38. Granström TB, Izumori K, Leisola M (2007) Appl Microbiol Biotechnol 74:273–276
39. Gosselin G, Bergogne M-C, Imbach J-L (1991) Nucleic Acid Chem 4:41–45
40. Gelas J, Horton D (1975) Carbohydr Res 45:181–195
41. Okabe M, Sun RC, Zenchoff GB (1991) J Org Chem 56:4392–4397
42. Sun RC, Okabe M (1995) Org Synth Coll 72:48–52
43. Gao J, Zhen ZB, Jian YJ, Wu Y (2008) Tetrahedron 64:9377–9383
44. Zhen ZB, Gao J, Wu Y (2009) Chin J Chem 27:205–208
45. Kireev AS, Breithaupt AT, Collins W, Nadein ON, Kornienko A (2004) J Org Chem 70:742–745
46. Luchetti G, Ding K, d'Alarcao M, Kornienko A (2008) Synthesis 3142–3147
47. Ferrier RJ, Middleton S (1993) Chem Rev 93:2779–2831
48. Martinez-Grau A, Marco-Contelles J (1998) Chem Soc Rev 27:155–162
49. Dalko PI, Sinaÿ P (1999) Angew Chem Int Ed 38:773–777
50. Madsen R (2007) Eur J Org Chem 399–415
51. Bernet B, Vasella A (1979) Helv Chim Acta 62:1990–2016
52. Bernet B, Vasella A (1979) Helv Chim Acta 62:2400–2410
53. Bernet B, Vasella A (1979) Helv Chim Acta 62:2411–2431
54. Hyldtoft L, Poulsen CS, Madsen R (1999) Chem Commun 2101–2102
55. Hyldtoft L, Madsen R (2000) J Am Chem Soc 122:8444–8452
56. Hansen FG, Bungaard E, Madsen R (2005) J Org Chem 70:10139–10142
57. Poulsen CS, Madsen R (2002) J Org Chem 67:4441–4449
58. Håkansson AE, Palmelund A, Holm H, Madsen R (2006) Chem Eur J 12:3243–3253
59. Bercier A, Plantier-Royon R, Portella C (2007) Carbohydr Res 342:2450–2455
60. Génu-Dellac C, Gosselin G, Imbach J-L (1991) Carbohydr Res 216:249–255
61. Hénon E, Bercier A, Plantier-Royon R, Harakat D, Portella C (2007) J Org Chem 72:2271–2278
62. Bercier A, Plantier-Royon R, Portella C (2010) to be published
63. Kim E, Gordon DM, Walther Schmid W, Whitesides GM (1993) J Org Chem 58:5500–5507
64. Palmelund A, Madsen R (2005) J Org Chem 70:8248–8251
65. Hadad C, Majoral JP, Muzart J, Caminade AM, Bouquillon S (2009) Tetrahedron 50:1902–1905
66. Park S, Shin I (2007) Org Lett 9:1675–1678
67. Camponovo J, Hadad C, Ruiz J, Cloutet E, Gatard S, Muzart J, Bouquillon S, Astruc D (2009) J Org Chem 74:5071–5074
68. Barberousse V, Boubia B, Samreth S (2001) WO Patent 2001036437, Chem Abstr 134:353473
69. Mignon L, Goichot C, Ratel P, Cagnin G, Baudry M, Praly J-P, Boubia B, Barberousse V (2003) Carbohydr Res 338:1271–1282
70. Barberousse V, Bondoux M, Thomas D, Peyrou V (2006) WO patent 2006100413. Chem Abstr 145:377529
71. Thomas D, Bondoux M, Barberousse V, Peyrou V (2008) WO patent 2008007027. Chem Abstr 148:144970
72. Bellamy F, Horton D, Millet J, Picart F, Samreth S, Chazan JB (1993) J Med Chem 36:898–903

73. Bellamy F, Barberousse V, Martin N, Masson P, Millet J, Samreth S, Sepulchre C, Theveniaux J, Horton D (1995) Eur J Med Chem 30:101s–115s
74. Dubb JW, Henderson LS, Okada SS, Peyrou V, Samuels RE, Sheth S, Spera PA, Toomey JR, Wilson D (2006) WO patent 2006060647. Chem Abstr 145:1040
75. Barberousse V, Samreth S, Boubia B, Bellamy F, Peyrou V (2005) FR patent 2860234. Chem Abstr 142:336586
76. Bondoux M, Mignon L, Ou K, Renaut P, Thomas D, Barberousse V (2009) Tetrahedron Lett 50:3872–3876
77. Dalko M, Breton L (2002) WO patent 2002051828. Chem Abstr 137:79179
78. Cassin G, Simmonet JT (2007) WO patent 2007020536. Chem Abstr 146:258259
79. Cavezza A, Boulle C, Guéguiniat A, Pichaud P, Trouille S, Ricard L, Dalko-Csiba M (2009) Bioorg Med Chem Lett 19:845–849
80. Mani K, Havsmark B, Persson S, Kaneda Y, Yamamoto H, Sakurai K, Ashikari S, Habuchi H, Suzuki S, Kimata K, Malström A, Westergren-Thorsson G, Fransson LA (1998) Cancer Res 58:1099–1104
81. Mani K, Belting M, Ellervik U, Falk N, Svensson G, Sandgren S, Fransson LA (2004) Glycobiology 5:387–397
82. Jacobsson M, Malmberg J, Ellervik U (2006) Carbohydr Res 341:1266–1281
83. Johnsson R, Mani K, Ellervik U (2007) Bioorg Med Chem Lett 17:2338–2341
84. Jacobsson M, Ellervik U (2002) Tetrahedron Lett 43:6549–6552
85. Jacobsson M, Ellervik U, Belting M, Mani K (2006) J Med Chem 49:1932–1938
86. Johnsson R, Mani K, Ellervik U (2007) Bioorg Med Chem 15:2868–2877
87. Jacobsson M, Mani K, Ellervik U (2007) Bioorg Med Chem 15:5283–5299
88. Keinicke L, Madsen R (2005) Org Biomol Chem 3:4124–4128
89. Lucchetti G, Ding K, Kornienko A, d'Alarcao M (2008) Synthesis 3148–3154
90. Stütz AE (1999) Iminosugars as glycosidase inhibitors: nojirimycin and beyond. Wiley-VCH, Weinheim, Germany. ISBN 978-3-527-29544-9
91. Compain P, Martin OR (2007) Iminosugars, from synthesis to therapeutic applications. Wiley, Chichester. ISBN 978-0-470-03391-3
92. Tsou E-L, Yeh Y-T, Liang P-H, Cheng W-C (2009) Tetrahedron 65:93–100
93. Laroche C, Plantier-Royon R, Szymoniak J, Bertus P, Behr J-B (2006) Synlett 223–226
94. Laroche C, Behr J-B, Szymoniak J, Bertus P, Schütz C, Vogel P, Plantier-Royon R (2006) Bioorg Med Chem 14:4047–4054
95. Pearson MSM, Floquet N, Bello C, Vogel P, Plantier-Royon R, Szymoniak J, Bertus P, Behr J-B (2009) Bioorg Med Chem 17:8020–8026
96. Pearson MSM, Plantier-Royon R, Szymoniak J, Bertus P, Behr J-B (2007) Synthesis 3589–3594
97. Yoshikawa M, Murakami T, Shimada H, Matsuda H, Yamahara J, Tanabe G, Muraoka O (1997) Tetrahedron Lett 38:8367–8370
98. Yoshikawa M, Morikawa T, Matsuda H, Tanabe G, Muraoka O (2002) Bioorg Med Chem 10:1547–1554
99. Ozaki S, Oe H, Kitamura S (2008) J Nat Prod 71:981–984
100. Oe H, Ozaki S (2008) Biosci Biotechnol Biochem 72:1962–1964
101. Mohan S, Pinto BM (2007) Carbohydr Res 342:1551–1580
102. Muraoka O, Yoshikai K, Takahashi H, Minematsu T, Lu G, Tanabe G, Wang T, Matsuda H, Yoshikawa M (2006) Bioorg Med Chem 14:500–509
103. Liu H, Pinto BM (2005) J Org Chem 70:753–755
104. Nasi R, Sim L, Rose DR, Pinto BM (2007) Carbohydr Res 342:1888–1894
105. Simons C (2001) Nucleoside mimetics: their chemistry and biological properties. Gordon and Breach Science, The Netherlands. ISBN 90-5699-324-0
106. Okano K (2009) Tetrahedron 65:1937–1949
107. Du J, Choi Y, Lee K, Chun BK, Hong JH, Chu CK (1999) Nucleosides & Nucleotides 18:187–195

108. Akagi M, Omae D, Tamura Y, Ueda T, Kumashiro T, Urata H (2002) Chem Pharm Bull 50:866–868
109. Stewart AJ, Evans RM, Weymouth-Wilson AC, Cowley AR, Watkin DJ, Fleet GWJ (2002) Tetrahedron Asymmetry 13:2667–2672
110. Zeitsch KJ (2000) The chemistry and technology of furfural and its many by-products. Elsevier, Amsterdam
111. Mamman AS, Lee J-M, Kim Y-C, Hwang T, Park N-J, Hwang YK, Chang J-S, Hwang J-S (2008) Biofuels Bioprod Bioref 2:438–454
112. Joule JA, Mills K (2000) Heterocyclic chemistry, 4th edn. Blackwell Science, Oxford
113. Kamm B, Kamm M, Schmidt M, Hirth T, Schulze M (2006) Lignocellulose-based chemical products and product family trees. In: Kamm B, Gruber PR, Kamm M (eds) Biorefineries industrial processes and products. Wiley-VCH, Weinheim
114. Aycock DF (2007) Org Process Res Dev 11:156–159
115. Lande S, Westin M, Schneider M (2008) Mol Cryst Liq Cryst 484:367–378
116. Lichtenthaler FW (2002) Acc Chem Res 35:728–737
117. Zhu J, Bienaymé H (eds) (2005) Multicomponent reactions. Wiley-VCH, Weinheim
118. Lee D, Sello JK, Schreiber SL (2000) Org Lett 2:709–712
119. Burke MD, Schreiber SL (2004) Angew Chem Int Ed 43:46–58
120. Burke MD, Berger EM, Schreiber SL (2003) Science 302:613–618
121. Holder NL (1982) Chem Rev 82:287–332
122. Gopinath R, Barkakaty B, Talukdar B, Patel BK (2003) J Org Chem 68:2944–2947
123. Tian Q, Shi D, Sha Y (2008) Molecules 13:948–957
124. Mannam S, Sekar G (2008) Tetrahedron Lett 49:1083–1086
125. Achmatowicz O, Bukowski P, Szechner B, Zwierzchowska Z, Zamojski A (1971) Tetrahedron 27:1973–1996
126. Harries JM, Li M, Scott JG, O'Doherty GA (2004) Approach to 5,6-dihydro-2H-pyran-2-one containing natural products. In: Harmata M (ed) Strategies and tactics in organic synthesis, vol. 5. Elsevier, London
127. Honda T (1997) Oxidative ring transformation of 2-furylcarbinols in natural products. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol. 19. Elsevier Science BV, Amsterdam
128. Georgiadis MP, Albizati KF, Georgiadis YM (1992) Org Prep Proced Int 24:95–118
129. Couladouros EA, Strongilos AT (2002) Angew Chem Int Ed 41:3677–3680
130. Ciufolini MA, Hermann CYW, Dong Q, Shimizu T, Swaminathan S, Xi N (1998) Synlett 105–114
131. Esser P, Pohlmann B, Scharf H-D (1994) Angew Chem Int Ed Engl 33:2009–2023
132. Schenck GO (1953) Liebigs Ann Chem 584:156–176
133. Bolz G, Wiersdorff (1972) BASF DE 2111119 A1
134. Cottier L, Descotes G, Nigay H, Parron J-C, Grégoire V (1986) Bull Soc Chim Fr 844–850
135. Hoffmann N (2008) Chem Rev 108:1052–1103
136. Oelgemöller M, Jung C, Mattay J (2007) Pure Appl Chem 79:1939–1947
137. Albini A, Fagnoni M (2008) ChemSusChem 1:63–66
138. Martel J, Tessier J, Demoute J-P (1981) Roussel-UCLAF EP 23454 B1
139. Feringa BL, de Lange B (1988) Tetrahedron 44:7213–7222
140. Moradei O, Paquette LA, Peschko C, Danheiser RL (2003) Org Synth 80:66–73
141. Marinkovic S, Brulé C, Hoffmann N, Prost E, Nuzillard J-M, Bulach V (2004) J Org Chem 69:1646–1651
142. Fleischer J, Bauer K, Hopp R (1971) DE 2109456 A1
143. Oertling H, Reckziegel A, Bertram H-J (2007) Chem Rev 107:2136–2164
144. Feringa BL, De Lange B, Jansen JFGA, de Jong JC, Lubben M, Faber W, Schudde EP (1992) Pure Appl Chem 64:1865–1871
145. Hoffmann N, Bertrand S, Marinkovic S, Pesch J (2006) Pure Appl Chem 78:2227–2246
146. Griesbeck AG, Hoffmann N, Warzecha K-D (2007) Acc Chem Res 40:128–140

147. Näsman JH, Johnson AT, White JD (1989) Org Synth 68:162–174
148. Cao R, Liu C, Liu L (1996) Org Prep Proced Int 28:215–216
149. Badovskaya LA, Latashko VM, Poskonin VV, Grunskaya EP, Tyukhteneva ZI, Rudakova SG, Pestunova SA, Sarkisyan AV (2002) Chem Heterocycl Compd 38:1040–1048
150. Vinicius M, De Souza N (2005) Mini Rev Org Chem 2:139–145
151. Carreira EM, Fettes A, Marti C (2006) Org React 67:1–216
152. Denmark SE, Heemstra JR, Beutner GL (2005) Angew Chem Int Ed 44:4682–4698
153. Casiraghi G, Zanardi F, Appendino G, Rassu G (2000) Chem Rev 100:1929–1972
154. Pringsheim H, Noth H (1926) Ber Dtsch Chem Ges 53B:114–118
155. Kaufmann WE, Adams R (1923) J Am Chem Soc 45:3029–3044
156. Huang Z, Qiu L (1992) Shiyou Huagong 21:35–38
157. Wang J, Wang G, Yan W (1998) Jingxi Huagong 15:37–40
158. Lachman I, Spaczynska B (1982) Polish Technol Rev 8–9
159. Bremmer JGM, Keeys RKF (1947) J Chem Soc 1068–1070
160. Wang A, Chen X, Kang H (2000) Huagong Shikau 14:6–8
161. Wojcik BH (1948) J Ind Eng Chem (Washington, DC) 40:210–216
162. Cho BT, Kang SK, Kim MS, Ryu SR, Duk K (2006) Tetrahedron 62:8164–8168
163. Naimi-Jamal MR, Mokhtari J, Dekamin MG, Kaupp G (2009) Eur J Org Chem 3567–3572
164. De Vries JG, van Bergen TH, Kellogg RM (1977) Synthesis 246–247
165. Gelbard G, Louis-Andre O, Cherkaoui O (1991) React Polym 15:111–119
166. Nowicki J, Maciejewski Z (1985) Przemysl Chemiczny 64:20–22
167. Theurillat-Moritz V, Guidi A, Vogel P (1997) Tetrahedron Asymmetry 8:3497–3501
168. Theurillat-Moritz V, Vogel P (1996) Tetrahedron Asymmetry 7:3163–3168
169. Jotterand N, Vogel P, Schenk K (1999) Helv Chim Acta 82:821–847
170. Jotterand N, Vogel P (1999) Tetrahedron Lett 40:5499–5502
171. Jotterand N, Vogel P (1999) J Org Chem 64:8973–8975
172. Jotterand N, Vogel P (1999) Synlett 1883–1886
173. Jotterand N, Vogel P (2001) Curr Org Chem 5:637–661
174. Eschinazi HE (1952) Bull Soc Chim Fr 967–969
175. Tyran LW (1957) US 2776981 19570108. Chem Abstr (1957) 51:43497
176. Aliev Ya Y, Sultanov AS (1959) Khim Khlopchatnika Akad Nauk Uzbek SSR 105–145. Chem Abstr 55:64997
177. Meszaros L, Schobel G (1968) Acta Phys Chem 14:127–137
178. Gardos G, Pechy L, Redey A, Scaszar ME (1976) Hung J Ind Chem 4:125–138
179. Coca J, Morrondo ES, Sastre H (1982) J Chem Technol Biotechnol 32:904–908
180. Lejemble P, Maire Y, Gaset A, Kalck P (1983) Chem Lett 1403–1406
181. Lejemble P, Gaset A, Kalck P (1984) Biomass 4:263–274
182. Jung KJ, Gaset A, Molinier J (1988) Biomass 16:89–96
183. Sugita M, Tsuji H, Okoshi T (2009) PCT Int Appl WO 2009069714 A120090604. Chem Abstr (2009) 150:563626
184. Kalnins P, Hillers S, Tarvid M (1951) Latv PSR Zinat Akad Vestis 443–452
185. Akimoto M, Echigoya E (1977) J Chem Soc Faraday Trans 1 73:193–202
186. Rivasseau J, Canesson P, Blanchard M (1980) J Phys Chem 84:2791–2795
187. Mori K, Miyamoto A, Murakami Y (1985) J Catal 95:482–491
188. Mahaim C, Carrupt P-A, Hagenbuch J-P, Florey A, Vogel P (1980) Helv Chim Acta 63:1149–1157
189. Metral J-L, Vogel P (1985) Helv Chim Acta 68:334–337
190. Metral J-L, Lauterwein J, Vogel P (1986) Helv Chim Acta 69:1287–1309
191. Miller LL, Thomas AD, Wilkins CC, Weil DA (1986) Chem Commun 661–663
192. Thomas AD, Mill LL (1986) J Org Chem 51:4160–4169
193. Khonke FH, Slawin AMZ, Stoddart JF, Williams DJ (1987) Angew Chem 99:941–943
194. Ellwood P, Mathias JP, Stoddart JF, Kohnke FH (1988) Bull Soc Chim Belg 97:669–678

195. Ashton PR, Isaacs NS, Kohnke FH, Stagno D'Alcontres G, Stoddart JF (1989) Angew Chem 101:1269–1271
196. Ashton PR, Isaacs NS, Kohnke FH, Mathias JP, Stoddart JF (1989) 101:1266–1269
197. Ashton PR, Mathias JP, Stoddart JF (1991) Polymer Prepr 32:419–420
198. Girreser U, Giuffrida D, Kohnke FH, Mathias JP, Philp D, Stoddart JF (1993) Pure Appl Chem 65:119–125
199. Ashton PR, Mathias JP, Stoddart JF (1993) Synthesis 65:119–125
200. Ashton PR, Girreser U, Giuffrida D, Kohnke FH, Mathias JP, Raymo FM, Slawin AMZ, Stoddart JF, Williams DJ (1993) J Am Chem Soc 115:5422–5429
201. Ahston PR, Brown GR, Smith DR, Stoddart JF, Williams DJ (1993) Tetrahedron Lett 34:8337–8340
202. Ahston PR, Brown GR, Brown GR, Isaacs NS, Giuffrida D, Kohnke FH, Mathias JP, Slawin AMZ, Smith DR, Stoddart JF, Williams DJ (1992) J Am Chem Soc 114:6330–6353
203. Carrupt P-A, Vogel P (1979) Tetrahedron Lett 19:4533–4536
204. Bessière Y, Vogel P (1980) Helv Chim Acta 63:232–243
205. Tamariz J, Vogel P (1984) Tetrahedron 40:4549–4560
206. Tornare J-M, Vogel P (1985) Helv Chim Acta 68:1069–1077
207. Dienes Z, Antonsson T, Vogel P (1993) Tetrahedron Lett 34:1013–1016
208. Dienes Z, Vogel P (1995) Bioorg Med Chem Lett 5:547–550
209. Vogel P (1998) Curr Org Chem 2:255–280
210. Hoffmann HMR (1972) Angew Chem Int Ed 11:324
211. Noyori R, Makino S, Okita T, Haykawa Y (1975) J Org Chem 40:806–807
212. Noyori R, Haykawa Y (1983) Org React 29:163–344
213. Sato T, Noyori R (1978) Bull Chem Soc Jpn 51:2745–2746
214. Rigby JH, Pigge FC (1997) Org React 53:351–478
215. Cowling AP, Mann J, Usmani AA (1981) J Chem Soc Perkin Trans 1:2116–2119
216. Foehlisch B, Gehrlach E, Herter R (1982) Angew Chem 94:144
217. Foehlisch B, Gehrlach E, Geywitz B (1987) Chem Ber 120:1815–1824
218. Vidal-Pascual M, Martinez-Lamenca C, Hoffmann HMR (2006) Org Synth 83:61–69
219. Rashatasakhon P, Harmata M (2009) Tetrahedron Lett 50:2109–2110
220. Noyori R, Sato T, Hayakawa Y (1978) J Am Chem Soc 100:2561–2563
221. Sato T, Noyori R (1980) Tetrahedron Lett 21:2535–2538
222. Noyori R, Sato T, Kobayashi H (1983) Bull Chem Soc Jpn 56:2661–2679
223. Sato T, Hayakawa Y, Noyori R (1984) Bull Chem Soc Jpn 57:2515–2525
224. Bunn BH, Cox PJ, Simpkins NS (1983) Tetrahedron 49:207–218
225. Hoffmann HMR, Dunkel R, Mentzel M, Reuter H, Stark CBW (2001) Chem Eur J 7:4771–4789
226. Lampe TFJ, Hoffmann HMR (1996) Chem Commun 1931–1932
227. Dunkel R, Hoffmann HMR (1999) Tetrahedron 55:8385–8396
228. Kim H, Hoffmann HMR (2000) Eur J Org Chem 2195–2201
229. Vakapoulos A, Hoffmann HMR (2001) Org Lett 3:177–180
230. Bowers KG, Mann J, Walsh EB, Howarth OW (1987) J Chem Soc Perkin Trans 1:1657–1666
231. Cowling AP, Mann J, Usmani AA (1981) J Chem Soc Perkin Trans 1:2116–2119
232. Dunkel R, Mentzel M, Hoffmann HMR (1997) Tetrahedron 53:14929–14936
233. Lee JC, Cho SY, Cha JK (1999) Tetrahedron Lett 40:7675–7678
234. Rashatasakhon P, Harmata M (2009) Tetrahedron Lett 50:2109–2110
235. Stark CBW, Pierau S, Wartchaw R, Hoffmann HMR (2000) Chem Eur J 6:684–691
236. Schwenter ME, Vogel P (2000) Chem Eur J 6:4091–4103
237. Sharpless KB, Kolb HC, Van Nieuwenhze MS (1994) Chem Rev 94:2483–2547
238. Schwenter ME, Vogel P (2001) J Org Chem 66:7869–7872
239. Vogel P, Gerber-Lemaire S, Carmona AT, Meilert KT, Schwenter ME (2005) Pure Appl Chem 77:131–137
240. Gerber-Lemaire S, Carmona AT, Meilert KT, Vogel P (2006) Eur J Org Chem 891–900

241. Meilert KT, Pettit GR, Vogel P (2004) Helv Chim Acta 87:1493–1507
242. Gerber-Lemaire S, Vogel P (2004) Eur J Org Chem 5040–5046
243. Favre S, Gerber-Lemaire S, Vogel P (2007) Org Lett 9:5107–5110
244. Reymond J-L, Vogel P (1990) Tetrahedron Asymmetry 1:729–736
245. Forster A, Kovac T, Mosimann H, Renaud P, Vogel P (1999) Tetrahedron Asymmetry 10:567–571
246. Vogel P, Cossy J, Plumet J, Arjona O (1999) Tetrahedron 55:12521–13642
247. Warm A, Vogel P (1986) J Org Chem 51:5348–5353
248. Vogel P, Fattori D, Gasparini F, LeDrian C (1990) Synlett 173–185
249. Vogel P (2001) De novo synthesis of monosaccharides. In: Fraser-Reid B, Tatsuka K, Thiem J (eds) Glycoscience II. Springer, Heidelberg, pp 1023–1179, Chapter 4.4
250. Vogel P (2006) Organic synthesis of sugars. CRC Press LLC, Boca Raton, FL, pp 629–725
251. De Guchteneere E, Fattori D, Vogel P (1992) Tetrahedron 48:10603–10620
252. Fattori D, Vogel P (1992) Tetrahedron 48:10587–10602
253. Durgnat J-M, Vogel P (1993) Helv Chim Acta 76:222–240
254. Huenenberger P, Allemann S, Vogel P (1994) Carbohydr Res 157:175–187
255. Auberson Y, Vogel P (1989) Angew Chem 101:1554–1555
256. Wagner J, Vogel P (1991) Tetrahedron Lett 32:3169–3170
257. Wagner J, Vogel P (1991) Tetrahedron 47:9641–9648
258. Baudat A, Picasso S, Vogel P (1996) Carbohydr Res 281:277–284
259. Emery F, Vogel P (1994) J Carbohydr Chem 13:555–563
260. Pechy P, Gasparini F, Vogel P (1992) Synlett 676–678
261. Jeanneret V, Gasparini F, Pechy P, Vogel P (1992) Tetrahedron 48:10637–10644
262. LeDrian C, Vieira E, Vogel P (1989) Helv Chim Acta 72:338–347
263. LeDrian C, Vionnet J-P, Vogel P (1990) Helv Chim Acta 73:161–168
264. Allemann S, Vogel P (1994) Helv Chim Acta 77:1–9
265. Lysek R, Schuetz C, Vogel P (2005) Bioorg Med Chem Lett 15:3071–3075
266. Lysek R, Schuetz C, Vogel P (2005) Helv Chim Acta 88:2733–2768
267. Lysek R, Favre S, Vogel P (2007) Tetrahedron 63:6558–6572
268. Jeganathan S, Vogel P (1991) Carbohydr Res 222:151–161
269. Emery F, Vogel P (1995) J Org Chem 60:5843–5854
270. Wagner J, Vogel P (1989) Chem Commun 1634–1635
271. Neff D, Chen Y, Vogel P (1991) Helv Chim Acta 74:508–516
272. Chen Y, Vogel P (1992) Tetrahedron Lett 33:4917–4920
273. Chen Y, Vogel P (1994) J Org Chem 59:2487–2496
274. Reymond J-L, Pinkerton AA, Vogel P (1991) J Org Chem 56:2128–2135
275. Bimwala RM, Vogel P (1992) J Org Chem 57:2076–2083
276. Ferritto R, Vogel P (1994) Tetrahedron Asymmetry 5:2077–2092
277. Ferritto R, Vogel P (1995) Tetrahedron Lett 36:3517–3518
278. Cossy J, Ranaivosata J-L, Bellosta V, Ancerewicz J, Ferritto R, Vogel P (1995) J Org Chem 60:8351–8359
279. Ferritto R, Vogel P (1996) Synlett 281–282
280. Baudat A, Vogel P (1996) Tetrahedron Lett 37:483–484
281. Frerot E, Marquis C, Vogel P (1996) Tetrahedron Lett 37:2023–2026
282. Kraehenbuehl K, Picasso S, Vogel P (1997) Bioorg Med Chem Lett 7:893–896
283. Baudat A, Vogel P (1997) J Org Chem 62:6252–6260
284. Marquis C, Picasso S, Vogel P (1999) Synthesis 1441–1452
285. Warm A, Vogel P (1987) Helv Chim Acta 70:690–700
286. Moritz V, Vogel P (1992) Tetrahedron Lett 33:5243–5244
287. Takayama H, Iyobe A, Koizumi T (1986) Chem Commun 771–772
288. Corey EJ, Loh TP (1993) Tetrahedron Lett 34:3979–3982
289. Akai S, Naka T, Omura S, Tanimoto K, Imanashi M, Takebe Y, Matsugi M, Kita Y (2002) Chem Eur J 8:4255–4264

290. Tojo S, Isobe M (2005) Synthesis 1237–1244
291. Guan YK, Li YL (2005) Chirality 17:113–118
292. Ryu DH, Kim KH, Sim JY, Corey EJ (2007) Tetrahedron Lett 48:5735–5737
293. Sakakura A, Kondo R, Matsumura Y, Akakura M, Ishihara K (2009) J Am Chem Soc 131:17762–17764
294. Mukaiyama T, Iwasawa N (1981) Chem Lett 29–32
295. Acena JL, Arjona O, Plumet J (1996) Tetrahedron Asymmetry 7:3535–3544
296. Arjona O, Iradier F, Medel R, Plumet J (1999) Tetrahedron Asymmetry 10:3431–3442
297. Acena JL, Arjona O, Leon MA, Plumet J (2000) Org Lett 2:3683–3686
298. Arjona O, Menchaca R, Plumet J (2001) Tetrahedron 57:6751–6755
299. Arjona O, Menchaca R, Plumet J (2001) Org Lett 3:107–108
300. Schindler CS, Carreira EM (2009) Chem Soc Rev 38:3222–3241

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Cellulose and Derivatives from Wood and Fibers as Renewable Sources of Raw-Materials

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Abstract Cellulose is the most important biopolymer in Nature and is used in preparation of new compounds. Molecular structure of cellulose is a repeating unit of β-D-glucopyranose molecules forming a linear chain that can have a crystallographic or an amorphous form. Cellulose is insoluble in water, but can dissolve in ionic liquids. Hemicelluloses are the second most abundant polysaccharides in Nature, in which xylan is one of the major constituents of this polymer. There are several sources of cellulose and hemicelluloses, but the most important source is wood. Typical chemical modifications are esterifications and etherifications of hydroxyl groups. TEMPO-mediated oxidation is a good method to promote oxidation of primary hydroxyl groups to aldehyde and carboxylic acids, selectively. Modified cellulose can be used in the pharmaceutical industry as a metal adsorbent. It is used in the preparation of cellulosic fibers and biocomposites such as nanofibrils and as biofuels.

Keywords Applications · Cellulose · Chemical modification · Hemicelluloses · TEMPO

Contents

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Abbreviations

AMIMCl	1-Allyl-3-methylimidazolium chloride
BMIMCl	1-Butyl-3-methylimidazolium chloride
CUA	Cellouronic acid
DP	Degree of polymerization
IL	Ionic liquids
NMMO	*N*-Methylmorpholine-*N*-oxide
SSCF	Simultaneous saccharification and cofermentation
SSF	Simultaneous saccharification and fermentation
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl

1 Cellulose and Hemicelluloses

Cellulose is the most common organic polymer (biopolymer) in Nature. Wood pulp is the most important raw material source in the processing of cellulose, most of which is used in the production of paper and paperboard. Other products can be prepared from cellulose such as cellulose esters and ethers. These cellulose derivatives are used for coatings, laminates, optical films, and sorption media, as well as for property-determining additives in building materials, pharmaceuticals, foodstuffs, and cosmetics. The molecular structure of cellulose implies a repeating unit of β-D-glucopyranose (β-D-Glc*p*) that is covalently linked through acetal function between the equatorial OH group of C-4 of one glucopyranose unit and the C-1 carbon atom of the next glucopyranose unit (β-1,4-glucan) (Fig. 1). As a result,

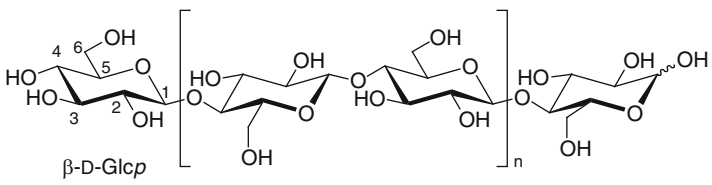

Fig. 1 Structure of cellulose

cellulose is an extensive, linear-chain polymer with a large number of hydroxyl groups present in the thermodynamically preferred $^4C_1$ chair conformation. It is a linear stiff-chain homopolymer characterized by its hydrophilicity, chirality, biodegradability, and broad chemical modifying capacity [1].

Some modified celluloses derived from wood pulp contain carbonyl and carboxyl groups resulting from the processes used in the isolation and purification of cellulose. The secondary hydroxyl groups of β-1,4-glucan cellulose reside in positions C-2 and C-3, the primary alcohol group at C-6. As a result of the supramolecular structure of cellulose, the solid state is represented by areas of both high order (crystalline) and low order (amorphous). The degree of polymerization (DP) is a measure of the chain length of cellulose. It varies with the origin and treatment of the raw material [1].

Cellulose can occur in two major and two minor crystallographic forms. In cellulose I, the modification in which cellulose is found throughout Nature, the chains are all oriented in the same direction (parallel). They are bound together by strong hydrogen bonds in one of the two transverse directions, but by only weak forces in the second one. In addition, there are hydrogen bonds between adjacent glucose residues within each chain. Crystalline, native cellulose has a rigid structure, which is both a chain lattice and a layer lattice, impermeable to water. Cellulose II is formed when the lattice of cellulose I is destroyed either by swelling with strong alkali or by dissolution. It is the thermodynamically more stable of two modifications. In cellulose II, the chains are antiparallel, and there are hydrogen bonds between the chains not only within but also between the layers. Other polymorphic forms of cellulose have been reported as cellulose III and IV. However, some authors consider that these are not true polymorphic forms, but are rather disordered versions or mixtures of celluloses I and II [2].

The high order structure and crystallinity of cellulose makes it recalcitrant to acid and base-catalyzed hydrolysis. Hydrogen bonding between cellulose chains is so intense that water cannot disrupt it through complexation with the hydroxyl groups. Cellulose is difficult to process because of the strong intra- and intermolecular hydrogen bonding. This makes cellulose insoluble in common solvents and thus limits its applications. A good cellulose solvent is capable of interacting with the hydroxyl groups, so as to eliminate, at least partially, the strong intermolecular hydrogen bonding between the polymer chains. The solubility increases by chemical reactions leading to covalent bond formation. Solvents capable of that are usually called *derivatizing solvents* [3].

Ionic liquids (IL) were shown to be good cellulose solvents over a wide range of DP values without implying covalent interaction [4]. Dissolution of cellulose in ionic liquids allows the comprehensive utilization of cellulose by combining two major green chemistry principles: using environmentally preferable solvents and biorenewable feed-stocks. Examples of ionic liquids are hydrophilic ionic liquids such as 1-butyl-3-methylimidazolium chloride (BMIMCl) and 1-allyl-3-methylimidazolium chloride (AMIMCl). Microwave heating significantly accelerates the dissolution process. Cellulose can be easily regenerated from its ionic liquid solutions by addition of water, ethanol, or acetone. After its regeneration, the

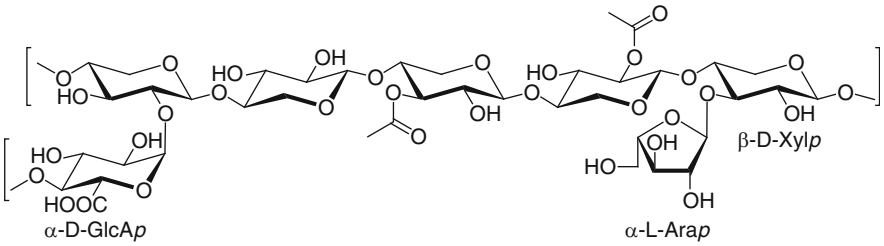

Fig. 2 Example of hemicellulose structure

ionic liquids can be recovered and reused. Fractionation of lignocellulosic materials and preparation of cellulose derivatives and composites are typical applications that will find commercial applications soon [5].

Hemicelluloses (also called polyoses) are the second most abundant polysaccharides in Nature after cellulose. Their structure (e.g., see Fig. 2) and chemical properties are presented in [6].

2 Sources of Cellulose and Hemicelluloses

Cellulose is a natural fiber found in all five continents. Quality and yield depend on the kind of plant, the variety, the soil, and the climate. Another important source is the recycling of agro-fiber-based products such as paper, waste wood, and point source agricultural residues. Recycling paper products back into paper requires a wet processing for the removal of inks, salts, and adhesives. Recycling the same products into composites can be done by using dry processing whereby all components are incorporated into the composite. In the latter process the need for costly separation procedures [7] is suppressed.

Lignocellulosic materials have in their composition cellulose, hemicelluloses, and lignin, among other minor compounds. Lignin is a phenolic polymer, which will not be described here. In Table 1 the percentage of cellulose and hemicelluloses in several common plants and sources [8] are presented.

Biosynthesis of cellulose by bacteria is another potentially useful source of this fiber. An example is *Acetobacter xylinum*, which possesses a cellulose synthase permitting high rate of in vitro synthesis of cellulose. Cellulose synthase has been purified and genes that encode the catalytic subunit have been cloned. In higher plants, a family of genes has been discovered that show interesting similarities and differences from the genes in bacteria that encode the catalytic subunit of the synthase. Genetic evidence now supports the concept that members of this family encode the catalytic subunit in these organisms, with various members showing tissue-specific expression [9].

Table 1 Percentage of cellulose and hemicelluloses in different plants and sources [8]

Source	Cellulose (%)	Hemicelluloses (%)
Hardwood	43–47	25–35
Softwood	40–44	25–29
Monocotyledon stems	25–40	25–50
Wheat straw	30	50
Corn cobs	45	35
Corn stalks	35	25
Bagasse	40	30
Low or nonlignified fibers plants	70–95	5–25

3 Cellulose and Derivatives

3.1 Wood to Paper

The most important application of cellulosic materials is the production of paper. Thus wood is transformed first into pulp by mechanical or chemical modification. Hardwood trees such as eucalyptus, birch, and softwood trees like pinus and spruce are used. In the Kraft process woodchips are treated with sodium hydroxide and sodium sulfide to promote the breakdown of the linkage between lignin, some hemicelluloses, and cellulose. This produces cellulose containing residual lignin. The latter is removed by a bleaching process that can apply several oxidizing agents. White pulp is pure cellulose that is used to produce paper.

3.2 Chemical Modification of Cellulose

Cellulose can be transformed chemically into all used sorts of products that can be applied in several fields. Several reactions can be used such as esterifications and etherifications of the hydroxyl groups. The majority of water-soluble and organic solvent-soluble cellulose derivatives are obtained by these reactions. They lead to drastic changes in the original properties of cellulose. Others reactions are ionic and radical grafting, acetalation, deoxyhalogenation, and oxidation. Since the usual cellulosic materials originating from wood and cotton pulps have aldehyde and carboxyl moieties, they can be used for chemical modifications, although they are not abundant compared with the alcoholic functions (Fig. 3) [10].

3.2.1 TEMPO-Mediated Oxidation

Oxidation reactions of carbohydrate polymers transform hydroxyl groups into carbonyl and carboxyl groups, which modify the properties of the polysaccharides.

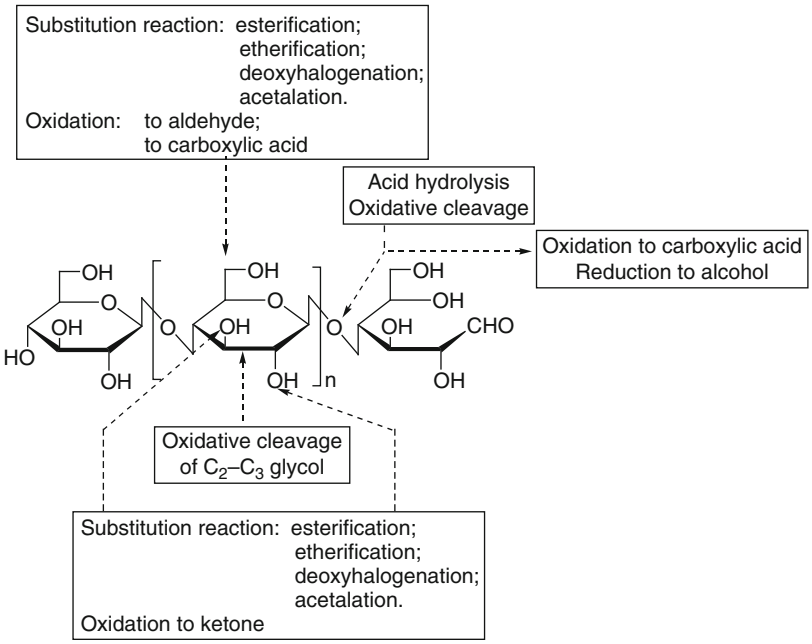

Fig. 3 Most applied reactions of cellulose

For instance, these modified polysaccharides can be used as calcium-sequestering agents [11].

Oxidation of secondary hydroxyl groups at C-2 and C-3 leads to acyclic dialdehydes and dicarboxylic derivatives. Selective oxidation of primary hydroxyl groups is more difficult, but can be achieved by stoichiometric as well as catalytic methods. Nitric acid oxidizes monosaccharides into aldaric acids. Oxidation of cellulose or starch by nitrogen dioxide (N_2O_4) yields 6-carboxy starch and 6-carboxycellulose respectively. Subsequent hydrolysis under well-controlled conditions (0.5–2 M HCl at 150 °C) of these materials yields D-glucuronic acid (D-Glc*A*p) [12].

Catalytic oxidation promotes the regioselective oxidation at C-6 primary hydroxyl groups of polysaccharides. With water-soluble and stable nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as catalyst, NaBr as cocatalyst, and NaOCl as oxidant, under aqueous conditions selective oxidation of primary alcohol groups into aldehydes and/or carboxylic acid groups can be realized in good yield [13] (Fig. 4).

In contrast to enzymatic or metal-catalyzed oxidation, the TEMPO-oxidation process is highly effective in the conversion of high molecular weight polysaccharides. Other advantages of this catalytic process are the high reaction rate and yield, the high selectivity, and little degradation of the polysaccharides [12].

The selective oxidation of chitosan and chitosan derivatives by TEMPO has been studied. The modifications on chitosan present difficulties due to the presence of the amine moieties [15]. Several types of cellulose have been submitted to

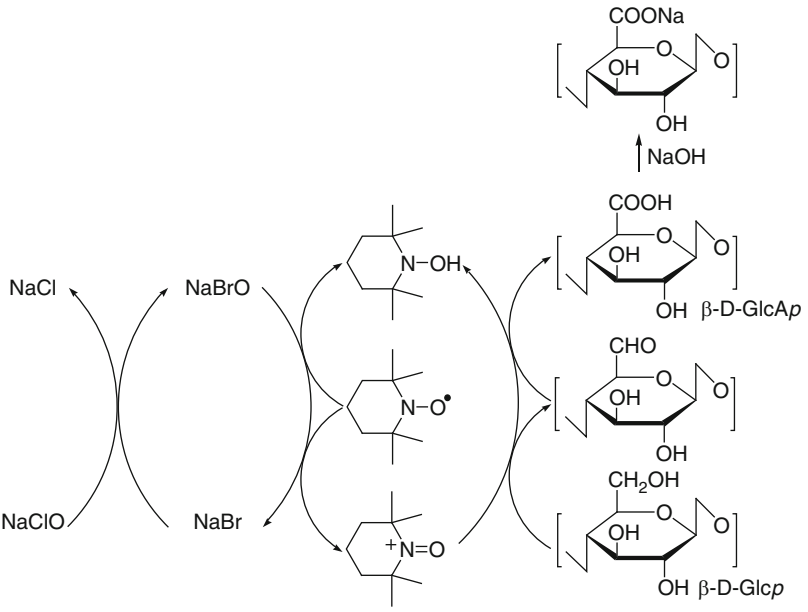

Fig. 4 TEMPO-mediated oxidation (H_2O, pH 10–11 of cellulose to form C6-carboxylate groups via C6-aldehyde groups [14]

Table 2 Conditions of TEMPO-mediated oxidation and oxidized pulp characteristics

		Experiment				
		1	2	3	4	5
Experimental conditions	TEMPO (mg/g) (odp)[a]	0.25	0.26	0.33	0.43	0.52
	NaBr (mg/g) (odp)	2.76	2.36	2.74	3.10	3.48
	NaClO (μL/g) (odp)	37	19	28	30	35
Results	Carboxyl content (μmol/g)	101	86.7	90.9	95.4	121
	Intrinsic viscosity (mL/g)	454	748	525 729[b]	755[b]	572

[a]Oven dry pulp
[b]Viscosity values obtained after borohydride reduction of the pulp

TEMPO-mediated oxidation. With regenerated and mercerized celluloses, the oxidation leads to water-soluble β-1,4-linked polyglucuronic acid sodium salt (cellouronic acid, CUA) quantitatively [16]. In contrast, with native celluloses having the cellulose I crystal structure, the cellulose slurries maintain the slurry states even after TEMPO-mediated oxidation. These modified celluloses form water-insoluble fibers [17]. This has enabled modification of the surface of cellulosic fibers.

This oxidation process is applied to industrial bleached kraft pulp of *Eucalyptus globulus*. Table 2 gives data for the optimization of the process and characteristics of the modified fibers so obtained.

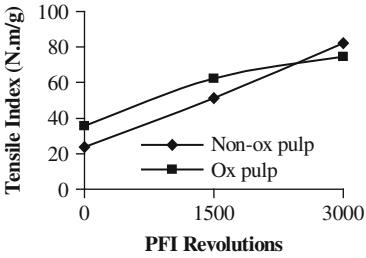

Fig. 5 Tensile index of the pulps for different refining levels

The determination of total carboxylic groups introduced in pulp fibers is based on conductometric titration. This shows that an increase of 11.6% in the carboxyl group content of pulp fibers gives rise to improvements of the swelling properties of the pulp and gives characteristics for making better paper. The oxidized pulp without refining exhibits an increase of 33% in the tensile strength (Fig. 5), 48% in tear index, and 60% in hand sheet internal cohesion [18].

Others reagents have been used to promote cellulose oxidation. A new TEMPO-mediated oxidation with catalytic amounts of TEMPO and NaClO, and $NaClO_2$ as the primary oxidant under aqueous conditions at pH 3.5–6.8, was used to prepare water-soluble β-(1 → 4)-linked polyglucuronic acid Na salts (CUAs) with high molecular weight in good yield. When regenerated cellulose with original DP of 680 was oxidized by the 4-acetamide-TEMPO/NaClO/$NaClO_2$ system at pH 5.8 and 40 °C for 3 days, CUA with weight average DP (DPw) of 490 was obtained quantitatively. No peaks other than six signals from β-(1 → 4)-linked anhydroglucuronic acid units of CUA were detected in the ^{13}C NMR spectra of the soluble oxidized products. Although the products prepared under the above conditions contained about 20% of unoxidized cellulose particles, the non-CUA fraction was separable from CUAs by filtration or salt-induced precipitation. The DPw values and yields of CUAs after filtration or salt-induced precipitation treatment were 250–380 and 45–71%, respectively [19].

3.2.2 Enzymatic Modifications

Multicomponent cellulases, purified endoglucanases, and cellobiohydrolases have been shown to modify pure natural cellulose (softwood pulp). Changes in structure and properties of the cellulose caused by enzymatic treatment depend on the composition, the type of enzyme, and the treatment conditions. The reactivity of cellulose toward some dissolving and derivatization processes may be improved if submitted first to enzymatic hydrolysis. Endoglucanases decreased the average degree of polymerization (DP) and improved the alkaline solubility of cellulose. Variation in the supramolecular structure of cellulose as analyzed by infrared spectroscopy correlates with its reactivity. This is explained in terms of change in conformation of the fibers due to the suppression of some hydrogen bonds [20].

3.3 Pharmaceutical Applications

Chemical modification of cellulose provides products that can be used in the pharmaceutical industry. They are inexpensive, recyclable, and biocompatible. The combination of polymer and pharmaceutical sciences has led to the introduction of polymers in the design and development of drug delivery systems. Polymeric delivery systems control or sustain drug delivery. Polysaccharides fabricated into hydrophilic matrices remain popular biomaterials for controlled-release dosage forms and the most abundant naturally occurring biopolymer is cellulose. Alternatively, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, microcrystalline cellulose, and hydroxyethyl cellulose can be used for the production of time controlled delivery systems. Cellulose acetate, cellulose phthalate, and hydroxymethyl cellulose phthalate are also used. Targeting of drugs to the colon, following oral administration, can be accomplished by using polysaccharides such as hydroxypropylmethyl cellulose and hydroxypropyl cellulose in hydrated form. They act as binders that swell when hydrated by gastric media and thus delay absorption [21].

3.4 Metal Absorbents

Too many industries currently produce varying concentrations of heavy metal laden waste streams with undesired consequences for the environment. Increasing emphasis has been placed on environmental impact minimization. This has led to the search for natural, inexpensive materials able to remove metals from factory effluents. Heavy metal absorption capacities of modified cellulose materials are found to be significant and their levels of uptake are comparable, in many instances, to other naturally occurring absorbent materials and to commercial ion exchange type resins. Many of the modified cellulose adsorbents proved to be regenerable and reusable over a number of absorption/desorption cycles. This allows easy recovery of the absorbed heavy metals in a concentrated form [22].

3.5 Cellulosic Fibers and Biocomposites

Cellulosic fiber reinforced polymeric composites find applications in many fields ranging from the construction industry to the automotive industry. The reinforcing efficiency of natural fiber is related to the nature of cellulose and its crystallinity. The main components of natural fibers are cellulose (α-cellulose), hemicelluloses, lignin, pectins, and waxes. For example, biopolymers or synthetic polymers reinforced with natural or biofibers (termed biocomposites) are a viable alternative to glass fiber composites. The term biocomposite is now being applied to a staggering range of materials derived wholly or in part from renewable biomass resources [23].

Regenerated cellulose fiber can be used for preparation of biocomposites. It can be obtained with an inexpensive and less toxic aqueous solution of NaOH/urea or NaOH/thiourea, which dissolves cellulose pulps directly and quickly. Cellulose pulps can be dissolved directly in NaOH/thiourea/urea aqueous solvent with corresponding weight ratio of 8:6.5:8 to obtain transparent spinning solution. The coagulation bath conditions (including coagulation bath composition, concentration, temperature, and time) and stretching conditions (including jet stretch ratio and draw ratio) are found to play an important part in affecting the formation and tensile properties of the prepared fibers [24].

3.6 Cellulose Nanopaper

Cellulose nanofibrils offer interesting potential as a native fibrous constituent of mechanical performance exceeding the plant fibers in current use for commercial products. Nanocomposites are prepared from a water suspension of cellulose nanofibrils and with water-based thermoplastic latex. Cellulose nanopaper films are carefully prepared from cellulose nanofibrils in water suspension. The best mechanical properties resulting from high molar mass nanofibrils are obtained from carboxymethylation pretreatment of wood pulp fibers followed by mechanical disintegration using a high shear homogenizer. Conventional *micropaper* shows inferior toughness. The superiority of nanopaper is likely to be caused by higher fibril strength, more favorable interfibril adhesion characteristics, and much smaller and more homogeneously distributed defects. Together with the fact that these materials originate from renewable sources and that their properties can be modified almost at will, they become extremely important in nanotechnologies [25].

3.7 Biofuels

Biofuels are an attractive alternative to current petroleum-based fuels as they can be utilized as transportation fuels with little change to current technologies and have significant potential to improve sustainability and reduce greenhouse gas emissions. Liquid (ethanol, biodiesel) or gaseous (methane or hydrogen) biofuels are derived from organic materials such as starch, oilseeds and animal fats, or cellulose. Ethanol is now the most widely used liquid biofuel obtained by fermentation of sugars, starches, or cellulosic biomass. Production of bioethanol from biomass is one way to reduce both the consumption of crude oil and pollution. Conversion technologies for the production of ethanol from cellulosic biomass resources such as forest materials, agricultural residues, and urban wastes are under development. They still have to demonstrate their commercial competitiveness [26]. To convert cellulose and hemicelluloses to ethanol, the polysaccharides must be broken down to corresponding monomers before microorganisms can utilize them. Because the

native cellulose fraction of the lignocellulosic materials is recalcitrant to enzymatic saccharification, a pretreatment is normally required to make them convertible into bioethanol by the microorganisms [27]. Conventional production of ethanol from cellulose via fermentation involves a complex process of pretreatment that includes (1) cellulase production, (2) hydrolysis of cellulose and hemicelluloses into hexoses and pentoses, followed (3) by their fermentation. Current strategies to produce fuel ethanol from cellulose, referred to as *second-generation* biofuels, utilize simultaneous saccharification and fermentation (SSF) or simultaneous saccharification and cofermentation (SSCF). Both SSF and SSCF require extensive pretreatment of the cellulosic feedstock by steam-explosion and/or acid treatment, followed by addition of exogenously produced cocktails of cellulolytic enzymes to hydrolyze cellulose chains and release the glucose monomers required for fermentation [28].

4 Conclusion

One of the most abundant natural and renewable sources of carbon is cellulose, fibers that have been used for centuries in the production of paper. Chemists have found ways to modify these water-insoluble fibers, applying various chemical or biochemical treatments that generate high value materials (modified cellulose). The latter find applications in medicine, in material sciences, and in the nanotechnologies, including production of absorbers to remove undesired and toxic contaminants from factory effluents. Chemical and biochemical modifications of cellulose generate materials that can be used to produce ethanol (biofuel) by fermentation.

References

1. Klemm D, Heublein B, Fink HP, Bohn A (2005) Angew Chem Int Ed 44:3358–3393
2. Hon DN-S (1989) Cellulose: chemistry and technology. In: Schniewind AP (ed) Concise encyclopedia of wood and wood-based materials chemical composition. Pergamon, New York
3. Heinze T, Petzold K (2008) Cellulose chemistry: novel products and synthesis paths. In: Belgacem MN, Gandini A (eds) Monomers, polymers and composites from renewable resources. Elsevier, Amsterdam
4. Spear SRP, SK HJD, Rogers RD (2002) J Am Chem Soc 124:4974–4975
5. Zhu S et al (2006) Green Chem 8:325–327
6. Martel F, Estrine B, Plantier-Royon R, Hoffmann N, Portella C (2010) Development of Agriculture Left-Overs: Fine Organic Chemicals from Wheat Hemicellulose- Derived Pentoses. In: Top Curr Chem. doi: 10:1007/128_2010_54
7. Dufresne A (2008) Cellulose-based composites and nanocomposites. In: Belgacem MN, Gandini A (eds) Monomers, polymers and composites from renewable resources. Elsevier, Amsterdam
8. Peters D (2006) Biotechnol J 1:806–814
9. Delmer DP (1999) Annu Rev Plant Physiol Plant Mol Biol 50:245–276

10. Isogai A (2001) Chemical modification of cellulose. In: Hon DN-S, Shiraishi N (eds) Wood and cellulosic chemistry. Marcel Dekker, New York, pp 599–626
11. Besemer AC, Bekkum H (1996) Calcium sequestering agents based on carbohydrates. In: Bekkum H, Roper H, Voragen AGJ (eds) Carbohydrates as raw materials III. VCH, Weinheim
12. Bragd PL, Bekkum H, Besemer AC (2004) Top Catal 27:49–66
13. de Nooy AEJ, Besemer AC, Bekkum H (1995) Carbohydr Res 269:89–98
14. Saito T, Isogai A (2006) Colloids Surf A Physicochem Eng Asp 289:219–225
15. Bordenave N, Grelier S, Coma V (2008) Biomacromolecules 9:2377–2382
16. Isogai A, Kato Y (1998) Cellulose 5:153–164
17. Saito T, Yanagisawa M, Isogai Al (2005) Cellulose 12:305–315
18. Duarte AP et al (2006) O Papel 76–82
19. Hirota M, Tamura N, Saito T, Isogai A (2009) Carbohydr Polym 78:330–335
20. Cao Y, Tan H (2002) Carbohydr Res 337:1291–1296
21. Kamel S et al (2008) Pharmaceutical significance of cellulose: a review. EXPRESS Polym Lett. doi:10.3144/expresspolymlett.2008.90
22. O'Connell DW (2008) Bioresour Technol 99:6709–6724
23. John MJ, Thomas S (2008) Carbohydr Polym 71:343–364
24. Zhang S (2009) Fibers Polym 10(1):34–39
25. Henriksson M et al (2008) Biomacromolecules 9:1579–1585
26. Demirbas A (2005) Energy Sources 27:327–337
27. Chandrakant P, Bisaria VS (1998) Crit Rev Biotechnol 18:295–331
28. Carere CR (2008) Int J Mol Sci 9:1342–1360

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DOI: 10.1007/128_2010_60

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Olive Pomace, a Source for Valuable Arabinan-Rich Pectic Polysaccharides

Manuel A. Coimbra, Susana M. Cardoso, and José A. Lopes-da-Silva

Abstract Cell wall polysaccharides account for nearly one third of olive pomace dry matter produced by the environment friendly biphasic system. These polysaccharides are mainly cellulose, glucuronoxylans, and arabinan-rich pectic polysaccharides, in equivalent proportions. The structural features of pectic polysaccharides are unique concerning the arabinan moiety due to the occurrence of a β-(1→5)-terminally-linked arabinose residue. This odd feature tends to disappear with olive ripening and can be used as a diagnostic tool in the evaluation of the stage of ripening of this fruit, as well as a marker for the presence of olive pulp in matrices containing pectic polysaccharides samples. These pectic polysaccharides have the ability to form elastic gels with calcium. The critical gelling calcium and galacturonic acid concentrations are higher than that observed for commercial citrus low-methoxyl pectic material. Nevertheless, they present a syneresis occurring for much higher calcium concentration and, consequently, show a much larger zone in which homogeneous gels are formed. In addition, olive pomace pectic polysaccharides gels are more resistant to temperature than the low-methoxyl pectin/calcium gels. These properties show that olive pomace can be a potential source of gelling pectic material with useful properties for particular applications.

Keywords Arabinan, Food additives, Gelling pectic material, L-Arabinosides, Olive pomace, Olive processing, Pectic extract

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Contents

1 Olive Fruits as Raw Materials

The olive tree (*Olea europaea)* is the only species of the Oleacea family that produces an edible fruit. The origins of cultivation of this plant are thought to have started about 5,000–6,000 years ago in the eastern Mediterranean and adjacent zones [1]. Olive tree cultivation has now spread throughout many regions of the world with Mediterranean-like climates such as South Africa, Chile, Australia, and California, and in areas with temperate climates such as New Zealand and Córdoba Province in Argentina. There are approximately 850 million productive olive trees worldwide, covering an area of more than ten million hectares [2, 3]. Nevertheless, approximately 98% of the total surface area of olive tree culture and total productive trees are provided by the Mediterranean area. Annual worldwide olive production is estimated to be more than 18 million tons [2].

The olive fruit differs from all other drupes in its chemical composition due to its relatively low sugar content, 3–6% *vs*. ca. 12%, high oil content, 12–30% *vs*. 1–2%, and its characteristic strong bitter taste which, among others, is caused by oleuropein (**1**) [4].

Approximately 90% of the total annual worldwide olive fruit harvested is used for olive oil production whilst table olives represent about one tenth of this production [3]. For table olive production, most of the olive fruits have to be processed in order to remove or, at least, reduce the bitter oleuropein component. The olives are usually submitted to fermentation or cured with lye or brine. One of the few exceptions to this rule is the Greek Thrubolea variety that is allowed to ripen on the tree [3]. For olive oil production, the olives are harvested as soon as the fruit reaches its maximum size because by that time the oil content also reaches the maximum amount. For most of the cultivars this stage corresponds to the change in the surface color from slightly pink to purple–pink or black [3].

2 Olive Polysaccharides

The plant cell wall consists of cellulose microfibrils within a matrix of non-cellulosic polysaccharides, glycoproteins, and phenolics, the types and composition of which are related to the maturity and function of the cell and the plant type. Most non-cellulosic cell wall components are held into the microfibrillar array by a combination of cross-links, including covalent and non-covalent ones [4].

The olive pulp cell wall is mainly composed of arabinan-rich pectic polysaccharides [5]. It also contains significant amounts of cellulose, glucuronoxylans, and xyloglucans whilst mannans and glycoproteins are minor components [5, 6]. The structure of the arabinan-rich pectic polysaccharides consists of a (1→4)-linked α-D-galacturonic acid (α-D-GalA) backbone that is interrupted in places by single residues of 2-*O*-linked-α-L-rhamnopyranose. α-L-Arabinofuranose (α-L-Ara) residues occur as side chains, linked to the C-4 of the L-rhamnose residues. D-GalA can bind calcium ions, allowing the pectic polysaccharide chains to assemble by calcium bridges. These Ca^{2+}-D-GalA complexes are commonly found in the middle lamella, playing an important role in cell–cell adhesion and cohesion. In accordance with that, modification and/or degradation of the pectic polysaccharides are closely associated to the cell separation and softening events occurring during the ripening of the fruit [7] (Fig. 1).

The changes taking place in olive pulp cell wall polysaccharides throughout ripening have been described for different cultivars. In general, these studies concluded that pectic polysaccharides are widely affected, becoming more soluble and more branched, showing a decrease in its degree of methyl esterification [7–11]. The reported changes in cell wall polysaccharides of olive pulp during ripening may be attributed to the increased activity of a diversity of cell wall enzymes such as polygalacturonase, pectinmethylesterase, and cellulase [11]. Still, in addition to the degrading processes, the synthesis of new polysaccharides during ripening has also been suggested to occur in Hojiblanca [10, 12] and Negrinha do Douro varieties [11]. The cell wall polysaccharide composition from olives of Negrinha do Douro variety, for the green and the black ripening stages, are shown in Fig. 2.

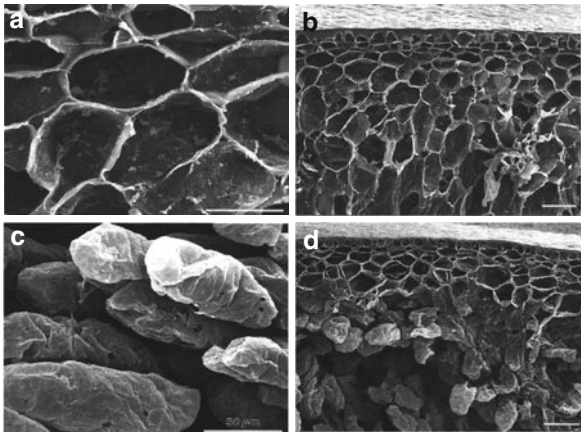


Fig. 1 SEM photographs of broken surface olives, adapted from Mafra et al. [7]: (**a**) details of green olive thin-walled parenchyma cells tightly packed (cell adhesion); (**b**) overview, showing tissues fracturing through the cells of epicarp and mesocarp with no cell separation at the middle lamella; (**c**) details of the black olive parenchyma showing cell separation (by the dissolution of pectic polysaccharides); (**d**) overview, showing a smaller region of broken cells in comparison to (**b**), involving only the epicarp and the first layers of the mesocarp

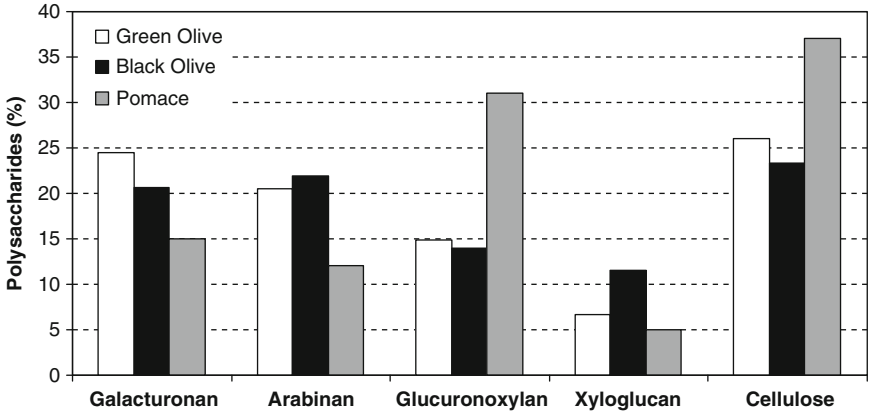


Fig. 2 Relative polysaccharide composition of olive pulp in (**a**) green and (**b**) black stages of ripening (data from [7]) and (**c**) of two-phases olive pomace (data from [13])

3 The Olive Pomace

Most industries involved in olive oil production are now using a two-phase extraction system. During the extraction process there is little or no addition of water to the extraction medium that minimizes both the consumption of water and the production of polluting liquid wastes [14, 15]. The main steps of this extraction process are schematized in Fig. 3. As for the previous technological processes, the formation of the olive paste by crushing olives and slow mixing of the latter at moderate temperatures (milling and malaxation) provoke the release of the oil from the fruit tissues, the joining of oil-droplets, and the formation of a continuous oil-phase which can be easily separated from the solid-phase. Yet the use of a "dual-phase decanter" allows the separation of the oil from the pastes with the production of only one by-product, which consists of vegetable matter and water. This residue is commonly known as "wet olive pomace," although several variant names are also in use [16–20]. The wet olive pomace, or two-phases olive pomace, is typically composed of water (60–70%), lignin (13–15%), cell wall polysaccharides (18–20%), olive oil (2.5–3.0%), and mineral solids (2.5%) [21]. Among their low molecular weight organic components, the major components are sugars (3%), peptides (1.5%), volatile fatty acids (C2–C7) (1%), polyalcohols (0.2%), polyphenols (0.2%), and pigments (0.5%) [21, 22].

The high moisture and organic matter contents of this residue turns it into a low-value commercial product. Therefore, several attempts have been made to valorize the wet pomace, although with relatively low economical success, including its use as natural fertilizer, as a combustible, and animal food additive [17, 23]. More valuable are their phenolic constituents, which represent 98% of the total phenols in the olive fruit [24–28]. The total amount of phenolic compounds that can be extracted from the two-phases olive pomace is approximately 150 mg g^{-1} (as expressed in oleuropein equivalents) of the dried, defatted and dehulled two-phases olive pomace [16]. The methanolic extract obtained from this residue has a complex

phenolic profile (Fig. 4), comprising compounds that are usually found in the olive phenolic extracts, namely verbascoside (**2**), rutin (**3**), caffeoyl-quinic acid (**4**), luteolin-7-glucoside (**5**), and 11-methyl-oleoside (**6**), together with uncommon ones, such as hydroxytyrosol 1′-β-glucoside (**7**), luteolin-7-rutinoside (**8**), oleoside (**9**), 6′-β-glucopyranosyl-oleoside (**10**), 6′-β-rhamnopyranosyl-oleoside (**11**), 10-hydroxy-oleuropein (**12**), and an oleuropein glucoside (**13**). These extracts still contain many already unknown compounds, and thus further studies are needed to reveal the total phenolic composition of the two-phases olive pomace residue, as well as their biological importance, in order to add value to the residue.

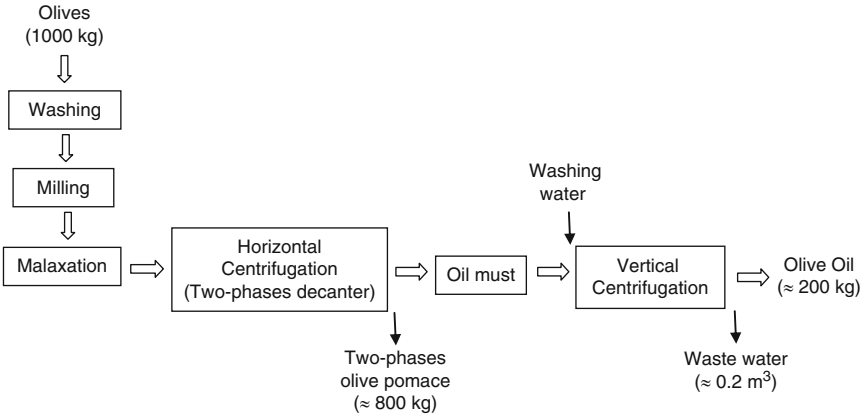


Fig. 3 Two-phase olive processing

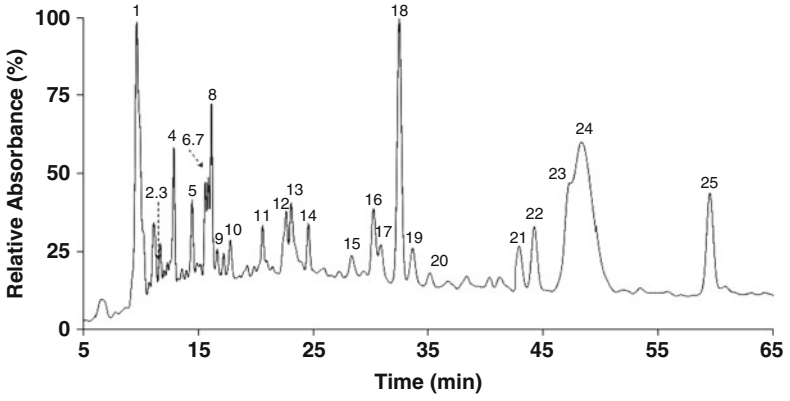
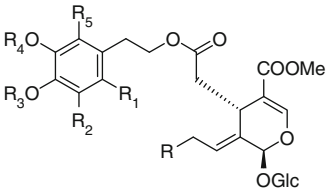
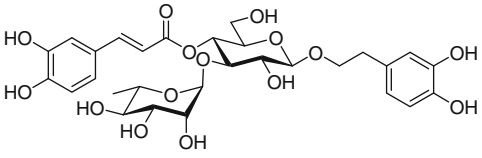


Fig. 4 Chromatographic profile at 280 nm of a methanolic extract from olive pomace (adapted from [16]). 1: hydroxytyrosol-1′-β-glucoside; 6: caffeoyl-quinic acid; 8: oleoside; 11: oleuropein aglycone derivative; 13: 11-methyl-oleoside and 4-hydroxyphenylacetic acid; 15: 10-hydroxy-oleuropein and unknown compound; 16: rutin; 17: luteolin-7-rutinoside; 18: luteolin-7-glucoside and verbascoside; 19: luteolin-7-rutinoside (isomer); 20: oleuropein glucoside; 21: luteolin-4-glucoside; 22: 6′-β-glucopyranosyl-oleoside; 25: 6′-β-rhamnopyranosyl-oleoside. The remaining numbers in the figure correspond to still unidentified compounds

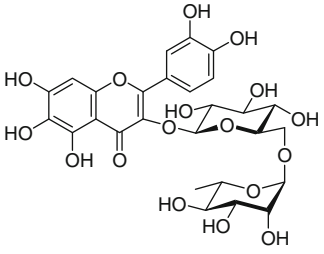
The occurrence in the olive pomace of unique oleuropein oligomers (**14**) with a degree of polymerization of up to five oleuropein monomers was reported by Cardoso et al. [27]. Their bioactivities have, however, not yet been studied. In fact, besides the evidence that olive phenolic compounds can have antioxidant, cardioprotective, antimicrobial, antihypertensive, anti-inflammatory, and chemo-preventive properties [26], the majority of the studies have focused on hydroxytyrosol (**15**). This compound has revealed remarkable pharmacological and antioxidant activities and thus has been further studied for its bioavailability and metabolism in humans in order to establish its health-beneficial effects [29–31].



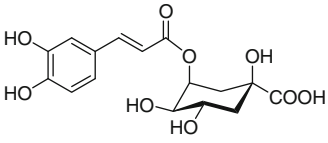
1: oleuropein (R= H; R_1, R_2, R_3, R_4 and R_5 = H)
12: 10-hydroxy-oleuropein (R= OH; R_1, R_2, R_3, R_4 and R_5 = H)
13: oleuropein glucoside (R= H; R_3 or R_4 = Glc; R_1, R_2 and R_5 =H)
14: oleuropein oligomer (R= H; R_1 or R_2 or R_3 or R_4 or R_5 = oleuropein residue(s))



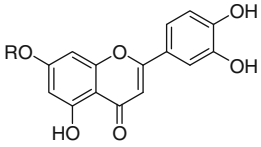
2: verbascoside



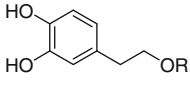
3: Rutin



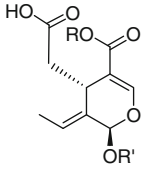
4: caffeoyl-quinic acid



5: luteolin-7-glucoside (R = Glc)
8: luteolin-7-rutinoside (R = Rha-Glc)

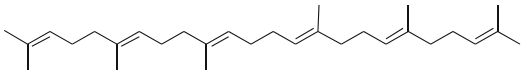


7: hydroxytyrosol 1´-β-glucoside (R = Glc)
15: hydroxytyrosol (R = H)

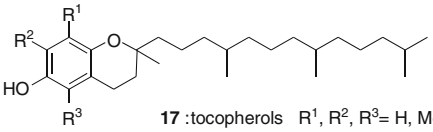


6: 11-methyl-oleoside (R = CH_3; R´ = Glc)
9: oleoside (R = H; R´ = Glc)
10: 6´-β-glucopyranosyl-oleoside (R = H; R´ = Glc-Glc)
11: 6´-β-rhamnopyranosyl-oleoside (R = H; R´ = Glc-Rha)

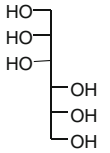
Continued efforts are still ongoing to find economical ways to isolate and purify these compounds [20]. Furthermore, one sees in olive pomace a source for squalene (**16**) [32], tocopherols (**17**) [33], D-mannitol (**18**) [34], triterpenes oleanolic (**19**) and maslinic (**20**) acids [35], pectic polysaccharides [13], hemicelluloses [36], and oligosaccharides [34].



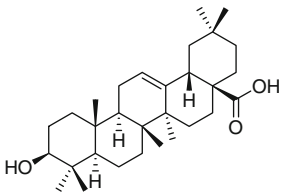
16: squalene



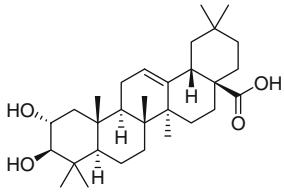
17 :tocopherols R^1, R^2, R^3= H, Me



18 : D-mannitol



19: Oleanoic acid



20: Maslinic acid

4 Olive Pomace Pectic Polysaccharides

As showed by Cardoso et al. [13, 37], the two-phase olive pomace contains considerable amounts of arabinan-rich pectic polysaccharides, as evaluated in an ethanolic insoluble residue (Fig. 2). These polymers contain a typical (1→5)-L-Ara*f* backbone, substituted at *O*-3 with a linkage composition of 5:4:3:1 for (1→5)-L-Ara*f*, T-L-Ara*f*, (1→3,5)-L-Ara*f*, and (1→3)-L-Ara*f*, respectively. These pectic arabinans are also characterized by the occurrence of T-β-L-Ara*f* (1→5)-linked to (1→3,5)-L-Ara*f* residues and of branched and linear blocks in their backbone. The proposed structure for the olive pomace pectic polysaccharides is represented in Fig. 5. It implies arabinan structures composed by an estimated average of 13 Ara residues linked to the rhamnogalacturonan backbone. This is found for distinct olive cultivars [37, 38].

The occurrence of a terminally-linked β-L-Ara*f* in the arabinan pectic polysaccharides has only been described for the olive fruit. This odd feature can be used as a marker of authenticity for the presence of olive pulp in matrices containing pectic polysaccharides. Moreover, as showed by Cardoso et al. [38], ripening of the olive fruit causes the disappearance of the characteristic arabinan terminally-linked β-L-Ara*f* residue (Fig. 6), and thus its quantification can be useful for the evaluation of the ripeness of the olive fruit.

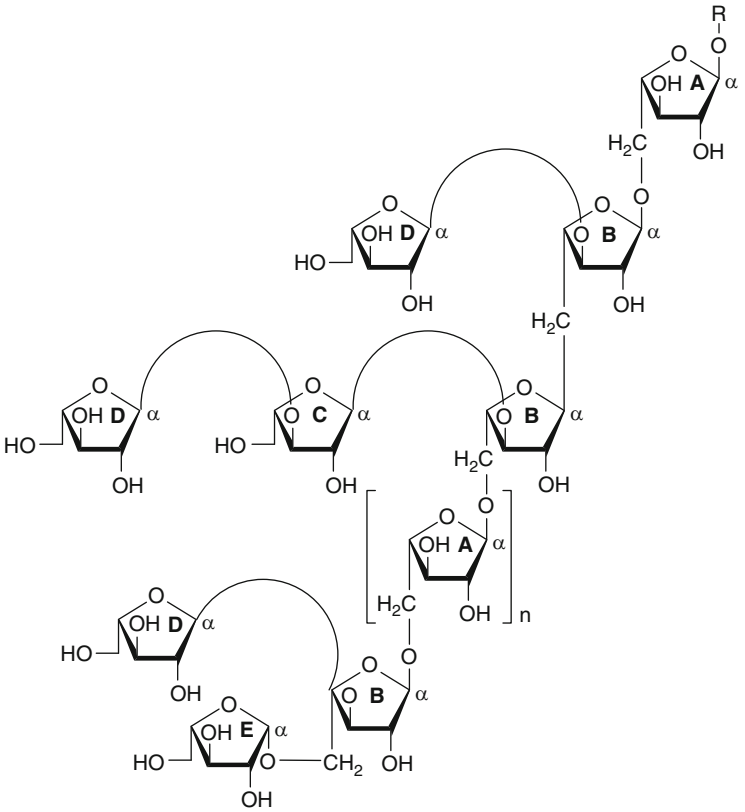


Fig. 5 Olive pectic polysaccharide structures (monosaccharide unit is L–arabinofuranose) [38]

The gelation potential of olive pomace pectic polysaccharides, which are low-methoxyl polymers (43% of methyl esterification), was exploited by Cardoso et al. [13, 39]. An important observation is that the main physico-chemical characteristics of olive pomace pectic polysaccharides are divergent from those of commercially available citrus low-methoxyl pectin (LMP) (Table 1). The two-phase olive pomace pectic extract (OPE) has a lower D-GalA content, a higher content in neutral sugars, mainly L-Ara, a higher degree of acetylation, and a lower molecular weight.

Despite the high neutral sugars content, the OPE form elastic gels on addition of calcium. The phase diagram representing the physical state of the OPE/calcium system, with respect to the D-GalA and calcium concentrations in 0.1 mol/L NaCl at pH 7, is shown in Fig. 7. The same data are represented for the commercially available LMP. For OPE, the minimum amount of polymer able to gel, independently of the calcium added to the system (critical D-GalA concentration, C_0), and the minimum amount of calcium required for gelation to occur whatever the polymer concentration (critical calcium concentration, C_1) were established as 2.8 g/L and 4.0 mmol/L, respectively, while those of the LMP were of approximately 1.0 g/L and

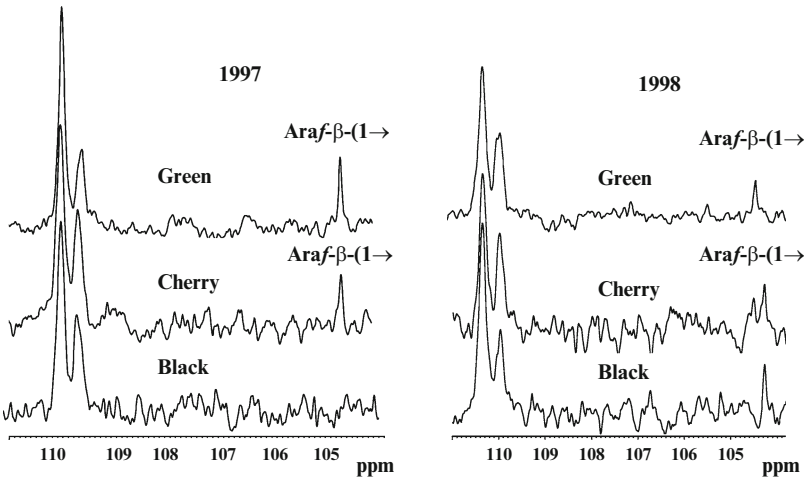


Fig. 6 Quantitative ^{13}C NMR spectra of pectic fractions obtained from olive pulp at three ripening stages for two harvest years, showing the disappearance of terminally-β-linked L-Ara*f* from olive pectic polysaccharide side chains [38]

Table 1 Main physico-chemical characteristics of the pectin samples [39]

	OPE[a, b]	LMP[a, c]
Degree of methyl-esterification	43.2%	34.8%
Degree of acetylation	11%	<1%
Galacturonic acid content	44.1%	88.7%
Total neutral sugars	38.8%	4.8%
Ash	1.1%	1.7%
Calcium	0.33 mg/g	2.5 mg/g
Intrinsic viscosity (pH 7, 0.1 mol/L NaCl)	1.04 dL/g	2.62 dL/g
Average (viscosimetric) molecular weight (M_v)	1.45×10^4	5.13×10^4

[a]Dry weight basis (when applied)
[b]Olive pomace pectic extract
[c]Low-methoxyl pectin

2.5 mmol/L, respectively. The higher C_0 and C_1 values of OPE are ascribed to their high content of neutral sugars as side chains, which can hinder the interaction between pectic chains and, consequently, the establishment of effective junction zones. In addition, the side chains can make difficult the access of calcium to the joint points, therefore contributing to a larger demand for calcium for gelation to occur. Notably, syneresis in the OPE/calcium systems occurs for much higher calcium concentration and, consequently, these systems show a much larger zone in which homogeneous gels are formed. This fact can also be a consequence of the high number of side chains, as the occurrence of biphasic gels has been attributed to the existence of a large number of intramolecular links leading to contraction of the

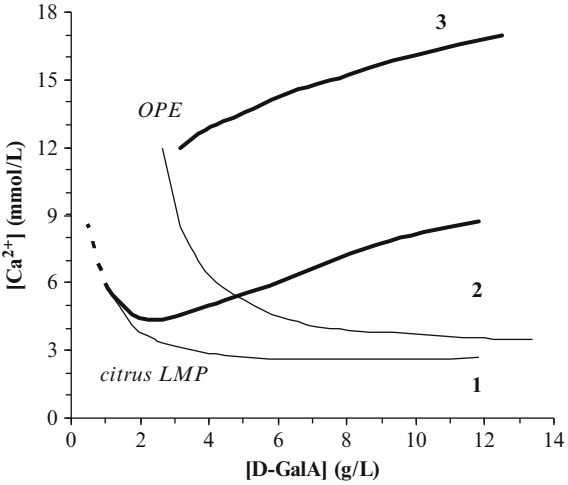


Fig. 7 Phase diagrams for olive pomace pectic extract (OPE) and citrus low-methoxyl pectin (LMP) in 0.1 mol/l NaCl at pH 7 and 20°C, as a function of D-GalA and calcium concentrations. *Dotted lines* denote less defined transitions (adapted from [13])

polymer chains [40] and exclusion of solvent. In this sense, the late appearance of syneresis in the OPE/calcium systems can also be related to a possible obstruction of the side chains on the formation of the intramolecular cross-links.

The kinetics of the formation of the olive OPE/calcium gels follows the general behavior described for the gelation process of pectins and other biopolymers. At distinct temperatures and pH 7, as also pH 3, there is a rapid increase of the storage modulus $G'(t)$ in the first 1–2 h, followed by its slower evolution to an asymptotic value. In contrast, the values of the loss modulus (G'') do not vary significantly as a function of time. In general, maturation at pH 7 originates stronger gels in comparison to those formed at pH 3. The opposite trend occurs when the maturation temperature increases [13].

Cardoso et al. [13] also compared the dependency of the viscoelastic properties of mature OPE/calcium gels upon the polymer and calcium concentrations to those of the LMP. They showed that, for these variables, both pectin systems exhibited a power law dependence of the G'. At pH 7, for the different concentrations of non-esterified carboxyl groups available in the pectin (D-GalA*), the PPE/calcium and citrus LMP/calcium systems exhibited similar dependencies on the calcium concentration (Fig. 8a), with a power law dependence of 2.9–3.3. Still, the gelling ability of OPE/calcium systems was more dependent on the polymer concentration than the citrus pectin. For the different calcium concentrations tested, the corresponding exponents of power law dependency were approximately 3.0 and 1.9 for OPE/calcium and citrus LMP/calcium systems, respectively (Fig. 8b). These results also confirm the lower capability of the pectic olive extracts to form, under similar ionic conditions, elastically effective junctions zones.

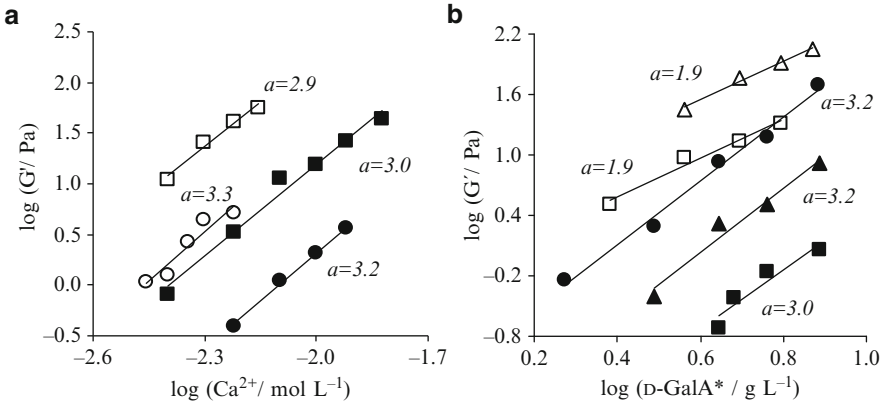


Fig. 8 Storage modulus (G') measured for 20 h cured gels of OPE and citrus LMP, at 20°C in 0.1 mol/L NaCl at pH 7, as a function of the (**a**) calcium concentration and (**b**) non-esterified carboxyl groups available in the pectin (D-GalA*). Results are shown for different D-GalA* concentrations (A): *open squares* [D-GalA*] = 6.4 g/L; *open circles* [D-GalA*] = 2.9 g/L; *filled squares* [D-GalA*] = 5.8 g/L; *filled circles* [D-GalA*] = 3.1 g/L and for different Ca^{2+} concentrations: *filled squares, open squares* [Ca^{2+}] = 4 mmol/L; *filled triangles, open triangles* [Ca^{2+}] = 6 mmol/L; *filled circles* [Ca^{2+}] = 10 mmol/L. *Filled symbols* denote the OPE system and *open symbols* represent the LMP pectin system. *a* represents the exponent of the power law, $G' \propto [\text{D-GalA*}]^a$ (adapted from [13])

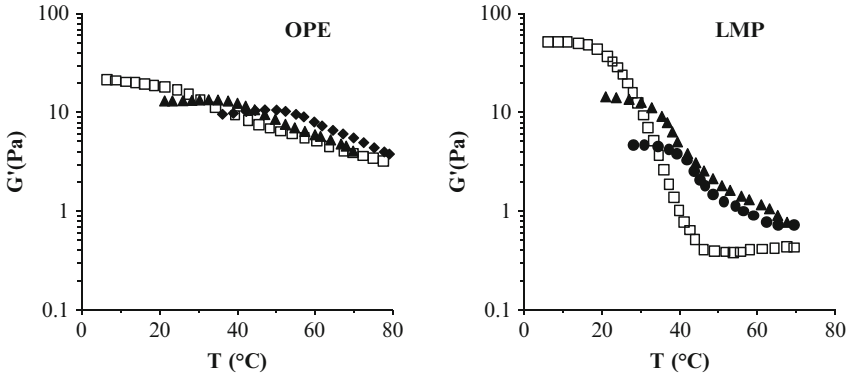


Fig. 9 Temperature sweeps (1 Hz, 2°C/min) of OPE- and LMP-calcium systems ([GalA] = 10 g/L, [Ca^{2+}] = 8.2 mmol/L (OPE) or [Ca^{2+}] = 4.4 mmol/L (LMP) at pH 7) after maturation for 20 h at distinct temperatures (*open squares* 5°C; *filled triangles* 20°C; *filled circles* 27.5°C; and *filled diamonds* 35°C). Heating was performed from the respective maturation temperature to 70 or 80°C (adapted from [39])

Interestingly, the OPE/calcium gels were shown to be more resistant to temperature than the LMP/calcium gels [39]. Indeed, in contrast to the LMP/calcium gels, the OPE/calcium gels show significant G′ values even at 70–80°C (Fig. 9), suggesting that the interactions responsible for the stabilization of these gels

structure are more resistant to high temperatures. Although the structural features of the olive pomace pectins renders difficult the interaction of chains through ionic links, it is possible that significant non-ionic interactions, such as hydrophobic and hydrogen links, can be responsible for the formation of stable associations at high temperature (thermostability of the junction zones), a behavior analogous to what is observed in high-methoxyl pectin gelation [41, 42].

5 Conclusion and Perspectives

Olive pomace dry matter produced by the two-phase system is a source of arabinan-rich pectic polysaccharides that should be exploited. They contain a diagnostic terminally-linked β-(1→5)-arabinose residue in the α-linked backbone; they are able to form elastic gels with calcium, which present syneresis for high calcium concentration and are more resistant to high temperature than commercially available low-methoxyl pectin/calcium gels. These properties make them valuable as food ingredients for specific applications.

References

1. Fernández-Díez MJ (1971) The olive. In: Hulmed AC (ed) The biochemistry of fruits and their products. Academic, London, pp 255–279
2. http://www.asemesa.es
3. Mafra I, Coimbra MA (2004) Improving the texture of processed fruit: the case of olives. In: Kilcast D (ed) Texture in food vol 2: solid foods. CRC Woodhead Publishing Ltd, Cambridge, pp 410–431
4. Vincken J-P, Schols HA, Oomen RJFJ et al (2003) If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. Plant Physiol 132:1781–1789
5. Coimbra MA, Waldron KW, Selvendran RR (1994) Carbohydr Res 252:245–262
6. Coimbra MA, Rigby NM, Selvendran RR, Waldron KW (1995) Carbohydr Polym 27: 277–284
7. Mafra I, Lanza B, Reis A et al (2001) Physiol Plant 111:439–447
8. Huisman MMH, Schols HA, Voragen AGJ (1996) Carbohydr Polym 31:123–133
9. Vierhuis E, Schols HA, Beldman G et al (2000) Carbohydr Polym 43:11–21
10. Jiménez A, Rodríguez R, Fernández-Caro I et al (2001) J Agric Food Chem 49:409–415
11. Mafra I, Barros AS, Nunes C et al (2006) J Sci Food Agric 86:988–998
12. Jiménez A, Rodríguez R, Fernández-Caro I et al (2001) J Agric Food Chem 49:2008–2013
13. Cardoso SM, Coimbra MA, Lopes da Silva JA (2003) Carbohydr Polym 52:125–133
14. Di Giovacchino L, Solinas M, Miccoli M (1994) J Am Oil Chem Soc 71:1189–1194
15. Alfano G, Belli C, Lustrato G et al (2008) Bioresour Technol 99:4694–4701
16. Cardoso SM, Guyot S, Marnet N et al (2005) J Sci Food Agric 85:21–32
17. Roig A, Cayuela ML, Sánchez-Monedero MA (2006) Waste Manag 26:960–969
18. Konstantinou M, Kolokassidou K, Pashalidis I (2007) Adsorption 13:33–40
19. Alburquerque JA, Gonzálvez J, Tortosa G et al (2009) Biodegradation 20:257–270
20. Suárez M, Romero M-P, Ramo T et al (2009) J Agric Food Chem 57:1463–1472
21. Borja R, Rincón B, Raposo F et al (2002) Process Biochem 38:733–742

22. Martín García AI, Moumen A, Yáñez Ruiz DR et al (2003) Anim Feed Sci Technol 107:61–74
23. Banat F, Al-Asheh S, Al-Ahmad R et al (2007) Bioresour Technol 98:3017–3025
24. Servili M, Baldioli M, Selvaggini R et al (1999) J Am Oil Chem Soc 76:873–882
25. Romero C, Brenes M, Garcia P et al (2002) J Agric Food Chem 50:3835–3839
26. Obied HK, Allen MS, Bedgood DR et al (2005) J Agric Food Chem 53:823–837
27. Cardoso SM, Guyot S, Marnet N et al (2006) J Sci Food Agric 86:1495–1502
28. Artajo L-S, Romero MP, Suárez M et al (2007) Eur Food Res Technol 225:617–625
29. Miró-Casas E, Covas MI, Farre M et al (2003) Clin Chem 49:945–951
30. Visioli F, Caruso D, Grande S et al (2005) Eur J Nutr 44:121–127
31. Fernández-Bolaños J, Rodríguez G, Rodríguez R et al (2006) Grasas Aceites 57:95–106
32. Bondioli P, Mariani C, Lanzani A et al (1993) J Am Oil Chem Soc 70:763–766
33. Ibanez E, Palacios J, Senorans FJ et al (2000) J Am Oil Chem Soc 77:187–190
34. Fernandez-Bolaños J, Rodriguez G, Gomez E et al (2004) J Agric Food Chem 52:5849–5855
35. Kuno N, Shinohara G (2002) Patent No WO02/012159 (14.02.2002)
36. Derriche R, Berrahmoune KS (2007) J Food Eng 78:1149–1154
37. Cardoso SM, Silva AM, Coimbra MA (2002) Carbohydr Res 337:917–924
38. Cardoso SM, Ferreira JA, Mafra I et al (2007) J Agric Food Chem 55:7124–7130
39. Cardoso SM, Coimbra MA, Lopes da Silva JA (2003) Food Hydrocoll 17:801–807
40. Allain C, Salomé L (1990) Macromolecules 23:981–987
41. Lopes da Silva JA, Gonçalves MP (1994) Carbohydr Polym 24:235–245
42. Lopes da Silva JA, Rao MA, Fu J-T (1998) Rheology of structure development and loss during gelation and melting. In: Rao MA, Hartel RW (eds) Phase/state transitions in foods. Marcel Dekker, USA

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Oligomannuronates from Seaweeds as Renewable Sources for the Development of Green Surfactants

Thierry Benvegnu and Jean-François Sassi

Abstract The development of surfactants based on natural renewable resources is a concept that is gaining recognition in detergents, cosmetics, and green chemistry. This new class of biodegradable and biocompatible products is a response to the increasing consumer demand for products that are both "greener", milder, and more efficient. In order to achieve these objectives, it is necessary to use renewable low-cost biomass that is available in large quantities and to design molecular structures through green processes that show improved performance, favorable ecotoxicological properties and reduced environmental impact. Within this context, marine algae represent a rich source of complex polysaccharides and oligosaccharides with innovative structures and functional properties that may find applications as starting materials for the development of green surfactants or cosmetic actives. Thus, we have developed original surfactants based on mannuronate moieties derived from alginates (cell-wall polyuronic acids from brown seaweeds) and fatty hydrocarbon chains derived from vegetable resources. Controlled chemical and/or enzymatic depolymerizations of the algal polysaccharides give saturated and/or unsaturated functional oligomannuronates. Clean chemical processes allow the efficient transformation of the oligomers into neutral or anionic amphiphilic molecules. These materials represent a new class of surface-active agents with promising foaming/emulsifying properties.

Keywords Alginate, Depolymerization, Mannuronate monomers, Oligomannuronates, Surfactants

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Contents

Abbreviations

CMC Critical micellization concentration
DP Degree of polymerization
HLB Hydrophilic–lipophilic balance
MSA Methanesulfonic acid

1 Oligomannuronates as Innovative Renewable Raw Materials

1.1 Alginate as a Source of Mannuronate

Alginates were first described by Standford in 1881 [1]. They were originally discovered thanks to industrial developments related to iodine production. Alginates are quite widespread in Nature. They can be found as major cell-wall components (up to 45% of the dry matter) in marine brown algae (*Phaeophyceae*). They also occur in acetylated form as exopolysaccharides produced by bacteria belonging to *Pseudomonas* and *Azotobacter* genders.

Alginate production by fermentation is technically feasible, but so far has not reached economical significance [2]. Industrially, alginates are only extracted from marine brown seaweeds (alginophytes) such as *Macrocystis* sp. (giant kelp), *Lessonia* sp., *Durvillaea* sp., *Laminaria* sp., and *Ecklonia* sp.

Macrocystis, *Lessonia*, and *Durvillaea* are endemic to the Pacific, whereas *Laminaria* is harvested and/or cultivated in the northern Atlantic and in Asia. *Ecklonia* sp. is mainly found on African coasts and in Asia.

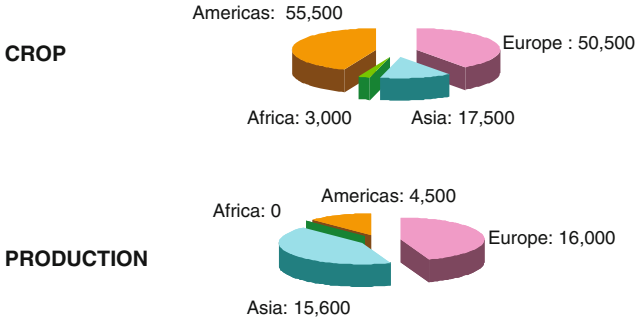


Fig. 1 Alginophyte WW crop and alginate WW production (tons dry weight) (data from FAO, 2001) [3]

Either seaweed harvested in the wild or cultivated seaweed is used as a raw material for alginate industrial extraction. Since brown seaweeds are among the fastest growing vegetable organisms on Earth and can be easily cultivated, alginates can be considered as an unlimited renewable raw material. They are expected to meet the needs of a steadily growing industry and the principles of green engineering and sustainable development. In 2001, worldwide alginate trade represented 36,100 metric tons (Fig. 1) for an estimated value of US$ 213 million.

Currently, the main markets for alginates are technical applications (textile printing, paper), food (texturizing solutions), pharmaceuticals (antacids), medical (wound dressings, dental prints), and biotechnological applications (encapsulation of living cells). Calcium alginate was also manufactured as a specialty technical fiber with high tenacity and fire retardant properties [4].

The term "alginate" refers to a broad family of linear unbranched copolymers of (1,4)-linked β-D-mannuronate (M) and (1,4)-linked α-L-guluronate (G) residues with widely varying composition and sequence. Within the heteropolysaccharide chain, the G and M residues can be found both distributed in homopolymer blocks (e.g., MMMM or GGGG) comprising between 20 and 30 repeating units, and in longer statistical or strictly alternating segments (MGMG) (Fig. 2) [5].

Macromolecular properties of alginates are highly dependent on G/M ratio and sequential distribution of G and M residues within homopolymeric blocks. Polyguluronate blocks are stiffer than polymannuronate and alternating blocks, which in turn are more soluble at low pH [6].

The composition and sequential distribution of M and G residues within alginate polymer vary according to the seaweed species, and also according to seasons and growth conditions [5]. Indeed, alginates are tailor-made by Nature to give different mechanical properties to different seaweeds and even to different tissues within a given seaweed (Table 1) [7].

The industrial process for extraction from seaweed biomass is based on two basic solution properties of alginates and alginic acid: (1) alginic acid and calcium alginate are insoluble in water and (2) sodium alginate is soluble in water. The two

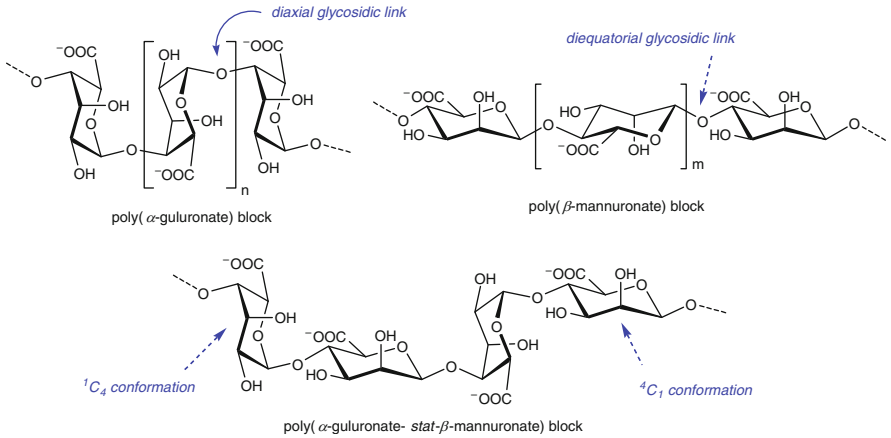


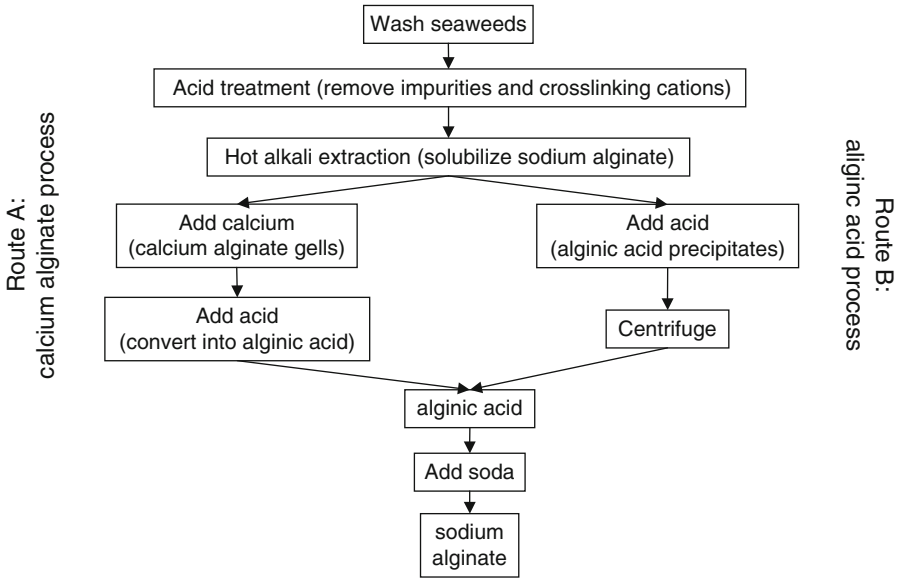
Fig. 2 Structure of the three constitutive blocks commonly found in natural alginates

Table 1 Average composition of seaweed alginates depending on their origin

Specie (part)	%MM	%MG	%GG
Laminaria hyperborea (stipe)	17	26	57
L. hyperborea (thallus)	36	38	26
Lessonia nigrescens	40	38	22
Lessonia trabeculata	25	26	49
Durvillaea antarctica	56	26	18
Laminaria digitata	43	32	25
Ecklonia maxima	38	34	28
Macrocystis pyrifera	38	46	16
Ascophyllum nodosum	44	40	16
Laminaria japonica	48	36	16

well-established routes for alginate extraction from algal material are summarized in Scheme 1.

Within the seaweed cell walls, alginate is ionically crosslinked by polycations originating from seawater (mainly calcium). The first step in alginate extraction is therefore acid treatment (so-called lixiviation) to convert alginate into insoluble alginic acid and remove cations and impurities (mineral salts, organic metabolites, contaminating polysaccharides such as fucoidan, laminaran, and also polyphenols when algae are not formylated). After solid/liquid separation and washing, sodium alginate is brought into solution by neutralization with alkali such as sodium carbonate. Then, by-products such as proteins, cellulose, and hemicellulose are separated and removed by flotation, filtering, or centrifugation. Alginic acid is precipitated from purified sodium alginate solution, dried, and finely ground. Final technical-grade sodium alginate is usually obtained by dry mixing alginic acid with sodium carbonate.



Scheme 1 Basic steps for alginate industrial extraction from seaweeds

In addition to sodium alginate, other soluble forms such as potassium alginate and ammonium alginate are commercially available.

1.2 Depolymerization of Polymannuronate Blocks

Alginate oligosaccharides prepared from alginate by acid hydrolysis or through the action of lyases represent attractive materials for the development of new industrial uses of alginate derivatives. Oligoalginates have been shown to present several potentially valuable biological properties for food, agricultural, and pharmaceutical applications [8–10].

Several authors have described the preparation of oligomannuronates by acid and/or enzymatic depolymerizations [11–19], but very few works deal with mannuronic acid in the monomeric form, probably because of the difficulty in hydrolyzing the α- or β-(1,4) links in alginates. Haug and co-workers have described the partial acid depolymerization of alginate to give homopolymer blocks of D-mannuronic acid (MM) and of L-guluronic acid (GG) with a degree of polymerization (DP) of approximately 20, in addition to heterogeneous blocks of both uronic acids (MG) [11–13]. The three types of blocks were separated by fractionated precipitation in accordance with their pH-dependant solubility in water (Table 2). Due to the difficulty of hydrolyzing the (1,4) linkages of the homopolymer blocks with an acid, very few groups have succeeded in preparing saturated oligouronates containing less than five osidic units. Shimokawa [20] and

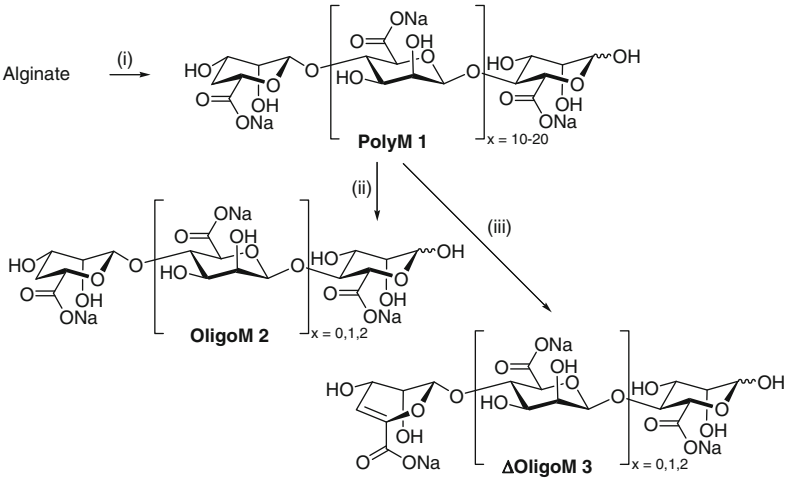
Table 2 Physical state of blocks as a function of pH

Physical state of blocks	pH < 2	2 < pH < 3	3 < pH
Precipitates	MM GG	GG	–
Solubles	MG	MG MM	MG GG MM

Matsubara [21] have reported the preparation of DP1–DP9 or DP1–DP12 saturated oligoguluronate mixtures in 11–12% yields. Nevertheless, there is no related methodology in the literature for the synthesis of these oligouronic acids on a multigram scale and with a low average DP and restricted polydispersity.

We initially investigated the synthesis of sodium mannuronate polymers **PolyM 1** by applying Haug's methodology on a semi-industrial scale (Scheme 2). The algae used as raw materials for the production of commercial alginates led to polysaccharides with different uronic acid compositions: *Laminaria digitata*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* are known to contain mannuronate-enriched alginates, whereas those present in *Laminaria hyperborea* are characterized by a lower mannuronate/guluronate ratio. Preliminary laboratory tests on various commercially available alginates permitted us to select S20NS alginate from *L. digitata* (higher M/G ratio, lower composition in MG blocks) for the pilot-plant transfer, thus allowing the preparation of sodium polymannuronates **PolyM 1** with an average DP of 16 and on a 100-g scale in a 23% yield [6, 22]. The production of these homopolymer mannuronate blocks was based upon the following steps: (1) acidification of an aqueous solution of alginate by 0.3 N HCl until pH 0.9 at 95–100°C; (2) after cooling, removal of two thirds of the liquid phase and centrifugation of the rest of the suspension to give a precipitate composed of MM and GG blocks; (3) solubilization of the homopolymers in water by addition of aq. 0.1 N NaOH until pH 4.5; (4) acidification to pH 2.85 with aq. 0.025 N HCl, and addition of diatomaceous earth; (5) filtration and neutralization of the filtrate (aq. 0.1 N NaOH); (6) ultrafiltration of the solution (8,000 D) and lyophilization to furnish sodium polymannuronates **PolyM 1**.

A first family of saturated oligomannuronates **OligoM 2** was produced by treating **PolyM 1** with acid in order to reduce the DP. Due to the insolubility of MM blocks at pH lower than 2.85, the reaction was performed at pH 3; heating to 100°C for 8 h led to a mixture of oligomannuronates with an average DP of 5 estimated by integration of the characteristic ^{1}H signals in ^{1}H NMR spectra (5.09–5.11 ppm, H-1α of reducing-end; 4.78 ppm H-1β of reducing-end; 4.53, 4.57 ppm, H-1 of nonreducing positions). Separation of oligouronic acids was generally performed by a high-performance liquid chromatography ion-pair method although the use of this powerful analytical approach in the case of charged oligomers could not be easily extended to a multigram scale. We therefore focused our attention on the development of other separation methodologies with the aim of reducing the polydispersity of our uronate mixtures. The most efficient purification method was found to be an ultrafiltration process with a 3,500 D cell membrane cut-off which permitted both elimination of the highest oligomers and the isolation of several grams of enriched DP 2–4 saturated uronates **OligoM 2** (Scheme 2) in the presence of sodium chloride.



Scheme 2 Depolymerization of alginate to polymannuronates **PolyM 1** and saturated **OligoM 2** and unsaturated **ΔOligoM 3** oligomannuronates. (i) aq. HCl, pH 0.9, 23%; (ii) aq. HCl, pH 3.0, 89%; (iii) enzyme (AL951), [24] pH 7.5, 22°C, 75%

A second family of unsaturated oligomannuronates **ΔOligoM 3** was additionally prepared through an enzymatic route (Scheme 2). Alginate lyases [19] depolymerize alginate through a β-elimination reaction that releases unsaturated oligosaccharides containing a 4-deoxy-L-*erythro*-hex-4-ene pyranosyluronate moiety at their nonreducing terminal uronate residues [23]. The enzyme specificity towards poly-M, poly-G, or poly-MG blocks is frequently difficult to control and complex mixtures of M- and/or G-containing uronides of various lengths are usually provided by enzymatic alginate hydrolysis [23]. Taking into account this problem, an enzymatic method, utilizing **PolyM 1** and the enzyme (AL951) [24] produced by *Pseudomonas alginovora*, was developed to prepare oligomannuronates (DP $\leq$ 4) incorporating 4,5-unsaturated nonreducing terminal residues. The enzymatic degradation of **PolyM 1** (50 g) was performed in an aqueous solution (5 L) containing enzyme (AL951) (enzyme/PolyM = 1 wt%) at 22°C for 24 h with a 0.02 N ionic strength (NaCl), and at pH 7.5 (aq. 1 N NaOH) in order to avoid the depolymerization of GG- and MG-/GM-blocks (Scheme 2). The depolymerization process was then stopped by adding dilute aq. HCl until pH 6.5. Purifying by an ultrafiltration process with a 3,500-D cell membrane cut-off permitted elimination of the enzyme in addition to oligomannuronates with a DP $>$ 5 and DP 2–4 unsaturated mannuronates (**ΔOligoM 3**, 75% yield) were isolated in the presence of salts (Scheme 2).

1.3 Direct Route to Oligomannuronates from Alginate

In order to reduce the cost of oligomannuronates and to make their manufacturing process easier, more direct routes to saturated and unsaturated oligomannuronates

were envisaged. The first time, two procedures were developed from alginate without requiring the intermediate step relative to the production of **PolyM 1**.

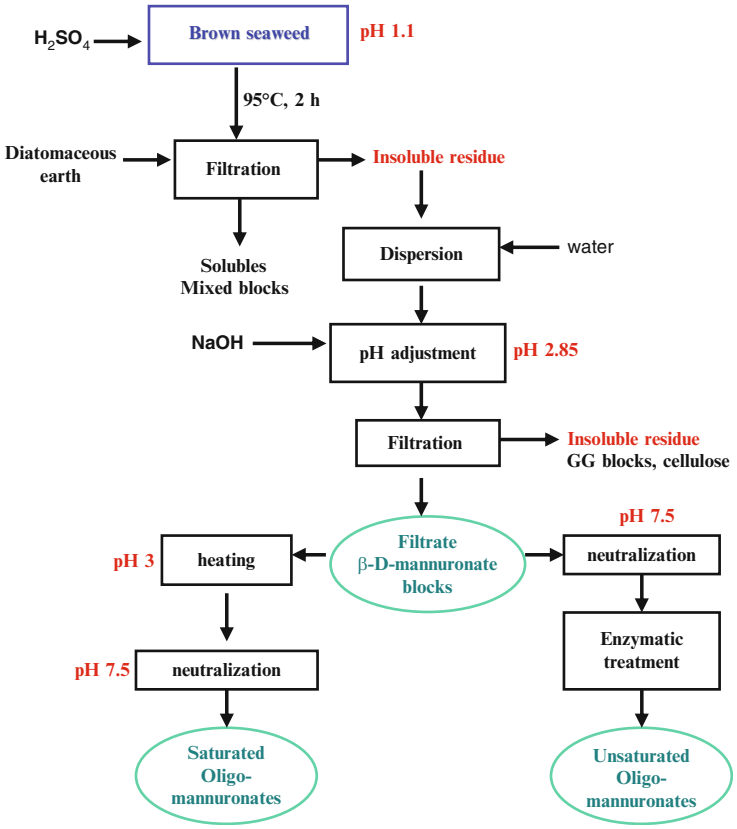
The process proposed for the production of saturated oligomannuronates **OligoM 2** is based on the partial acid hydrolysis of alginate. By heating an acidic (pH 1) suspension of alginate at 100°C, the MG mixed blocks are cleaved whereas MM and GG homopolymeric blocks remain under a precipitated form and are undamaged (Table 2). Then mixed blocks are eliminated by a simple solid/liquid separation. After increasing the pH to 2.85, MM blocks become water soluble and are recovered by (1) a second filtration to remove the insoluble GG blocks, and (2) a lyophilization of the resulting filtrate. This direct procedure allowed the isolation of **OligoM 2** (DP 2–4) in a 25% overall yield.

In the same way, unsaturated oligomannuronates **ΔOligoM 3** were produced directly from alginate by an enzymatic degradation. The enzymatic hydrolysis of alginate was achieved in an aqueous solution containing enzyme (AL951) (enzyme/alginate = 0.5 wt%) at 22°C for 24 h and at pH 7.5 (aq. 1 N NaOH). An ultrafiltration process with a 3,500-D cell membrane cut-off permitted the elimination of the enzyme, the guluronic homopolymeric and heteropolymeric sequences as well as oligomannuronates with a DP > 5. **ΔOligoM 3** (DP 2–4) were isolated in a 40% yield after lyophilization of the permeate.

1.4 Direct Route to Oligomannuronates from Marine Algae

Further simplification of the process used to make oligoalginates was developed directly from dried or fresh brown seaweeds as the starting materials instead of alginates. A new process was then implemented to produce various oligomannuronate mixtures from seaweeds [6]: oligosaccharide mixtures composed of (1,4)-linked β-D-mannuronic acid were prepared by (1) acid treatment (saturated oligomers) or (2) enzymatic hydrolysis (unsaturated oligomers).

The procedure is based on the following steps (Scheme 3). (1) The fresh or dried seaweed is stirred with sulfuric acid (pH 1.1, H_2SO_4 concentration: 0.15 mol/L) at 95°C for 2 h. Calcium and magnesium alginates are converted to alginic acid, alginate mixed blocks are hydrolyzed and the major part of algal molecules are solubilized (solubles: fucans, mannitol, proteins, peptides, amino acids). (2) A separation by filtration after adding diatomaceous earth is efficiently achieved between (a) the insoluble homopolymer MM, GG blocks and algal cellulose, and (b) the solubles and mixed MG, GM blocks. (3) The insoluble residue containing MM and GG blocks in addition to cellulose is dispersed into water and the pH is increased to 2.85 (aq. 30% NaOH). Homopolymer MM blocks are then released from the solid fraction. (4) A simple filtration provides an acid filtrate of **PolyM** blocks. (5) The heating of the filtrate at 95°C at pH 3 for 8 h leads to the formation of saturated **OligoM** oligomannuronates (overall yield: 14.4%: 7.5 g oligomers from 60 g *L. digitata* micronized flour). (6) In parallel, the **PolyM**-containing solution is neutralized (pH 7.5; aq. 1 N NaOH) and an enzymatic treatment



Scheme 3 Process for the production of saturated oligomannuronates **OligoM 2** and unsaturated oligomannuronates **ΔOligoM 3** from brown seaweed

(AL951) furnishes unsaturated **ΔOligoM** oligomannuronates (overall yield: 14.7%; 7.9 g oligomers from 60 g *L. digitata* micronized flour).

2 Chemical Transformations of Oligomannuronates into Surfactants

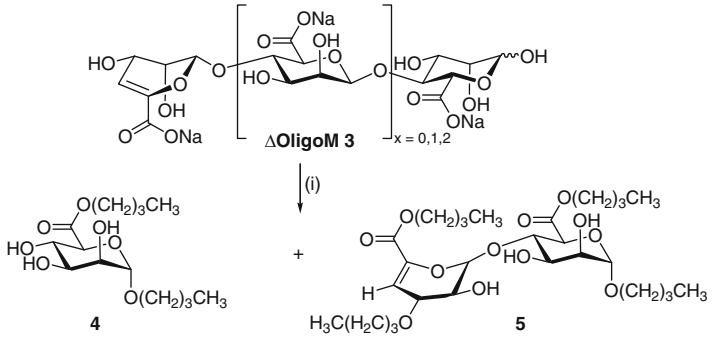
2.1 Synthesis of Butyl Monosaccharide and Disaccharide Mannuronates

Nowadays, no saturated or unsaturated mannuronic acid derivatives are commercially available in a monomeric form, thereby limiting their uses in chemical synthesis. In contrast to D-glucuronic, D-galacturonic, or L-iduronic acids, only the D-mannurone lactone form (D-mannofuranurono-6,3-lactone) was available for

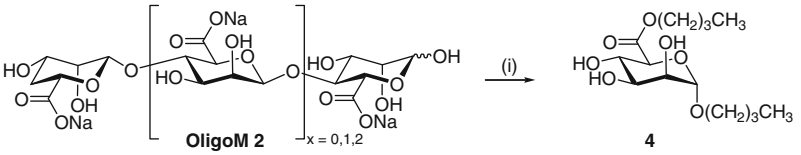
mannuronic acid but today this product is no longer marketed. Consequently, no major chemical derivatizations of this substrate such as esterification, amidification, etherification, or glycosylation reactions were fully investigated. However, the development of environmental friendly synthetic routes to mannuronate-based monomers, easily applicable on an industrial scale, could be of great importance for the design and the production of innovative high added value uronate derivatives.

Saturated and unsaturated oligomannuronates of DP 2–4 represent elective starting materials for the preparation of new mannuronate monomers. Acid treatment of oligomannuronates in butanol was found to be the most adequate strategy for the transformation of these oligomers into di- and/or monosaccharides. The use of other organic solvents was inappropriate mainly due to (1) the low dispersion of the substrates and the products in the reaction mixture, (2) the unreactivity of the substrates because of their insolubility in nonaqueous media, and (3) the difficulties in product purification and analysis. In order to overcome these problems, the use of butanol as the solvent to make the dispersion of sodium uronates easier, as well as acidic conditions to favor hydrolysis, esterification and/or glycosylations was envisaged.

Treatment of mannuronate oligomers **ΔOligoM 3** with methanesulfonic acid (MSA, 4 equiv.) in butanol at 50–55°C for 5 days with several successive azeotropic coevaporations (H_2O/BuOH) under reduced pressure, provided *n*-butyl (*n*-butyl α-D-mannopyranosiduronate) **4** and *n*-butyl [*n*-butyl 4-deoxy-β-D-*threo*-hex-4-ene pyranosid) uronate-(1→4)-*n*-butyl α-D-mannopyranosid] uronate **5**, in 35% and 25% yield, respectively (Scheme 4) [25]. They could be easily separated by column chromatography (CH_2Cl_2–CH_3OH: 98/2, v/v). These two derivatives are the result of four *one pot* reactions: acid hydrolysis of glycosidic linkages, esterification, glycosylation, and allylic ether formation reactions. First, the acid hydrolysis of the β-(1,4) linkages of the oligomers gave uronates in a monomeric or a dimeric form, thereby reducing the number of reaction products. Second, acidification of the carboxylate functions followed by esterification with butanol afforded the corresponding butyl esters. Next, Fisher glycosylation with the short alcohol



Scheme 4 Chemical transformation of unsaturated oligomannuronates of DP 2–4 into monomer **4** and dimer **5** mannuronates. (i) MSA, BuOH, 50–55°C, 5 days, **4**: 35%; **5**: 25%



Scheme 5 Chemical transformation of saturated oligomannuronates of DP 2–4 into monomannuronate **4**. (i) MSA, BuOH, 112–115°C, 24 h, 50%

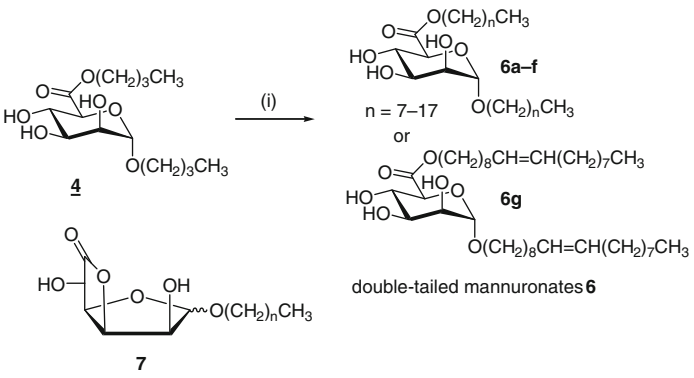
stereoselective provided alkyl glycosides **4** and **5** in a pyranoside form and with an α-configuration (the low-field resonance $\delta = 100.5$ ppm observed for the anomeric carbon is indicative of an α-configuration). Finally, the half-chair conformation $^{1}H_{2}$ of the 4,5-unsaturated residue of **5** which is derived from the altrose series was evidenced by 2D-COSY ^{1}H NMR, ^{1}H-^{13}C correlation spectra and t-ROESY experiments [$J_{H1'H2'} = 2.3$ Hz, $J_{H2'H3'} = 6.9$ Hz, distance between H1′ and H3′, δ(H1′–H3′) δ(H1–H5), and δ(H2′–H3′) δ(H3–H4)], demonstrating the inversion of the allylic alcohol C3′ configuration during the reaction of BuOH with the carbocation intermediate.

MSA appeared to be the best acid catalyst since it is an easy-to-handle liquid, often recyclable and less aggressive than sulfuric acid or HF. It is considered as readily biodegradable, ultimately forming sulfates and carbon dioxide [26]. More importantly, it provides tautomerically and anomerically pure butyl products unlike other acid conditions such as hydracids (HCl) or acid resins which furnish a mixture of pyranoside/furanoside alkyl glycosides. Additionally, in contrast to hydracids, MSA does not exhibit oligomerization processes.

The same acidic reaction conditions were further applied to saturated oligomers **OligoM 2** which result from the acidic depolymerization process (Scheme 5). In refluxing butanol for 24 h and in the presence of MSA (4 equiv.), these saturated oligomannuronates were efficiently transformed into *n*-butyl (*n*-butyl α-D-mannopyranosiduronate) **4** (50% yield) [22]. The scaling up of this chemical process was clearly demonstrated on several hundred grams of reagents. The use of polymannuronates instead of oligomers gave the same monomeric compound **4** but the reactions proceeded more slowly and the monomannuronate **4** was generally isolated in a lower yield (30%). Noteworthy, the replacement of MSA by sulfuric acid successfully led to the formation of the monomer product but additional nonidentified impurities were observed as well as a more intense black coloring of the reaction mixture.

2.2 Double-Tailed Surfactants

The controlled introduction of two lipophilic alkyl chains into mannuronate monomers represents a convenient way of producing double-tailed surfactants (Scheme 6). Indeed, the incorporation of longer hydrophobic alkyl chains than the two butyl groups brings a more amphiphilic character to the glycosides.



Scheme 6 Synthesis of double-tailed mannuronates **6** and chemical structure of by-product lactone **7**. (i) MSA, $CH_3(CH_2)_nOH$ or $CH_3(CH_2)_7CH{=}CH(CH_2)_8OH$, 65°C, 2–5 mbar, **6a** ($n = 7$): 67%; **6b** ($n = 9$): 58%, **6c** ($n = 11$): 72%, **6d** ($n = 13$): 61%, **6e** ($n = 15$): 58%, **6f** ($n = 17$): 50%, **6g**: 45%

The presence of these hydrophobic alkyl chains with various lengths allows the amphiphilic molecules to cover a wide Hydrophile–Lipophile Balance (HLB) range suitable for different types of colloidal systems [27, 28]. Long alkyl chains give the surfactants predominantly hydrophobic properties and corresponding low HLB numbers. This means that they will be able to stabilize colloidal systems such as water-in-oil emulsions. Decreasing the alkyl chain length produces more hydrophilic surfactants (high HLB values) which can conversely stabilize oil-in-water emulsions.

These alginate-derived molecules are a new class of uronate amphiphiles that complete the family of sugar-based surfactants [29]. The ester and acetal functionalities of these products reproduce the main structural characteristics of both sugar ester-type (sucrose and sorbitan esters) [30] and alkyl glycoside-type (alkylpolyglycosides, APGs) [30] surfactants. Noteworthy, the carboxylate moiety of the mannuronate residue makes the introduction of a single ester function easier than in the case of sucrose esters which generally consist of mono-, di-, tri-, or tetraester mixtures.

With the aim of proposing simple environmentally benign procedures, simultaneous transesterification and transglycosylation reactions were carried out with *n*-butyl (*n*-butyl α-D-mannopyranosiduronate) **4** in fatty alcohols (octyl, decyl, dodecyl, tetradecyl, hexadecyl alcohols) without any additional solvent, at 65°C, in the presence of MSA and at low pressure (2–5 mbar) in order to eliminate the butanol formed in the reaction. The main products are the *n*-alkyl (*n*-alkyl α-D-mannopyranosiduronates) **6** which are the thermodynamically and kinetically products. A minor compound was defined as the *n*-alkyl D-mannofuranurono-6,3-lactone **7** (Scheme 6) which was previously synthesized from commercial D-mannofuranurono-6,3-lactone [31]. The kinetics of the reaction was then evaluated by GC studies [22] and permitted to define the best reaction conditions relative to the amounts of MSA, fatty alcohols and time conversion. The best yields for the

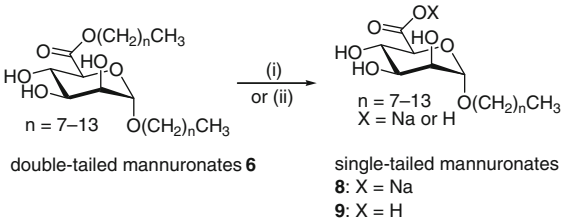
synthesis of compounds **6** (45–72%) were obtained when monomer **4** was treated with 1 equiv. of MSA, 8 equiv. of fatty alcohol, at 65°C for 6 h. A small amount of noncharacterized by-products (5%) and lactone **7** (15% yield) were detected by GC in addition to a nonidentified intermediate which disappeared with the formation of the desired compounds **6**. Thus, this one-pot two step procedure provided original double-tailed sugar surfactants characterized by the presence of two similar alkyl chains connected to the carbohydrate moiety by both ester and glycosidic connecting linkages with a high stereocontrol at the anomeric position.

A process in semi-continuous mode was additionally performed from several hundred grams of saturated oligomannuronates **2** which were first hydrolyzed, glycosylated, and esterified with butanol in a 5-L reactor. After neutralizing the reaction mixture and removing on silica gel the salts and the degradation products formed during these reactions, subsequent transesterification and transglycosylation reactions with 4 equiv. of dodecanol were carried out under the conditions described in this subsection (1 equiv. MSA, 65°C, 3 mbar, 6 h) to provide the corresponding dodecyl mannuronate **6c** in quite satisfactory yield. After the work-up (neutralization, purification on silica gel), dodecanol was added to the isolated double-tailed surfactant in order to obtain a self-emulsifying base (surfactant **6c**/dodecanol) characterized by a 50/50 ratio in weight. This base was then discolored by hydrogen peroxide (7.5% in weight) in the presence of 1N NaOH (1.3%) at 65°C to give a final yellow product characterized by a 5.4 pH value compatible with applications in cosmetics.

2.3 Single-Tailed Surfactant Acids and Salts

Since nonionic carbohydrate-derived surfactants are available in sufficient quantities and at competitive costs at present, anionic versions of these chemicals such as carboxylates, sulfates, and phosphates are less common. Until now there have been only a few products (anionic derivatives of APG) established in the market [29]. Indeed, the derivatization of neutral sugar-based surfactants to furnish anionic compounds is not easy at a competitive cost with the sulfate (alcohol ether sulfates, AES) and sulfonate (linear alkylbenzene sulfonates, LAS) surfactants derived from petrochemicals. Within this context, uronate derivatives possessing carboxylate functionality represent attractive starting materials for the preparation of negatively charged surfactants.

Direct transformation of double-tailed mannuronates into single-tailed sodium uronates was simply achieved through saponification (Scheme 7). Aqueous 0.1 N NaOH (1.1 equiv.) was slowly added to a solution of *n*-alkyl (*n*-alkyl α-D-mannopyranosiduronate) **6a–c** (1 equiv.) in CH_2Cl_2. After stirring for 1 h at room temperature, the reaction medium was concentrated under reduced pressure at 30°C and the residue was diluted with hot CH_3OH. Silica gel was added and the mixture was concentrated under reduced pressure and dried under vacuum. The residue was suspended in hot CH_2Cl_2, filtered, and rinsed several times with



double-tailed mannuronates **6**

single-tailed mannuronates
8: X = Na
9: X = H

Scheme 7 Synthesis of single-tailed sodium mannuronates **7** and mannuronic acids **8**. (i) aq 0.1 N NaOH, CH_2Cl_2 [**8a** ($n = 7$): 90%; **8b** ($n = 9$): 88%; **8c** ($n = 11$): 89%]; (ii) aq 0.1 N NaOH, CH_2Cl_2 then aq. 5% HCl, 0°C [**9a** ($n = 7$): 90%; **9b** ($n = 9$): 88%; **9c** ($n = 11$): 89%; **9d** ($n = 13$): 88%]

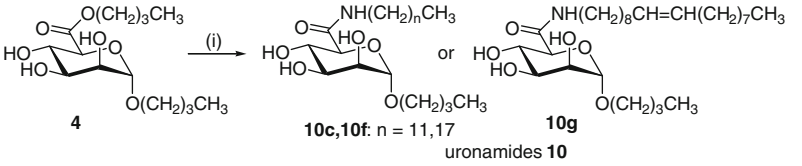
CH_2Cl_2 until total elimination of the formed fatty alcohol. The silica gel was finally rinsed with 2-propanol/EtOAc/H_2O (6:3:1). After concentration and drying, purification by dialysis at 100 D afforded sodium *n*-alkyl α-D-mannopyranosiduronates **8**.

The acid form of these single-tailed surfactants was achieved by successive basic (0.1 N NaOH, 1.1 equiv., in water/CH_2Cl_2; 1 h) and acidic (1 N HCl at 0°C until pH 1) treatment of compounds **6a–d** to give the corresponding α-D-mannopyranosidic acids **9a–d**. These carboxylic acids are more hydrophobic than the corresponding sodium salts, thereby modifying the interfacial properties and particularly the CMC values (see Sect. 3.1) that should decrease with this increasing hydrophobicity. These results are of much interest as this is one of the very few examples where *O*-glycosiduronic acids have been synthesized without protecting groups and with a high stereocontrol.

2.4 *Uronamide-Type Surfactants*

An advantageous property of amides over esters is that they are less sensitive to hydrolysis under alkaline conditions. Commercial fatty acid glucamides having a linear headgroup derived from D-glucitol coupled via an amide linkage to an alkyl chain are manufactured by reductive amination of glucose with methylamine, followed by aminolysis of an activated fatty acid [30]. In contrast, the synthesis of glycosamides with a cyclic carbohydrate residue has not widely been investigated for industrial developments. Nonetheless, the physicochemical properties (water solubility, interfacial properties) could be significantly changed by the presence of a cyclic structure instead of a linear acyclic carbohydrate moiety.

Starting from *n*-butyl (*n*-butyl α-D-mannopyranosiduronate) **4**, uronamides **10** bearing a short butyl chain at the anomeric position and a longer alkyl (dodecyl, octadecyl, and oleyl) chain amide-linked to the sugar head were prepared following a one-step solvent-free procedure (Scheme 8). The aminolysis reaction was performed at 65°C to make the fatty amine liquid and under reduced pressure (3 mbar) to eliminate the butanol formed all through the reaction. To avoid significant



Scheme 8 Synthesis of uronamides **10**. (i) $CH_3(CH_2)_nNH_2$ or $CH_3(CH_2)_7CH{=}CH(CH_2)_8NH_2$, 65°C, 3 mbar, **10c** ($n = 11$): 76%, **10f** ($n = 17$): 82%, **10g**: 78%

amounts of unreacted fatty amines, which could be considered as irritable agents in cosmetics, the butyl mannuronate **4** was used in slight excess, enabling the complete consumption of the amine. Chemically pure uronamides **10** can then be isolated in excellent yields (76–82%) after column chromatography on silica gel. They can also be used as mixtures composed of uronamides and starting butyl mannuronate, without any further treatment of the reaction media, for industrial applications.

As for double-tailed mannuronates, a semi-continuous mode was developed for the production of dodecyl and octadecyl uromanides **10c**, **f**, in a 5-L reactor. The crude reaction mixture resulting from the production of butyl mannuronate **4** was neutralized until pH 7 (1 N NaOH) and partly concentrated before adding the fatty amine at 70°C. The resulting mixture was stirred under progressive reduced pressure (400 to 1 mbar) at 60°C until gas evolution stopped. Water was then added and the solution was stirred at 70°C for 15 min. The organic products flocculate and solidify at room temperature which permits the easy removal of the salt-containing aqueous phase. This solvent-free process represents a promising way of manufacturing nonionic surfactants derived from mannuronates without requiring the standard steps of solid–liquid separations.

3 Physicochemical Properties of Alginate-Derived Surfactants and Potential Applications

3.1 CMC and Surface Tension Measurements

Surfactants are amphiphilic molecules that exhibit various interactions depending on their molecular structures. The polar part engages in electrostatic interactions (hydrogen bonding, dipole interactions, ionic bonding, etc.) with surrounding molecules, e.g., water and ions. The nonpolar part, on the other hand, associates with neighboring nonpolar structures via hydrophobic and van der Waals interactions. When added to an aqueous solution, surfactant molecules minimize their energy by creating a monolayer on the air–water surface [32, 33]. The hydrophobic parts of the surfactants are directed towards the less polar air, while the hydrophilic parts of the surfactants are directed towards the polar water molecules. Upon

Table 3 CMC and surface tension values of anionic and neutral single-tailed compounds **8a**, **c**, **9c**, **d** in comparison with standard neutral surfactants **PEG** and **APG**

Compounds	CMC (mmol/L)	CMC (g/L)	γCMC (mN/m)
8a (C8)	1.62	0.49	30.2
8c (C12)	0.28	0.11	29.5
9c (C12)	0.13	0.05	30.5
9d (C14)	0.16	0.06	29.7
PEG	0.08	0.04	33
APG	0.26	0.09	30

increased surfactant concentration, the surface becomes increasingly saturated by surfactant molecules, which decreases the surface tension of the solution (value obtained lower than the initial 73 mN/m surface tension between water and air).

One of the most widely used indexes for evaluating surfactant activity is the critical micelle concentration (CMC). The CMC is in effect the solubility of a surfactant within an aqueous phase, or the minimum surfactant concentration required for reaching the lowest interfacial or surface tension values γ. At concentrations above the CMC, surfactants associate readily to form micelles that can be spherical, oblate, tablet shaped, or rod-like, with a hydrophilic surface and a hydrophobic interior. Such micellar structures usually have hydrodynamic radii, ranging from 200 to 400 Å. The CMC value is estimated from the inflection point in the γ vs log C curve.

Studies of the CMC value and the surface tension of aqueous solutions of salt and acid single-tailed compounds **8a**, **c** and **9c**, **d** have been carried out [22]. They give a measure of the amount of surfactant needed for a given application. Surface tension experiments were not investigated from double-tailed surfactants because of their low water solubility. The values were measured with a drop tensiometer. A syringe with a U-shaped needle was lowered into a sample cell containing an aqueous solution of surfactant, and an air bubble was produced from the syringe. The dynamic surface tension was measured by filming the rising bubble and analyzing the contour of the bubble according to axiasymmetric drop-shape analysis (ADSA) with a Tracker instrument from IT Concept [34]. The surface tension was thus determined at room temperature for several concentrations of surfactants in pure water (Fig. 3). As shown in Table 3, four different amphiphilic molecules differing in their hydrophilic moieties (anionic or nonionic), and the length of the linear hydrophobic chains were studied. Compounds with C14–C18 fatty chains were not soluble enough in water at room temperature to exhibit surface tension properties under standard conditions. The data also include values obtained with industrial nonionic surfactants, such as alkylpolyglucoside (APG) and polyethylene glycol (PEG) derivatives possessing a dodecyl alkyl chain, in order to carry out a comparison.

Compounds **8a**, **c** and **9c**, **d** reduce the surface tension to values (29–30 mN/m) that compare favorably with those obtained with commercial nonionic surfactants (PEG, APG) [35]. The surface tensions were found to be independent of the sugar

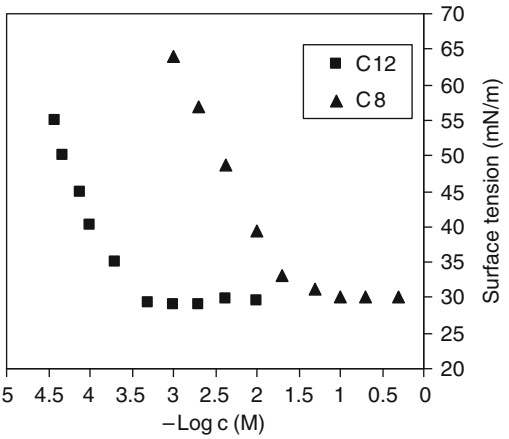


Fig. 3 Example of the γ vs log C curve for anionic single-tailed compounds **8a** (C8) and **8c** (C12)

residue hydrophilicity and the hydrophobic character of the alkyl chains, unlike the CMC values. In all cases, carboxylate derivatives exhibited higher CMC values than their neutral counterparts. This is clearly due to the presence of the negative charge, which renders the amphiphilic molecule more soluble in water. As expected, the CMC values decrease with increasing alkyl chain length.

3.2 Foaming Behavior

Good foaming behavior is also an attractive property for the use of surfactants in body-cleansing products, such as shower gels and foam baths or hair shampoos. Ether sulfates are the most important class of surfactants used for cosmetic cleansing formulations. The foamability and foam stability of the C12 sodium alkyl α-D-mannuronate **8c** and the C12–C14 acid forms **9c**, **d** was studied by a fairly simple test in comparison with a sodium dodecyl sulfate (SDS) reference. Foam was formed by bubbling air into a round-bottomed flask containing an aqueous solution of surfactant, and it was continuously transferred into a beaker until a given volume of foam was reached [36–39]. The air flow was then stopped and the foam stability was recorded. The foaming performance was then evaluated as the time required to obtain the desired volume (2 L) of foam, and the foam stability corresponds to the foam residual volume observed with time. Surfactant concentrations of 0.1 or 0.2 g/L were chosen for studies above CMC (Table 4).

Anionic surfactant **8c** did not exhibit good foaming properties in so far as the foam rapidly collapsed before its transfer into the beaker. It is noteworthy that mannuronic acids **9c**, **d** are high-foaming anionic surfactants that are comparable with the ether sulfate derivative. In particular, similar values are observed for

Table 4 Time required to obtain 2 L of foam for compounds **8c** and **9c**, **d** in comparison with SDS

Compounds	C (g/L)	t (mn)
8c (C12)	0.1	–[a]
9c (C12)	0.1	3
9d (C14)	0.2	12
SDS	1	1.5

[a]The collapse of foam occurred before its transfer into the beaker

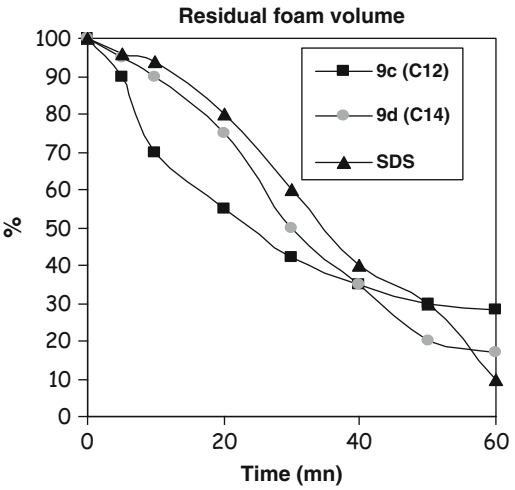


Fig. 4 Foam stability with time for mannuronic acids **9c**, **d** in comparison with SDS

surfactant **9c** and SDS both in regard to foam performance (Table 4) and foam stability (Fig. 4). Applications of this dodecyl α-D-mannopyranosiduronic acid in foaming formulations (shampoos, body washes) are currently envisaged with an industrial partner.

3.3 Emulsification Properties

Surfactants are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids, and gases, thereby permitting them to mix or disperse readily as emulsion in water or other liquids [32, 33]. Emulsification thus involves the formation of a liquid–liquid interface. Microemulsions are macroscopically homogeneous and thermodynamically stable mixtures of a surfactant, oil and water. On a microscopic level, they consist of oil-domains separated from water-domains by a surfactant film. Microemulsions are good solvent of both organic and inorganic compounds, which is a useful property, for example, in catalyzing the hydrolysis of fat compounds. Macroemulsions (often called just emulsions), on the other hand, are not thermodynamically stable. Here,

surfactants are used to retard the breakdown process. The emulsification properties of a surfactant can be predicted relatively well using the HLB concept. The hydrophilic–lipophilic balance (HLB) concept [27, 28] is a numerical value that can be calculated and/or determined experimentally for each surfactant, since each structural constituent is given a numerical value. This HLB number indicated for which properties the surfactant may be useful. For instance, the following applications have been suggested: w/o emulsifier (HLB of 3–6), wetting agent (7–9), o/w emulsifier (8–18), detergent (13–15), and solubilizer (15–18). A low value indicates a hydrophobic surfactant, while a high number indicates a hydrophilic surfactant.

Double-tailed ester-type surfactants **6a–c**, **f**, **g** and uronamides **10c**, **f**, **g** were studied as to their properties as emulsifying agents. Three systems were studied; sunflower oil–water, paraffin oil (Marcol 82)–water, and capric/caprylic triglycerides (Oleon)–water. In order to determine the w/o or o/w type of emulsions formed in the presence of the surfactants, the drop-dilution method was used. To a small portion of the emulsion (surfactant/water/oil: 5/47.5/47.5 in weight) placed on a slide, a drop of water with a pin point is added and stirred slightly. If the water blends with the emulsion, it is an oil-in-water emulsion, but if oil blends with the outside phase it is a water-in-oil emulsion. As indicated in Table 5, ester-type and amide-type compounds **6a–c** and **10c**, based on C8 to C12 fatty alcohols and amines, are able to form o/w emulsions whereas surfactants **6f**, **g** and **10f**, **g** composed of stearic (C18) or oleic (C18:1) alkyl chains exhibit w/o emulsions.

HLB values of the surfactants **6a–c**, **f**, **g** and **11g** have also been evaluated experimentally by using the required HLB concept of the oil/water system [40]. The HLB system predicts the optimum emulsion stability when the HLB value of the surfactant systems matches the required HLB of the oil/water system. The required HLB is the value at which enhanced emulsion stability will be attained. Optimization of the performance can be achieved by only including surfactant systems with similar HLB values. Mixtures composed of a mannuronate-type surfactant and a commercial cosurfactant with a known HLB value (Span 85, Brij 72, Span 40, Span 20) were formulated with various surfactant/cosurfactant ratios (20, 40, 60, and 80 wt%) to create different HLB values of the system. Then, the performance was determined and plotted vs the HLB. A maximum appears in the plot and the

Table 5 Type of emulsion and HLB vales determined for compounds **6a–c**, **f**, **g**, and amides **10c**, **f**, **g**

Type	Surfactant	Emulsion	HLB
Esters	**6a** (C8–C8)	o/w	11
	6b (C10–C10)	o/w	11
	6c (C12–C12)	o/w	11
	6f (C18–C18)	w/o	5
	6g (C18:1–C18:1)	w/o	4
Amides	**10c** (C4–C12)	o/w	–[a]
	10f (C4–C18)	w/o	–[a]
	10g (C4–C18:1)	w/o	5

[a]Not determined

corresponding HLB maximum is equal to the required HLB. The resulting experimental HLB values (Table 5) were found to be in good agreement with the type of emulsion determined by the drop-dilution method.

Furthermore, highly stable emulsions were obtained with time (several months) and at high temperature (45°C) with vegetable (sunflower) oils, mineral (paraffin) oils, and fatty ester formulations including these new synthetic alginate-derived emulsifiers. Self-emulsifying bases resulting from surfactant **6c** or **10c**/dodecanol mixtures characterized by a 50/50 ratio are now under investigation for applications in cosmetics.

4 Conclusion and Perspectives

The results described here show that brown seaweeds could represent attractive renewable raw materials for the preparation of novel biocompatible surfactants. They open a new way of industrial development for a biomass which is often considered as a waste. Several novel mannuronate-based surfactants were synthesized and some of their surface properties were characterized and compared with those of commercial products. The syntheses are generally environmentally friendly using biodegradable reagents and most synthetic routes are solvent-free. These procedures should be possible to scale up for industrial production, and some processes have already been developed in a semi-continuous mode for the production of mannuronate-type surfactants in a multigram scale. It clearly appears that some of these amphiphilic compounds are good candidates for both trivial and more complex applications in such fields as cosmetics, health, and agrochemistry. In particular, mannuronate salts could represent potential alternatives to nonbiodegradable anionic surfactants derived from petrochemicals which represent the major part of surfactant market worldwide. Additionally, emulsifying formulations based on mannuronamide derivatives could substitute APG-based emulsifiers; studies relative to emulsion stability in the presence of electrolytes are under evaluation to assess the interest of these new oligomannuronate-derived products in cosmetics.

Furthermore, the results achieved sowed the seeds for future developments which are under investigation in ENSCR/CEVA laboratories: (1) the exploration of additional marine algae as sources of innovative carbohydrate-based materials and (2) the possibility of exploiting intact oligoalginates as original hydrophilic moieties for the production of new surfactants and glycopolymers.

Very recently, our attention was also focused on another family of algae, green seaweeds, as a source of natural polysaccharides. Green seaweeds are distributed worldwide and are very common in coastal areas. They overgrow as green-tides in eutrophicated areas as in Brittany or Venice lagoon and they create both ecological and economical problems. These marine algae contain on the dry weight basis 40–50% of cell wall polysaccharide with a majority of water soluble ulvan. Ulvan is a complex sulfated polysaccharide composed of different repeating chemical

sequences mostly based on disaccharides made of rhamnose, glucuronic acid, iduronic acid, xylose, and sulfate [41]. The unique chemical and physicochemical properties of ulvan make this family of polysaccharides attractive candidates for novel functional and biologically active polymers for food/feed, pharmaceutical, chemical, aquaculture, and agriculture domains [41]. A research program has already been initiated to transform these polymers into monomeric [42], oligomeric, or polymeric [43] surfactants using several chemical and/or enzymatic processes. These original derivatives exhibited attractive emulsifying, coemulsifying, and moisturizing properties that could be used for potential applications in cosmetics, pharmaceutics, or as dispersants for oil-spills.

Finally, oligoalginates are potential new starting materials for the synthesis of glycopolymers containing alginate-derived blocks. Synthetic carbohydrate polymers with biocompatible and biodegradable properties are already used in tissue engineering and controlled drug release devices [44, 45]. Within this context, amphiphilic block copolymers and brush copolymers based on oligomannuronates blocks could be prepared to form either core-shell type nanoparticles in aqueous medium, named macromolecular micelles, or biodegradable gels for drug delivery applications.

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References

1. Stanford ECC (1881) British Patent no 142
2. Sabra W, Zeng AP, Deckwer WD (2001) Appl Microbiol Biotechnol 56:315–325
3. McHugh DJ (2003) A guide to the seaweed industry. In: FAO fisheries technical paper no. 441, Rome FAO
4. Caramaro L (2005) Fibres et fils à usage technique. Techniques de l'Ingénieur N2510
5. Haug A (1964) Composition and properties of alginates. Thesis, Norwegian Institute of Technology, Trondheim
6. Brault D, Heyraud A, Lognoné V, Roussel M (2003) Methods for obtaining oligomannuronates and guluronates, products obtained and use thereof. WO 099870
7. Smidsrod O, Draget KI (1996) Carbohydr Eur 14:14–18
8. Darvill A, Bergmann C, Cervone F, De Lorenzo G, Ham KS, Sipro MD et al (1994) Biochem Soc Symp 60:89–93
9. Akiyama H, Endo T, Nakakita R, Murata K, Yonemoto Y, Okayama K (1992) Biosci Biotech Biochem 56:355–356
10. Yonemoto Y, Tanaka H, Yamashita T, Kitabatake N, Ishida Y, Kimura A, Murata K (1993) J Ferment Bioeng 75:68–70
11. Haug A, Larsen B, Smidsrod O (1966) Acta Chem Scand 20:183–190
12. Haug A, Larsen B (1962) Acta Chem Scand 16:1908–1918
13. Haug A, Larsen B, Smidsrod O (1967) Acta Chem Scand 21:691–704
14. Tsujino I, Saito T (1961) Nature 192:970–971
15. Preiss J, Ashwell G (1962) J Biol Chem 237:309–316

16. Gacesa P (1987) FEBS Lett 212:199–202
17. Boyen C, Bertheau Y, Barberon T, Kloareg B (1990) Enzyme Microb Technol 12:885–890
18. Zhang Z, Yu G, Guan H, Zhao X, Du Y, Jiang X (2004) Carbohydr Res 339:1475–1481
19. Yamasaki M, Ogura K, Hashimoto W, Mikami B, Murata K (2005) J Mol Biol 352:11–21
20. Shimokawa T, Yoshida S, Takeuchi T, Murat M, Ishii T, Kusakabe I (1996) Biosci Biotechnol Biochem 60:1532–1534
21. Matsubara Y, Iwasaki K, Maramatsu T (1998) Biosci Biotechnol Biochem 62:1055–1060
22. Roussel M, Benvegnu T, Lognoné V, Le Deit H, Soutrel I, Laurent I, Plusquellec D (2005) Eur J Org Chem 3085–3094
23. Heyraud A, Colin-Morel P, Gey C, Chavagnat F, Guinand M, Wallach J (1998) Carbohydr Res 308:417–422
24. Ifremer (1997) FR 97 03218
25. Roussel M, Lognoné V, Plusquellec D, Benvegnu T (2006) Chem Commun 3622–3624
26. Baker SC (1991) Nature 350:627–628
27. Griffin WC (1954) J Soc Cosmet Chem 5:249–256
28. Davis JT (1961) Interfacial phenomena. Academic, New York
29. Ruiz CC (2008) Sugar-based surfactants. CRC, New York
30. Benvegnu T, Plusquellec D, Lemiègre L (2008) Surfactants from renewable sources. In: Belgacem MN, Gandini A (eds) Monomers, polymers and composites from renewable resources. Elsevier Limited, Amsterdam
31. Roussel M, Moutard S, Perly B, Lefeuvre M, Benvegnu T, Plusquellec D (2003) Carbohydr Res 338:375–378
32. de Gennes PG, Taupin C (1982) J Phys Chem 86:2294–2304
33. Atwood D, Florence AT (1983) Surfactant systems: their chemistry, pharmacy and biology. Chapman and Hall, London
34. Rotenberg Y, Boruvka L, Neumann AW (1983) J Colloid Interface Sci 93:169–183
35. Kitamoto D, Yanagishita H, Shinbo T, Nakane T, Kamisawa C, Nakahara T (1993) J Biotechnol 29:91–96
36. Bikerman JJ (1938) Trans Faraday Soc 34:634–638
37. Ross J, Miles GD GD (1941) Oil & Soap 18:99–102
38. Domingo X (1995) Tenside Surf Det 32:310–316
39. Roussel M (2001) Ph.D. dissertation, University of Rennes 1, France
40. Pasquali RC, Sacco N, Bregni C (2009) Latin Am J Pharm 28:313–317
41. Lahaye M, Robic A (2007) Biomacromolecule 8:1765–1774
42. CEVA – Unpublished results
43. Ranson M, Gutierrez G, Brault D, Le Deit H, Pages-Xatart-Pares X, Alfos C (2007) WO 2007/045795
44. Ladmiral V, Melia E, Heddleton DM (2004) Eur Polym J 40:431–449
45. Simanek EE, McGarvey GJ, Jablonowski JA, Wong CH (1998) Chem Rev 98:833–862

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From Natural Polysaccharides to Materials for Catalysis, Adsorption, and Remediation

Françoise Quignard, Francesco Di Renzo, and Eric Guibal

Abstract Polysaccharides display most of the properties needed for applications in catalysis, adsorption or remediation. Requisites common to these applications are appropriate surface functions to ensure substrate–material interactions, accessibility of the functional groups, and proper shaping for easy manipulation. Natural polysaccharides are well known as supports for enzymatic catalysts and gelling agents in aqueous phase, due to the high level of dispersion of hydrocolloids. However, they suffer from diffusional limitations in the dry state, due to the low surface area of the dried materials generally used, xerogels or lyophilized solids. This chapter deals with the proper methods to prepare dry materials which retain the dispersion of the polymer hydrogel, namely polysaccharide aerogels. The materials whose properties are described here are stable in most organic solvents and present numerous and diverse surface functionalities (like hydroxy, carboxy, or amino groups). Shaping and appropriate drying of gelling polysaccharides provide a new opportunity to obtain materials from one of the less energy-intensive sources of biomass. Their application in catalysis and adsorption could open substantial markets for products of seaweed harvesting and coproducts of the seafood industry.

Keywords Aerogels, Catalysis, Polysaccharides, Porous materials, Remediation, Shaping, Textural properties

Contents

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1 Introduction

Hydrocolloid-forming polysaccharides are natural polyelectrolytes able to gelify water when added in tiny amounts. Hydrogels containing 1–2% polymer and 98–99% water can be shaped as self-standing spheres or films with good mechanical stability. This property is at the basis of their natural function as water-storage agents for living organisms as well as of their main application, in which about 45,000 tons per year of seaweed-extracted polysaccharides are used as thickening agents for the food industry. Polysaccharides display most of the properties needed for catalysis, adsorption, or remediation applications. Many requisites are common to these applications such as surface functions to ensure substrate–material interactions and diffusion properties.

1.1 Why Use These Biopolymers for Catalysis?

The first application of polysaccharide hydrogels in catalysis, as support for enzymatic catalysts, stems from their widespread use as growth media for bacterial growth. At present, alginates and carrageenans are the main enzyme supports in the brewing industry and for bio-labeled laundry powders. Natural polysaccharides, which have been known for many years as supports for enzymatic catalysts, have recently received increased attention as supports for metal catalysts. Many reasons may explain this increasing interest: (1) unlimited availability of the materials in Nature, industrial processes of extraction already running at the plant scale; (2) high binding ability of these materials for selected metals due to

the presence of numerous and diverse surface functionalities like hydroxy, carboxy, or amino groups; (3) physical and chemical versatility of these materials (which can be easily modified); (4) insolubility in most organic solvents; (5) easy degradation of the organic material at the end of the life cycle (less toxic degradation products than conventional resins); (6) possible conformational effects (in relation to the physical structure of these materials). However, their use in catalysis suffers from diffusion limitations due to the low surface area of the dried materials generally used, xerogels or lyophilized solids. Highly dispersed polysaccharides are known in aqueous phase, where the macromolecules are able to form a three-dimensional network. The interactions of the functional groups with water reduce their effectiveness as catalysts in aqueous media. Evaporative drying methods bring about the collapse of the gel structure and do not allow retention of the accessibility of the functional groups. The technique of supercritical drying allows shrinkage of the gels to be avoided and has already brought several breakthroughs in other fields of the materials science, e.g., for silica-based adsorbents, insulation materials, optical fibers, or the preparation of carbon precursors [1, 2]. In the case of polysaccharides, supercritical drying was originally used to prepare samples for electron microscopy [3]. As recent publications have reviewed the field on biopolymers and catalysis [4, 5], this chapter will focus on the aerogel formulation of polysaccharides for catalysis, a quite recent field of investigation.

1.2 Why Use These Biopolymers for Metal Recovery?

First investigations on metal biosorption focused on the use of biomass (algae, fungi, yeast, and bacteria) as raw materials. The in-depth study of sorption processes using these materials identified active and passive phenomena depending on the type of biomass and the composition of the solution/media. However, for "dead" biomass the analysis of saturated biosorbents by EDX analysis showed that, in most cases, the metals were located in the external part (or at the surface) of the cell membrane. Based on these observations a number of studies have contributed to identifying the main reactive compounds in the cell wall of these microorganisms such as alginate-based materials for algae and chitin (or chitosan) for fungal biomass. Alginate and chitosan are biopolymers that offer complementary reactive functional groups including carboxylic acid groups (mannuronic and guluronic acids) and amine groups, respectively. These reactive groups can be considered as the pendant of conventional weak acid (examples: Amberlite IRC-86, IRC-50, Lewatit CNP-105) and weak base (examples: Amberlite IRA-96, Lewatit VP-OC-1065) resins (synthetic polymers) for materials of biological origin (i.e., derived from renewable resources). The differences in the acid–base properties of these materials (pK_a between 3 and 4 for carboxylic acid groups in alginate, pK_a between 6.3 and 7 for amine groups in chitosan) may explain their complementary effects in terms of both fields of application and target metals to be sorbed.

2 Polysaccharides Hydrocolloids

Natural polysaccharides available on an industrial scale include polymers with anionic functions, like alginates (carboxylic groups) or carrageenans (sulfonic groups) derived from seaweed, or with cationic functions, like as chitosan (amino groups), obtained by deacetylation of chitin from seafood shells (Fig. 1).

2.1 Alginates

Alginates are a family of polysaccharides mainly extracted by brown algae. Alginate hydrogels are able to store up to 100 times their weight of water, trapped in a network of hydrophilic fibrils. This property accounts for their natural occurrence as water reservoirs in plants of the tidal area [6].

Monovalent metal ions form soluble salts with alginate whereas divalent or multivalent cations, except Mg^{2+}, form gels. The properties of the alginate gels are affected by the ratio and sequencing of uronic monomers [7], the concentration of cations in the maturation bath, and the time of maturation [3, 8]. The mechanical strength of the gels increases with their guluronic content. The guluronate sequences of the copolymer aggregate according to the so-called "egg-box

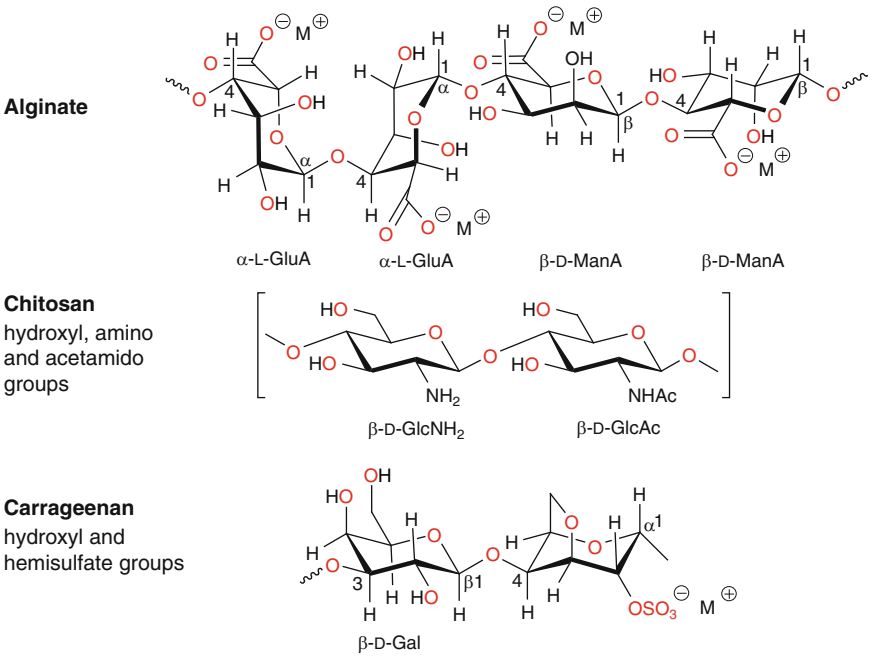


Fig. 1 Polysaccharides with different chemical functions

model," in which each calcium cation is coordinated to the carboxyl and hydroxyl groups of four guluronate monomers from two adjacent chains of the polymer [9–12]. This kind of energetically favorable aggregation, shared by other polysaccharide like pectates, confers a high rigidity to the gel and corresponds to a strong affinity of the guluronic residues for divalent cations [13–15]. In natural systems, guluronic acid is formed by enzyme-catalyzed epimerization of mannuronic acid [16–18]. This catalytic process accounts for the rigidification which occurs during the lifespan of seaweed, from recently grown flexible fronds to more rigid older woody stems [19].

A less frequently cited method to form a gel from alginate is to lower the pH of a sodium alginate solution. Proton exchange transforms the alginate in alginic acid and decreases repulsion forces between the chains. Alginic acid gels formed in this way have been known and used for a long time [20] but their structure has been poorly understood by comparison with the more extensively studied ionotropic gels. The effects of chemical composition and molecular weight on gel strength and gelling kinetics were studied [13]. Acidic gels with higher guluronic content, like the corresponding ionotropic gels, present a higher mechanical strength. Small-angle X-ray scattering (SAXS) suggested the formation of junction zones with a high degree of multiplicity [21, 22].

Alginates are widely applied in drug release systems [23] for the entrapment of biologically active materials [24] and as supports in biocatalysis.

2.2 Chitosan

Chitosan, a linear copolymer of linked β-(1,4)-glucosamine, is traditionally obtained by deacetylation of chitin (poly-β-(1,4)-acetylglucosamine) from seafood industry wastes (crab and shrimp shells and squid pens). Much lower amounts of chitosan are directly obtained from fungi [25]. The product of deacetylation of chitin is defined as chitosan when its degree of deacetylation is beyond 50%. The degree of deacetylation and molecular weight of chitosan influence all the physico-chemical properties (such as solubility and viscosity) and can determine the field of application of chitosan. For a given deacetylation degree, the type and origin of the parent chitin influence the gelling properties. Indeed, two polymorphic forms of chitin differ by the orientation of the polymer chains. The antiparallel orientation of chains in α-chitin accounts for the formation of more hydrogen bonds between chains than in β-chitin, characterized by a parallel orientation of the polymer chains [26]. This confers a higher rigidity and a lower reactivity to the most naturally abundant α-chitin [27]. The different reactivity of the two chitin polymorphs affects their deacetylation behavior. Chitosan from α-chitin is a highly crystalline block copolymer of glucosamine and acetylglucosamine while chitosan from β-chitin is an amorphous random copolymer [28]. Chitosan can form either chemical or physical hydrogel [29, 30]. Chemical hydrogels are obtained by crosslinking chitosan strands with irreversible covalent links. Physical hydrogels are stabilized

by various kinds of reversible links, as in ionically crosslinked hydrogels, polyelectrolyte complexes, or entangled gels. The latter – the simplest way to prepare a chitosan hydrogel – are formed by solubilization of chitosan in an acidic aqueous medium followed by precipitation in an alkaline solution.

Several methods have been used to modify chitosan either physically [31–33] or chemically [34–36] in order to improve its mechanical or chemical stability, hydrophilicity, and metal sorption capacity. The amino groups of chitosan provide it with the highest chelating ability among the natural polymers obtained from seafood wastes and natural substances [37, 38]. The high exchange capacity of the hydrogels has allowed the introduction of large amounts of transition metals with potential applications both in remediation and catalysis.

Chitosan finds wide applications in biomaterials, drug-delivery systems [39, 40], food additives, water clarification [41], and as support for cells, enzymes [42], and catalysts [4, 5].

2.3 Carrageenans

Carrageenans are a large family of sulfate-bearing polygalactoses extracted from red marine algae (Rhodophyceae). Their three major types, designated by means of the Greek letters κ, ι, and λ, differ by the number of sulfate groups and the frequency of anhydrogalactose groups. For instance, the structure of κ-carrageenan is constituted of linear chains of alternating (1→3)-linked α-galactose-4-sulfate and (1→4)-linked 3, 6-β-anhydrogalactose [42].

κ-Carrageenan presents better ionotropic and thermotropic gelation properties than the other types of carrageenan [43]. The details of the mechanism of the salt-induced gelation are still a matter of debate, although there is general agreement on a two-step mechanism implying a coil-to-helix transition followed by aggregation of helices. Potassium, rubidium, and cesium strongly promote the gelation of carrageenan [44, 45]. Carrageenans beads are often used as a matrix to entrap molecules of biological significance, such as food products, enzymes, and cells [46, 47]. The loaded beads can be used as immobilized biocatalysts or for controlled release of entrapped molecules of pharmaceutical interest.

3 Shaping

One of the advantages of the gel formulation is the easy preparation of materials with the most appropriate shape for each application. Some of the possible shapes in which hydrogels can be molded, extruded, or modeled by physical phenomena are represented in Fig. 2.

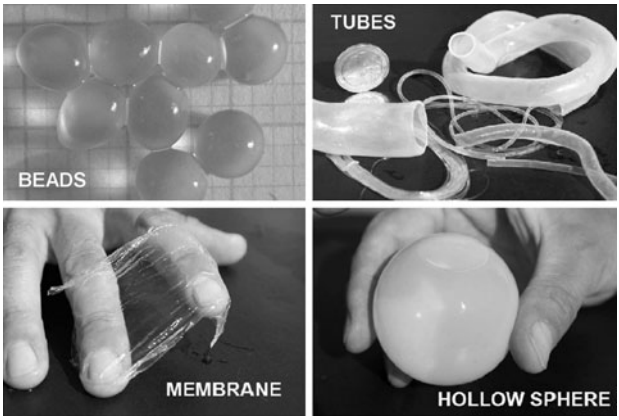


Fig. 2 Examples of different conditionings of chitosan

3.1 Gel Beads

Gel-bead conditioning provides many advantages. Bead preparation does not require any complex device, allows easy dosage of components and easy encapsulation of suspended matter, and produces objects of custom-tailored size. Typically, the procedure starts from a solution of the biopolymer in an appropriate solvent, water for Na-alginate, acidic solution for chitosan. This viscous solution is added dropwise in the coagulating bath. The coagulation may consist of a neutralization step with a concentrated alkaline solution (generally NaOH) for chitosan [48–50] or an ionotropic gelation of biopolymer drops, generally metal salts solutions [8, 51, 52], or hydrochloric acid for sodium alginate [53–56] and molybdate salts or polyphosphate for chitosan [57–59]. The viscosity of the solution has an important impact on the control of the shape of the beads. The water content of the beads is related to the concentration of the polymer solution and is usually very high, generally greater than 95%.

3.2 Fiber and Hollow Fiber

Cellulose derivative fibers are commercially available and extensively used for the preparation of dialysis modules. Cellulose acetate fibers functionalized with metal alkoxides for the immobilization of enzymes was described by Kurokawa and Hanaya [60]. In this example, the presence of metal alkoxides induced the gelification of cellulose acetate due to the coordination of the polyvalent metal on the hydroxyl groups on pyranose rings. The strength of the fiber strongly depends on alkoxide content.

Fibers and hollow fibers made of chitosan and alginate materials are less frequently cited. The preparation of fibers and hollow fibers obeys the same two-step procedure: (1) dissolving the biopolymer, followed by (2) the extrusion of the solution into a coagulation bath, as described for gel bead preparation. The fiber is extruded through a thin nozzle into a bath and falls from a spinneret directly into the coagulation bath or alternatively in air to stretch the extruded fiber and reduce its diameter before it enters into the coagulating bath [61].

Another technique for the preparation of chitosan hollow fibers consists of the coagulation of the chitosan fiber in an ionotropic gelation bath followed by forced extrusion of the noncoagulated core [62, 63].

The use of hollow fibers offers interesting perspectives in catalysis: the solution to be treated is circulating through the lumen of the fiber, while the reagent can be provided in a liquid or gas state at the outer side of the fiber. These conditions have not been widely investigated for catalytic applications. Only a few papers focus on the testing of catalytic hollow fibers made of biopolymers [64–66].

3.3 Membranes

Flat membrane materials are extensively used in separation processes, especially filtration. For manufacturing chitosan or alginate membranes, the biopolymer solution is casted on a glass, polystyrene, polyethylene, or polycarbonate surface. A partial evaporation of the solvent (50%) allows the maintaining of the rigid structure of the membrane prior to its neutralization or coagulation. Flat membranes have been prepared using chitosan and alginate alone [67–70] or combined with other polymers (natural or synthetic) or inorganic compounds [71–74].

4 From Hydrocolloids to Porous Materials

4.1 Drying

To what extent can the high dispersion of polysaccharide hydrogels be retained in their dried form? Drying of the hydrogels by evaporation of water usually brings about a complete collapse of their secondary structure. In Fig. 3a, beads of Ca-alginate hydrogel formed by dropping a 0.24 M solution of $CaCl_2$ solution in a 2% solution of Na-alginate (20% guluronic) are represented. The beads of the xerogel obtained by evaporation of the solvent at room temperature are represented in Fig. 3b. The average volume of the xerogel beads is 2.7% of the average volume of the hydrogel beads, corresponding to nearly complete shrinkage of the gel. It is clear that the polymer strands are compliant enough to be drawn together by the

capillary tension [75, 76]. Capillary tension depends on the presence of a liquid–vapor interface and disappears if the solvent is extracted in supercritical conditions.

The miscibility of water and liquid carbon dioxide is very poor and an intermediate solvent has to be used to allow the replacement of water by carbon dioxide. In a procedure initially developed to prepare representative samples for electron microscopy, water is replaced by ethanol through exchanges with alcoholic solutions of increasing concentration. The alcogel prepared by a final exchange with absolute ethanol (Fig. 3c) is introduced in a pressure vessel in which liquid CO_2 is admitted and replaces ethanol in the gel. The CO_2-impregnated gel is compressed and heated above the critical point of CO_2 (31.05°C, 73.8 bar). Release of pressure above the critical temperature allows CO_2 to be extracted without the formation of any liquid–vapor interface and a dried aerogel is formed (Fig. 3d).

In the case of alginate gels, the overall retention of gel volume in the supercritical drying process corresponds to retention of the microscopic structure of the hydrogel. A cross-section of Ca-alginate aerogel, as pictured in Fig. 4, presents an open framework of isolated fibrils with diameter around 5 nm and an average length

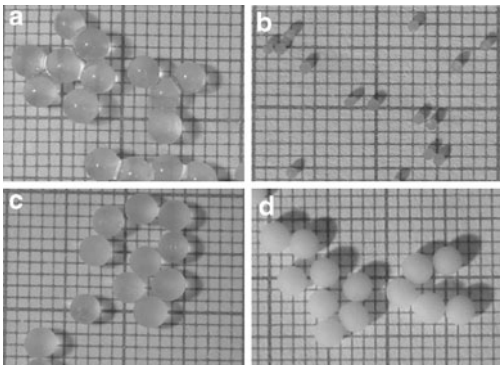


Fig. 3 Ca-alginate beads in different formulations: (a) hydrogel, (b) xerogel obtained by evaporative drying, (c) alcogel from ethanol solvent exchange, (d) aerogel from supercritical CO_2-dried alcogel. *Grid square side 1 mm*

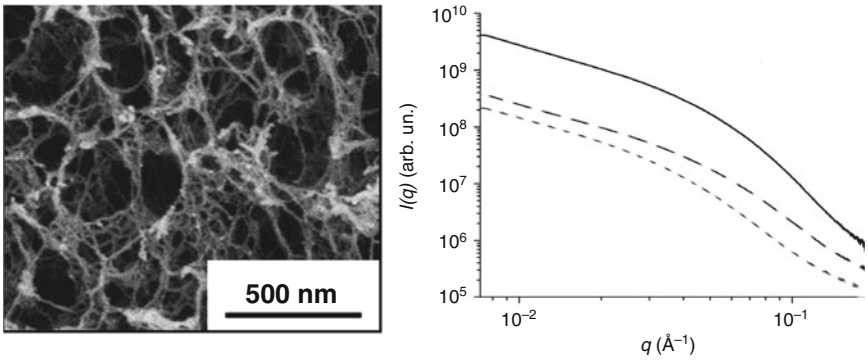


Fig. 4 *Left*: micrograph of a cross-section of Ca-alginate aerogel. *Right*: SAXS diagram of hydrogel (*dotted line*), alcogel (*dashed line*), and aerogel (*full line*) of Ca-alginate

near 200 nm. Contacts between fibrils occur at nodes connecting the ends of three fibrils. This kind of branching suggests that the fibrils have been formed as a network and the gel is not the result of the random aggregation of isolately-formed fibrils.

The fibrillar structure was already present in the hydrogel from which the aerogel has been formed, as has been demonstrated by SAXS experiments [77]. The results of this study are summarized in Fig. 4, in which the scattering patterns of a Ca-alginate hydrogel and the corresponding alcogel and aerogel are reported. The scattering intensity increases in the order hydrogel $<$ alcogel $<$ aerogel, in correspondence with the decrease of density of the continuous phase from water to ethanol and air. In the meantime, the slope of the curves in the region around 10^{-2} Å^{-1} remains constant at unit value, indicating that the morphology of the polymer aggregates is always cylindrical and is not altered by the drying process.

The different shrinkage of xerogel and aerogel is the origin of a different accessibility of their secondary structures, as can be assessed by nitrogen physisorption at 77 K [78]. The N_2 isotherm measured on an aerogel and a xerogel of chitosan are reported in Fig. 5. The isotherm of the aerogel is intermediate between types 2 and 4 of the IUPAC classification [79] corresponding to a solid with mesopores (pores with diameter between 2 and 50 nm) at the borderline with macroporosity (pores larger than 50 nm). The surface area of the aerogel can be measured by the BET method at 320 m^2 g^{-1}. The isotherm of the xerogel is barely measurable and corresponds to a solid with a surface area lower than 5 m^2 g^{-1}.

The effectiveness of the supercritical drying method depends on the mechanical properties of the polymer. The decrease in gel volume during the preparation of the aerogel is reported in Table 1 for several polysaccharide materials. The shrinkage of Ca-alginate hydrogels during the aerogel preparation induces a 7% decrease in bead size, corresponding to a 20% decrease in gel volume. The shrinkage takes place essentially in the alcohol exchange of the hydrogel and is negligible during the CO_2 supercritical drying of the alcogel [75]. Other polysaccharides present a more important shrinkage when submitted to the same procedure of supercritical drying.

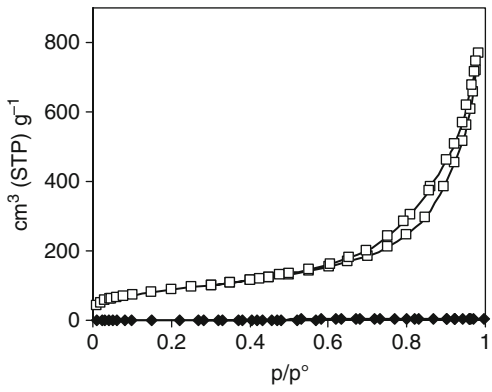


Fig. 5 N_2 physisorption isotherms of an aerogel (*empty squares*) and a xerogel (*filled lozenges*) of chitosan

Table 1 Typical textural data for polysaccharide aerogels

Aerogel	Shrinkage upon drying (vol%)	Porosity ($cm^3\ g^{-1}$)	Mesopore volume ($cm^3\ g^{-1}$)	Average mesopore size (nm)	Surface area ($m^2\ g^{-1}$)	Fibril diameter (nm)
Ca-alginate	20	39	1.16	40	570	4
Alginic acid	22	38	0.89	20	390	6
Chitosan from α-chitin	60	15	0.44	12	330	7
Chitosan from β-chitin	51	19	0.09	25	150	16
κ-Carrageenan	95	1	0.76	18	200	18

The likely explanation for this effect is the flexibility of the polymer structures in the gel. In the hydrogel, the entropic effect due to the presence of a solvent allows the polymer strands to be kept well apart. Upon drying, the enthalpic advantage of a reduction of the exposed surface is high enough to draw together the strands of the most flexible polymers, also in the absence of the tension of a solvent meniscus. The rigidity of the alginate fibrils is high enough to resist this effect, while chitosan hydrogels lose about half their volume during the drying procedure and carrageenan hydrogels undergo nearly complete shrinkage.

The differences in shrinkage behavior are paralleled by the porosity of the aerogels, whose values are reported in Table 1. If total porosity can be calculated from the volume/mass ratio of the material, the mesoporosity (pores with diameter between 2 and 50 nm) is measured by N_2 physisorption at 77 K. No significant microporosity (N_2-accessible pores with diameter smaller than 2 nm) is measured in the samples. Total porosity of the alginate aerogels is about 38 $cm^3\ g^{-1}$, while chitosan present half this pore volume and the pore volume of carrageenan is 40 times smaller. Alginate and chitosan aerogels present an extremely open structure and are essentially macroporous (pores with diameter larger than 50 nm). In these aerogels, mesoporosity is a minor phenomenon probably related to the zones of contact among fibrils.

The size distributions of the mesopores of several polysaccharide aerogels, as evaluated from the N_2 desorption curves by the method of Broekhoff and de Boer [80], are represented in Fig. 6. Alginate aerogels present a very broad mesopore size distribution with no solution of continuity with macroporosity. Chitosan aerogels present a lower mesopore volume and their texture is affected by the origin of the material. Aerogels prepared with chitosan issued by deacetylation of the parallel-chains of β-chitin present virtually no mesoporosity, while aerogels of chitosan prepared by deacetylation of the antiparallel chains of α-chitin present a limited mesoporosity with a diameter distribution centered at 12 nm.

The aerogel of carrageenan presents a completely different texture. The mesopore volume corresponds to the whole porosity of the material (see Table 1) and mesopores present a quite narrow diameter distribution centered at 18 nm. In this case, the pores correspond to residual cavities which are still accessible after a

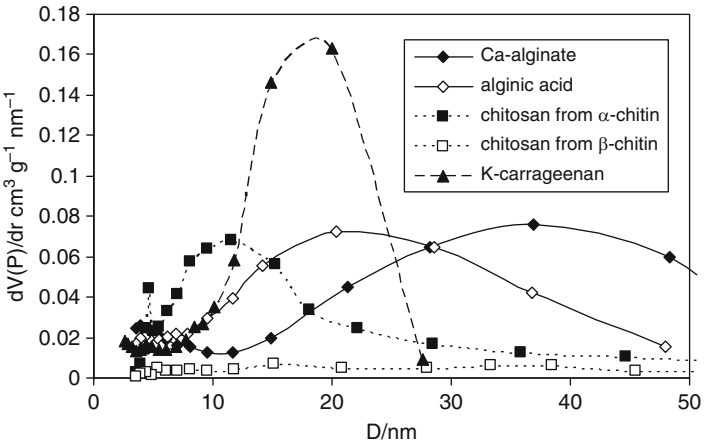


Fig. 6 Mesopore size distributions of several polysaccharide aerogels from the desorption isotherms of N_2 at 77 K

nearly complete collapse of the hydrogel structure. It is worth noting that the pore volume of these aerogels, despite being the lowest among the polysaccharide aerogels of this study, still corresponds to a gel in which the void volume is significantly higher than the volume occupied by the polymer.

The surface areas of the aerogels, reported in Table 1, provide direct information on their dispersion. If the surface area is attributed to the outer surface of the isolated fibrils which constitute the materials and the fibrils are ideally assumed to be cylindrical, the average diameter of the fibrils can be evaluated as $D = 4/(\rho S)$, where D is the diameter in μm, ρ is the density of the polymer (as a first approximation assumed to be equivalent to the density of the crystalline polymer [81–84]), and S the surface area in $m^2\ g^{-1}$. The surface area and the size of the fibrils are not directly correlated to the extent of shrinkage upon drying and seem to be strongly dependent on the type of polysaccharide and the method of gelling. The aerogels of alginic polymers present the highest surface area, with values of 570 $m^2\ g^{-1}$ for ionotropic Ca-alginate and 390 $m^2\ g^{-1}$ for pH-driven alginic acid gel. Aerogels of chitosan issued from α-chitin present a higher surface area (330 $m^2\ g^{-1}$) than aerogels of chitosan issued from β-chitin (150 $m^2\ g^{-1}$). κ-Carrageenan aerogels, despite their lower porosity, still present a significant surface area (200 $m^2\ g^{-1}$).

4.2 Control of the Structural Properties

4.2.1 Alginates

As the alginate aerogels are a faithful image of the texture of the parent hydrogels, the parameters which modify the dispersion of the hydrogels also affect the textural properties of the aerogel. As described previously, the properties of the alginate gels

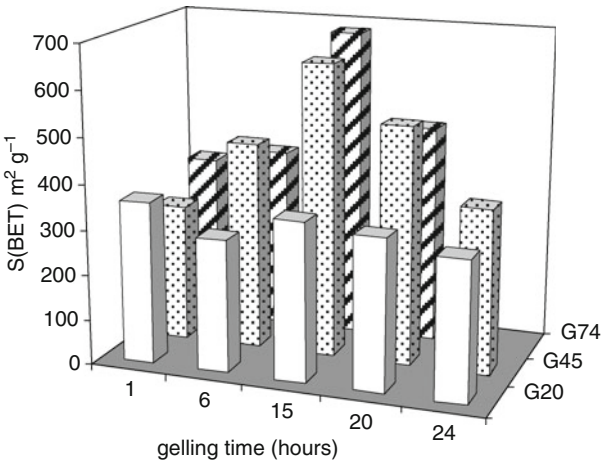


Fig. 7 Influence of the guluronate/mannuronate ratio and ripening time on the surface area of Ca-alginate aerogels

are affected by the ratio and sequencing of uronate monomers (mannuronate or guluronate) [7], the concentration of cations in the gelling bath, and the time of ripening in the gelling solution [3, 8].

The aerogel from guluronate-rich alginates presents a more compact nanostructure than the mannuronate-rich sample [75], in agreement with the better mechanical properties of guluronate-rich hydrogels [7]. Velings et al. studied the kinetics of gelling of a mannuronate-rich Ca-alginate and found that the hydrogels reached stable mechanical properties after some hours ripening in the gelling solution [8]. Indeed, the surface area of the aerogels evolves in different ways for alginates with different guluronate content. As an instance, the evolution of the surface area of Ca-alginate aerogels with the ripening time at room temperature in a 0.24 M Ca^{2+} solution is reported in Fig. 7 for three alginates with different guluronic ratios (20%, 45%, and 74% of total alginate). The surface of the aerogels of mannuronate-rich alginate rapidly reached a constant value of about 300 $m^2\ g^{-1}$. The surface area of the aerogels of the guluronate-rich alginates reached a maximum of more than 600 $m^2\ g^{-1}$ at 15 h ripening time and decreased towards 350 $m^2\ g^{-1}$ for longer ripening time. This result suggests that the organization of the guluronic-rich hydrogels passes through an intermediate state more dispersed or less stable that the final one.

The effect of the concentration of Ca^{2+} cations in the gelling solution on the surface area of the aerogel is reported in Fig. 8 for another mannuronate-rich (20% guluronate) alginate after 6 h ripening. The surface area increases with the Ca^{2+} concentration and reaches a shallow maximum at the concentration 0.24 mol L^{-1}.

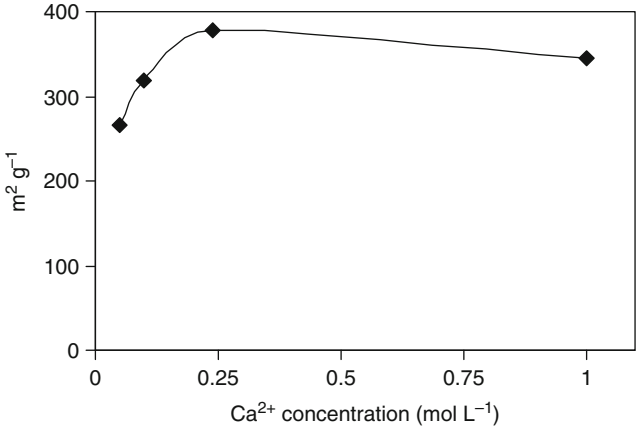


Fig. 8 Influence of the concentration of the Ca^{2+} gelling solution on the surface area of an alginate aerogel

4.2.2 Chitosan

The viscosity of the polymer solution largely controls the textural properties of the aerogels [85], and viscosity depends on the molecular weight, the deacetylation degree, and the natural source of the parent chitin. For a given degree of deacetylation, the type of chitin affects the distribution of the residual acetylated glucosamines, the crystallinity of the polymer, and its gelling properties [28]. Aerogels with less surface area are obtained from hydrogels of chitosan obtained by deacetylation of β-chitin from squid pen than by deacetylation of α-chitin from crab shell.

The textural properties of the aerogels can be modulated by controlling the medium of gelation. For example, replacing the alkali aqueous solution by an alkali hydroalcoholic solution allows an increase of the surface area from 125 $m^2\ g^{-1}$ to 220 $m^2\ g^{-1}$ when the ethanol fraction of the gelling solution increases from 0 vol.% to more than 70 vol.%. According to the study of Clayer and colleagues [86], the amphiphilic structure of the alcohol contributes to maintain the critical balance between the hydrophobic and hydrophilic interactions. The increase of the specific surface area can be connected to the assumption that alcohol, by decreasing strength and number of interfibril hydrogen bonds, prevents the collapse of the chains in the drying phase. The observation of the dispersion of the materials in the MEB pictures is in agreement with this assumption (Fig. 9).

A more important modification of the textural properties is observed when the gelification is induced by a cross-linking agent like, for instance, the "masked dialdehyde" TMP (1,1,3,3-tetramethoxypropane) [87].

In Fig. 10, the N_2 sorption isotherms of the aerogels of an alkali-gelled chitosan from β-chitin and the same chitosan gelled by TMP (2% molar cross-linker with respect to the number of amine groups) are compared. The aerogel of the

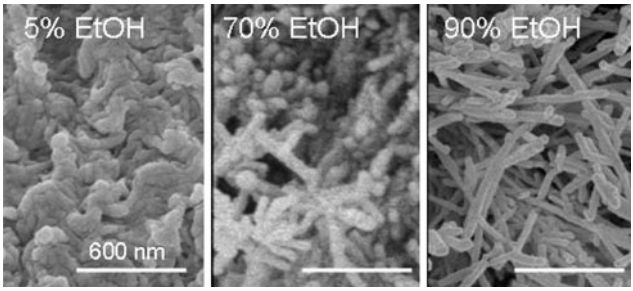


Fig. 9 MEB pictures of chitosan aerogels obtained in hydroalcoholic medium

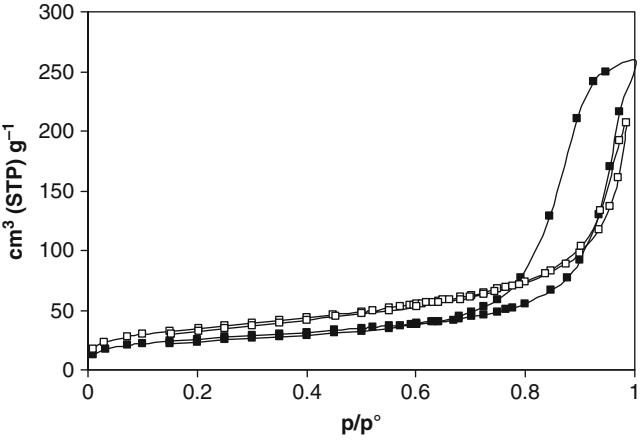


Fig. 10 N_2 sorption isotherms of aerogels of chitosan (*empty squares*) and cross-linked chitosan (*filled squares*)

cross-linked chitosan presents a mesopore volume of 0.32 cm^3 g^{-1} with a narrower pore size distribution centered around 160 Å (surface area of 95 m^2 g^{-1}) while the aerogel of the alkali-gelled chitosan presents a small mesopore volume with a broad pore size distribution which goes beyond the mesopore size limit of 500 Å (surface area of 120 m^2 g^{-1}).

4.3 Accessibility of the Functional Groups

Does the high surface area of the polysaccharide aerogels corresponds to good accessibility of their functional groups? The assessment of this property is based on the spectroscopic or chemical monitoring of the interaction of probe molecules with the functional groups.

4.3.1 Accessibility in the Gas Phase

FTIR of gas-phase probe molecules is an efficient tool with which to study the interaction of the functional groups with selective reactive gases. It provides information on the accessibility of the active sites. The FTIR spectroscopy of adsorbed NH_3 has been used to investigate the acidity of alginate aerogel films prepared in the presence of different cations or gelified in acidic medium [88, 89]. Ammonia was chosen as the probe molecule due to its ability to differentiate between Lewis and Brønsted acid sites. The spectra of ammonia adsorption on aerogels alginic acid or cobalt-alginate gels are reported in Fig. 11. In the adsorption of ammonia on the alginic acid aerogel, the characteristic band of the free carboxyl groups at 1,735 cm^{-1} disappears and is replaced by the C–O stretching bands of carboxylate at 1,620 and 1,575 cm^{-1}. This demonstrates that the carboxylic acids of the alginate are salified by NH_4^+ as effective Brønsted sites. The nearly complete disappearance of the carboxylic acid band indicates that nearly all the acid sites are accessible to the probe molecule.

Ammonia interacts with cobalt-alginate aerogel according to two distinct mechanisms: (1) molecular adsorption on electron-acceptor sites (Lewis acid) or (2) proton transfer from a Brønsted acid site and electrostatic bonding of the resulting ammonium cation to an anionic site of the adsorbent. In the case of the ionotropic gels formed by transition metal cations, a significant fraction of carboxylic groups are not salified and ammonia adsorption takes place both on the divalent cation and by proton transfer from the available Brønsted sites.

Transmission FT-IR spectroscopy has been used to monitor the deuteration by D_2O vapor at room temperature of wafers of aerogels of chitosan from β-chitin [90]. The deuterating agent is a probe of the accessibility of the materials to small polar molecules. The FTIR spectra of chitosan aerogel submitted to increasing pressures of D_2O are reported in Fig.12. The admission of D_2O vapor brings about a progressive decrease of the intensity of all O–H and N–H bands. At a D_2O pressure of 13 mbar, the O–H and N–H bands have virtually disappeared,

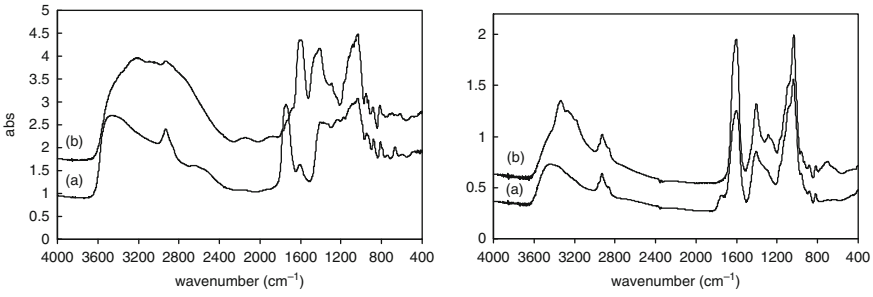


Fig. 11 FT-IR spectra of aerogel of alginic acid (*left*) and Co-alginate (*right*) exposed to NH_3 vapor at 30 mbar pressure

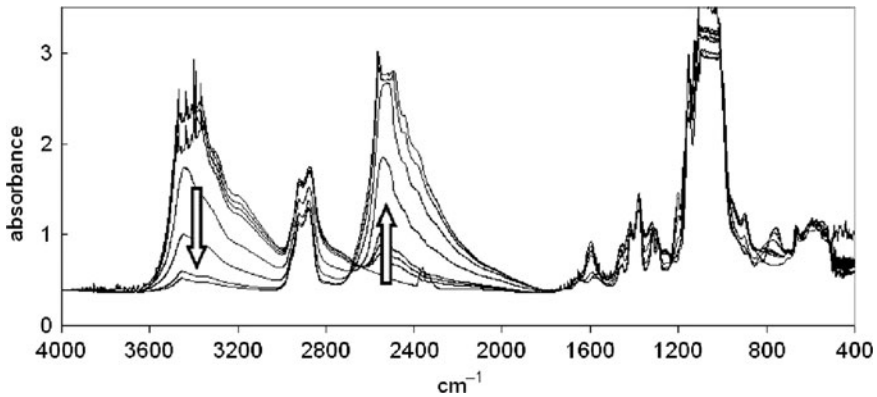


Fig. 12 FTIR spectra of chitosan aerogel submitted to increasing pressures of D_2O (0, 0.01, 0.24, 0.33, 3.7, 9.7, 12, and 13 mbars)

indicating a nearly complete deuteration of the alcohol and amine groups of the aerogel.

4.3.2 Accessibility in the Liquid Phase

n-Hexanol has being used as a polar probe molecule to estimate the potential of alginate aerogels as adsorbents for trace polar contaminants in hydrocarbon feedstocks. The amount adsorbed in the monolayer allows evaluation of the surface area of the adsorbent and confirms that immersion in hydrocarbon does not modify the size and the dispersion of the polysaccharide fibrils. The comparison of the surface density of adsorbate with the structure of the surface indicates that hexanol is adsorbed on alginic acid by the formation of hydrogen bonds between the alcohol heads and two hydroxyls of the polymer surface. In the case of alginates gelled by divalent cations, stronger adsorption sites allows completion of a monolayer at lower concentrations of the polar molecule [91].

These experiments confirmed that both the surface functions of the polysaccharide framework and the cations used as gelling agents are accessible to polar molecules in the liquid phase. Moreover, the immersion in organic low surface-tension solvents does not affect the texture of the aerogels.

The accessibility of the primary amine functions of chitosan has been quantified in organic solvent by formation of a salicylaldimine Schiff base upon treatment with salicylaldehyde. When contacting salicylaldehyde the aerogel beads of chitosan become yellow and their UV-visible diffuse reflectance spectra exhibit the absorption band at 318 nm, characteristic of the Schiff base. Quantitative GC analysis of the remaining salicylaldehyde in the solution allows calculation of the fraction of reacted amino groups, which corresponds to the fraction of accessible sites. In the aerogel formulation, up to 70% of the amine groups are accessible [92].

5 Perspectives Within Sustainable Development

5.1 Applications in Metal Remediation

5.1.1 Sorption Mechanisms

The sorption properties of these biopolymers may be explained by several mechanisms including (1) complexation/chelation, (2) electrostatic attraction/ion exchange, or (3) formation of ion pairs and binding. The kind of mechanism involved in metal binding depends on the metal, the pH, the composition of the solution, which, in turn, controls the surface properties of the sorbent (charge), and the speciation of the metal.

Chelation/Complexation Mechanisms

Pearson's theory (Hard and Soft Acid and Base Theory, i.e., HSAB) establishes the rules for the strength of interactions between ligands and metal ions [93]. According to the HSAB theory, soft acids preferentially react with soft bases, while hard acids have a marked preference for hard bases (for metal and ligand classifications see Table 2). Chitosan (amine groups) and alginate (carboxylic acid groups) are part of different groups offering complementary characteristics for the binding of metal ions. These properties allow prediction of the affinity of the biopolymer for chelation and complexation of metal ions.

Most of the studies dedicated to metal sorption using alginate pointed out the preponderance of ion exchange mechanisms (especially in acidic solutions);

Table 2 Summary of HSAB theory affinities

Hard acids	Borderline	Soft acids
Li^+, Na^+, K^+, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, UO_2^{2+}, VO^{2+},Al^{3+}, Sc^{3+}, La^{3+}, Cr^{3+}, Mn^{3+}, Si^{4+}, Ti^{4+}, Zr^{4+}, Th^{4+}, ...	V^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+},Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Bi^{3+}, ...	Cu^+, Ag^+, Au^+, Tl^+, Ga^+, Cd^{2+}, Hg^{2+}, Sn^{2+}, Tl^{3+}, Au^{3+}, In^{3+}, Pd^{2+}, Pt^{2+}, ...
	Preference for ligand atom	
$N \gg P$		$P \gg N$
$O \gg S$		$S \gg O$
$F \gg Cl$		$I \gg F$
Ligand classification	Ligand classification	
$F > O > N \approx Cl > Br > I > S$		$S \gg I \gg Br > Cl \geq O > F$
$OH^- > RO^- > RCO_2^-$		
$CO_3^{2-} \gg NO_3^-$		
$PO_4^{3-} \gg SO_4^- \gg ClO_4^-$		

however, in some cases, the contribution of chelation mechanisms was cited. For example, DeRamos et al. compared the sorption properties for alkaline-earth metal cations and lanthanide metals [94]. They showed by NMR analysis that alkaline-earth metal ions were only bound to guluronic acid moieties while for lanthanide metal ions (rare-earth metals, RE) both guluronic (G) and mannuronic (M) acid groups were involved in metal sequestration. These differences were attributed to the secondary structure of the biopolymer. In guluronic acid units the diaxial linkage pattern forms a buckled structure with deep cavities favorable for alkaline-earth metals; in contrast, mannuronic acid groups are arranged diequatorially, forming ribbon-like structure with shallow cavities (less favorable for the binding of alkaline-earth metal ions). The oxygen ligand atoms are also different on M and G acids units: one ring oxygen for M units and one hydroxyl oxygen for G dimmers. The hydroxyl oxygen is a stronger Brønsted-Lowry base than ether oxygen, giving more affinity for alkaline earth metal ions for G-rich alginate. The buckle-shape guluronate dimmers are characterized by a shorter distance between negatively charged carboxylate groups (compared to the ribbon-like structure of mannuronate chains); the higher charge density and stronger interaction with positively charged counterions explain the marked affinity for alkaline-earth metal cations. The flat structure of M chains induces a block charge density much lower and consequently less favorable for binding alkaline-earth metals. In this case (M-rich alginate and RE) the binding is more influenced by the charge density of the metal than by the conformation of the polymer; the main controlling parameters are the size of metal ions (and their hydration sphere) and the ease of packing of the alginate chains (which depends on the spatial distribution of G and M units).

For chitosan, though several studies commented on a possible contribution of –OH groups in the stabilization of metal binding on polymer chains, it is commonly accepted that most of the binding occurs on the free electronic doublet of nitrogen (on primary amino groups). The molecular mechanism involved in metal binding has been the subject of much controversy for the last few decades: two theories were opposed. The *bridging model* suggests the contribution of two amine groups (from the same or from different chains) via inter- or intramolecular complexation. The *pendant model* supposes that the metal is bound to a single amino group in a pendant fashion, with possible contribution of –OH groups (for example –OH group in the C3 position) or water molecule for the stabilization of the coordination sphere in the case of copper binding. This sorption mechanism is selective for transition metals over alkaline and alkaline-earth metal ions; this is attributed to the absence of *d* and *f* unsaturated orbitals (for alkaline-earth metals unlike to transition metals). The properties of chitosan for complexation of metal ions have been extensively reviewed by Varma et al. [95] and by Guibal [96]: these reviews provide more complete information on the sorption capacity obtained for chitosan and derivative materials. Sorption capacities strongly depend on the metal (Cu(II), Cd(II), Pb(II), Ni(II), Hg(II), Cr(III), or U(VI)) but generally vary between 0.5 and 2 mmol metal g^{-1}, for pH range 5–7 [96].

Ion Exchange/Electrostatic Attraction Mechanisms

Sodium alginate is soluble in water; the exchange of sodium ions with calcium ions allows the gelling of the biopolymer. Hydrochloric acid solutions have also been used for the "coagulation" of alginate solutions (for example HCl solutions at moderate concentration, i.e., 0.1 M) [53]. The process has been widely used for conditioning the biopolymer in the form of gel beads. This jellification can also be operated using other metal cations such as Cu^{2+} or Al^{3+}, the biopolymer solution being directly dropped into the gelling solution. However, in most cases the pre-formed alginate gel beads are used for metal sorption, which proceeds through the exchange of calcium cations with the target metal ions. The ion exchange may also proceed through the exchange of protons with metal cations when the pH ranges between 2 and 4 (consistent with the pK_a of M and G units, i.e., 3.38 and 3.65, respectively). The number of protons exchanged varies with the metal (and more specifically its speciation). For example, Ibanez and Umetsu [97] reported the sorption of Cr(III) on alginate gel beads: they observed that the tendency of Cr (III) to form hydroxocomplexes induced the exchange of one, two, or three protons per Cr(III) bound, depending on the predominant species under given experimental conditions. Calcium alginate beads have been extensively investigated for the sorption of metal ions such as Cu(II), Cd(II), Zn(II), Cr(VI), and Pb(II) [98–103]: sorption capacities as high as 1–2 mmol metal g^{-1} were obtained at pH in the range 4–7, depending on the metal.

In the case of chitosan, the ion exchange properties are brought by the protonation of amine groups under acidic conditions. These protonated amine groups can attract metal anions. The pK_a of amine groups strongly depends on the degree of acetylation and the yield of neutralization of the biopolymer [104]; however, with conventional commercial chitosan (i.e., 80–90% of deacetylation) the pKa approaches 6.4; in acidic media (below pH 5) almost all amine groups are protonated. The main drawback of this mechanism is due to the simultaneous dissolving of the biopolymer during amine protonation. Chitosan is soluble in almost all organic and inorganic acids at mild concentration, with the exception of sulfuric acid. So the biopolymer cannot be used in its raw form in acidic media (except H_2SO_4) without pre-treatment involving chemical cross-linking. Although a number of different cross-linking agents have been tested, including epichlorhydrine and ethylene diglycidyl ether, the cross-linker most frequently used remains glutaraldehyde. The Schiff's base reaction between amine groups of chitosan and aldehyde groups of the cross-linker leads to the formation of ceto-imine bonds that can be stabilized by reaction with borohydride. The new linkages between the different chains of biopolymer (glutaraldehyde being a dialdehyde can be bound to chitosan chains on both sides) contribute to stabilizing the polymer in acidic solutions (down to 0.1–1 M HCl concentration). However, the grafting of aldehyde groups induces a steric hindrance around amine groups. Additionally, this reaction reduces the availability of amine functions engaged in supplementary linkages; the amine groups are not available for interacting with metal cations and the sorption efficiency significantly reduces. Hence, this treatment is not appropriate for metal

binding when sorption proceeds through chelation (example of Cd(II) sorption using glutaraldehyde sorption on cross-linked chitosan, [105]). This negative impact is less important in the case of ion exchange mechanism as shown by Ruiz et al. [106] in the case of Pd(II) sorption using glutaraldehyde cross-linked chitosan flakes: increasing the cross-linking ratio hardly affected sorption capacity. The ion exchange mechanism is not controlled by the availability of nitrogen, and the modification of amine functions by aldehyde grafting did not change significantly the ability of the reactive groups to be protonated under selected experimental conditions. This process was tested for the recovery of Mo(VI), V(V), Pd(II), Pt (IV), Au(III), Cr(VI), As(V), or Se(V) with sorption capacities of 0.5–3 mmol metal g^{-1} for metal forming only mononuclear species and up to 7–8 mmol metal g^{-1} for metals forming polynuclear species (such as Mo(VI) of V(V)), at pH ranging between 2 and 4 (depending on the metal) [96].

The sorption properties can be significantly improved by modification of the biopolymers, changing either the physical form (raw particles, hydrogels, aerogels, fibers, films, hollow fibers, and so on (see Fig. 2) or by their chemical structure. Indeed, chitosan has been frequently chemically modified by grafting new functional groups [95, 96]. The chemical modification is targeted to (1) improve chemical stability in acidic solutions (cross-linking) [106], (2) increase the number of reactive groups (grafting of supplementary amine functions) [107], and (3) enhance the reactivity (grafting carboxylic groups) and selectivity (grafting sulfur compounds) of the polymer under less favorable experimental conditions (for example extending the pH range of use) [108]. The physical modification is generally used for enhancing diffusion properties, which in turn improves the sorption kinetics.

It could be interesting to observe that these biopolymers can also be used in a soluble form for the binding of metal ions and the subsequent removal of metal-loaded macromolecules by ultrafiltration processes [109, 110]; this is the basis of polymer-enhanced ultrafiltration. Biopolymers such as alginate and chitosan can be used as alternative materials to synthetic polymers such as acrylates and polyethyleneimine. The dissolved state of the polymer allows better accessibility and availability of reactive groups, and, at saturation of the polymer, it is generally possible to reach a higher rate of use of these reactive groups for metal or dye binding [111].

5.1.2 Controlling Parameters

The sorption properties (both at equilibrium and in kinetic terms) are controlled by many parameters related to (1) solution chemistry (i.e., pH, metal concentration, composition of the solution, and more specifically presence of ligands) or (2) polymer characteristics (i.e., particle size, porosity, pore size, conditioning, etc.). The comparison of experimental data in the abundant literature is thus difficult since experimental conditions are not systematically fully described. These biopolymers are poorly porous under their raw form (flakes, powder) [112],

and it is generally necessary to improve their diffusion properties by preparing hydrogels; this allows improving substantially the sorption kinetics [113]. The conditioning of the polymer under the form of gel beads allows expansion of the porous structure of the material (water content higher than 95%), and "breaking" of its original crystallinity. The main drawback of this conditioning is the decrease in the mass and volumetric density of sorption sites (due to high water content); drying the hydrogel under uncontrolled conditions (oven- or air-drying) induces an irreversible collapse of the porous structure and a loss of the beneficial effect of gel conditioning. Alternative drying (under supercritical CO_2 conditions) allows maintaining of the original porous structure of the hydrogel in the form of aerogels [114]. An alternative and less sophisticated drying method has been used, consisting of the saturation of the hydrogel with a "spacer" (here sucrose) before the drying step. This technique allowed maintaining of, at least partially, diffusion properties (after rehydration and sucrose release); kinetic profiles were comparable to those obtained with wet hydrogels [115].

One of the most critical parameter, apart the optimized conditioning of the biopolymer, is the speciation of the metal. This parameter strongly controls the affinity of the metal for the binding under selected experimental conditions. An emblematic example is shown by copper sorption on chitosan. Under conventional experimental conditions, the metal is bound by complexation on amine groups at near neutral pH (below metal solubility). However, the presence of ligands, such as citric acid (for example) leads to the formation of anionic complexes that can bind to protonated amine groups in acidic solutions [116]. In this case, a change in metal speciation leads to a change in the sorption mechanism (ion-exchange/electrostatic attraction vs complexation). In other cases, the speciation influences the potential of the sorbent for binding the metal. Molybdenum and vanadium form polynuclear complexes (heptamolybdate and decavanadate species) that are preferentially adsorbed on protonated amine groups [117, 118]. The sorption isotherms can be correlated to the formation of the polynuclear species in function of the pH: sorption begins with the appearance of these favorable species, as the joined effect of pH and metal concentration.

5.1.3 Is There Another Life for These Biopolymer-Metal Ions Composite After Metal Sorption?

These biopolymers can be used for the immobilization of metal ions not only with the final objective of metal recovery (and subsequent valorization by desorption or chemical/thermal destruction of the polymer matrix) but also for elaborating new materials or designing new applications. Depending on the metal immobilized on the biopolymer, it is possible to design new sorbents (immobilization of iron on alginate [119], of molybdate on chitosan [59], for As(V) removal, of silver on chitosan for pesticide removal [120]), supports for affinity chromatography [121], antimicrobial material [122], drug release material [123], neutron capture therapy [124], and photoluminescent materials [125]. These are only a few

examples of the possibilities offered by these biopolymers for supporting metal ions and designing new systems with advanced capacities and high added value.

5.2 *Applications in Catalysis*

Several properties of the polysaccharide aerogels make them promising materials for catalysis: their high surface area and the absence of diffusional limitations in their macroporous framework and the high density and the accessibility of their functional groups (up to 5.8 mmol g^{-1} amino groups for chitosan, 5.6 mmol g^{-1} carboxylic groups for alginic acid, 2.8 mmol g^{-1} sulfate groups for k-carrageenan). They can behave as organic solid acid or base catalyst depending on the nature of the polymer, and these functional groups are promising sites for further functionalization. With surface areas as high as 500 m^2 g^{-1}, polysaccharides can compete with inorganic solids as supports for organometallic or metal catalysts. As previously mentioned, shaping of the catalyst can easily be performed by adapting the method of contacting the polysaccharide solution and the gelling agent. To date, millimeter-size aerogel spheres have been used but formulations as membranes or hollow fibers are possible.

5.2.1 Chitosan as a Catalyst

The reaction of esterification of glycidol with lauric acid (Fig. 13) is known to occur in presence of a base or in presence of a Lewis acid. This reaction leads to the formation of monoglycerides, useful emulsifiers in most processed fatty or oily foods, as well as in cosmetic and pharmaceutical products. However, the nucleophilic attack of the oxygen atom of the carboxylic function whether on the α-C or β-C of the epoxide can produce two isomers. Generally the selectivity of the reaction reflects preferred α position

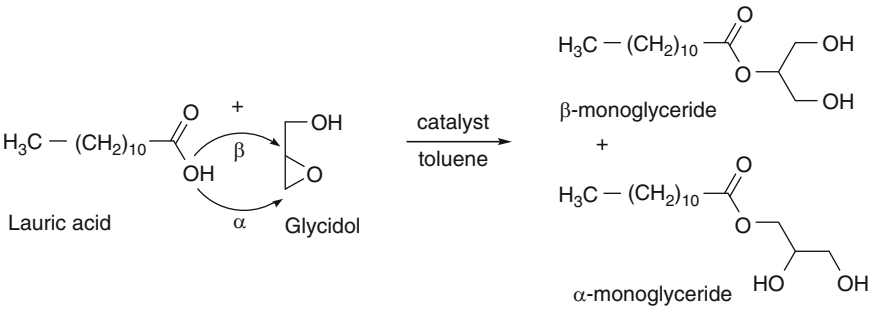


Fig. 13 Monoglyceride formation by fatty acid addition to glycidol

This reaction was chosen to probe whether the amine functionality borne by the chitosan framework can display intrinsic catalytic activity in base catalyzed reactions.

Different chitosan-based catalysts are compared in Table 3. The reactions were performed at 70°C due to the thermal stability of the catalyst. The first ones focus on the influence of the drying procedure in comparison with an uncatalyzed reaction and a known heterogeneous catalyst (Table 3, entries 1–5). Lyophilized chitosan (**C_1**) does not display any activity when the aerogel is as efficient as the functionalised silica (**C_3**). This result illustrates the accessibility to the amino groups of chitosan in its aerogel form.

Hybrids materials in which the chitosan gel serves as a host for an inorganic component have been tested. When silica is the inorganic part of the composite (C_4), a dramatic lost of selectivity is observed. Indeed, at 65% conversion, the selectivity drops from 65% to 15% (entries 6 and 3). The monoglyceride yield does not exceed 10% and the main reaction is thus glycidol polymerization. With successive runs, the selectivity increases, suggesting that some surface sites responsible for the secondary reaction have been blocked by glycidol or derivatives. Similar results have been reported with functionalized silica.

In order to reduce the secondary products (polymer or β-monoglyceride) hybrids were prepared with titanium oxide as the inorganic part. Indeed, titanium tetraisopropoxide ($Ti(OPr)_4$) is known to assist the regioselective opening of the epoxide cycle. Complexation of the oxygen atom of the cycle to the metal center, acting as a weak Lewis acid, increased both the yield and regioselectivity of this reaction [129, 130].The replacement of silica by titania avoids the polymerization of glycidol induced by the Bronsted acidity of the surface silanols. Confirming the synergetic effect expected from the Ti center and the amino groups of chitosan, the hybrid catalyst (C5) displays the best catalytic properties in terms of activity (5 h, 96% conversion) and selectivity (100%).

Table 3 α-Monoglyceride formation catalyzed by chitosan based catalysts

Catalyst	Run	Conversion% (time h)[a]	Selectivity[b] (%)	Reference
Without	1	0 (24)	0	[92]
Lyophilized chitosan (C1)	1	0 (24)	0	
Chitosan aerogel (C2)	1	65 (24)	65	
	2	98 (24)	71	
NH_2–SiO_2 (C3)	1	79 (24)[c]	89	[126]
Chitosan-SiO_2 (C4)	1	66 (24)	15	[127]
	2	45 (24)	22	
	3	46 (24)	22	
Chitosan-TiO_2 (C5)	1	96 (5)	100	[128]
	2	90 (7)	100	
	3	90 (7)	100	

[a]Glycidol conversion
[b]α-Monoglyceride selectivity, 70°C
[c]110°C

As previously described, the parameters which affect the properties of the hydrogel affect the texture of the aerogel. Chitosan aerogel surface area can be doubled but the benefit of such an increase, which is clear in the first catalytic test (98% conversion, 12 h), becomes a drawback in the successive re-uses of the catalyst (second run: 47% conversion). Therefore a balance has to be met between dispersion of the material (surface area) and the mechanical stability of the chitosan beads.

5.2.2 Polysaccharide Aerogels as Supports for Catalysts in Aqueous Phase

Considering the hydrophilic properties of the support, the effectiveness of polysaccharide aerogel microspheres as catalyst support was evidenced in the so-called Supported Aqueous Phase Catalysis [131]. The stability of the catalyst obtained was investigated in terms of textural stability and catalytic activity in the reaction of substitution of an allyl carbonate with morpholine catalyzed by the hydrosoluble Pd $(TPPTS)_3$ complex [132].

Direct impregnation by water can originate a capillary tension able to draw together the alginate fibrils and destroy the texture of the gel. Thus the best way to obtain the catalytic materials was to impregnate the aerogel beads with anhydrous ethanol for 1 h before contacting the beads with an aqueous solution of trisulfonated triphenyl phosphine palladium complex $Pd(TPPTS)_3$ synthesized in situ. After 30 min ripening at 40°C, the beads were dehydrated by immersion in two successive baths of anhydrous ethanol and dried under supercritical CO_2 conditions. This method of impregnation with the catalyst solution allowed retention of the textural properties of the materials. For example, the aerogel of an alginate with 74% guluronic residue retained its initial surface of 453–450 $m^2\ g^{-1}$ of alginate when loaded with 0.37 wt% Pd. The catalytic tests were performed in acetonitrile at 50°C with 1% Pd/methyl allyl carbonate molar ratio. Quantitative yield was reached within 30 min. The catalytic activity was not affected by the guluronic/mannuronic ratio of the alginate. Considering the hydrophilic properties of both the support and the catalyst, the effect of water addition was studied, as had been done for aqueous phase catalysts supported on freeze-dried polysaccharides [133, 134]. The activity increased with the absorbed amount of water, suggesting that the catalytic sites, not accessible in the dried material, became accessible due to the swelling of the support by water. Similar results in terms of conversion and recycling of the catalyst were obtained in ethanol, suggesting that the interaction of the palladium complex and the polysaccharide was sufficient to stabilize the Pd(O) species.

The same procedure of catalyst synthesis applied to other polysaccharides, such as *k*-carrageenan and chitosan, allowed data on the influence of the chemical structure of the support to be obtained. The differences in turnover numbers (moles of product per moles of Pd per hour), close to 500 for alginates, 190 for carrageenan, and only 40 for chitosan, indicated that the activities were correlated to the electrostatic properties of the support. Carrageenans only bear one sulfate group per two saccharide monomers, while alginate presents one carboxylic group

per monomer. In chitosan aerogels, 70% of the amino groups of the polysaccharide are accessible, and the sensitivity of the allylic substitution reaction to the pH of the medium could explain the very low activity obtained when the Pd complex was supported on this basic polysaccharide. This explanation does not exclude the possibility of a direct inhibition of the palladium complex by the amino groups of chitosan.

5.2.3 Polysaccharide Aerogels as Supports for Bifunctional Catalysts

Bifunctional catalysts can be obtained which couple the presence of a metal complex immobilized on the aerogel with the activity of the intrinsic functions of the polysaccharide. Metallophthalocyanine supported on chitosan is an example.

Selective allylic oxidation of olefins like isophorone is a very demanding chemical reaction [135].The keto-isophorone obtained by oxidation of β-isophorone (Fig. 14) is an important intermediate for the preparation of flavors and fragrances. In homogeneous catalysis, yields superior to 90% are reached [136, 137]. The heterogeneous analogs were always worse catalysts with, for example, a drop of the yield from 67% to only 6% when the Co(salen) complex was immobilized in silica [138].

The reaction is performed in the presence of an organic base like triethylamine, considered as a cocatalyst. The rule of the base is to deprotonate isophorone (IP), and thus the enolate is oxidized by oxygen (O_2) to keto-isophorone (KIP).

With its high surface area and the accessibility to the amino groups, chitosan aerogel appeared as a good candidate to play the double role of support for metal complexes and organic base. Silica supported metallophthalocyanine are efficient catalysts for the oxidation of aromatic compounds [139]. The immobilization of hydrosoluble metallophthalocyanines (MPcS with M = Fe or Co) on chitosan aerogels afforded new heterogeneous catalysts for the aerobic oxidation of β-isophorone [140].

The catalysts (MPcS@chitosan) were obtained by impregnation of chitosan aerogel beads with an aqueous solution of sulfonated metal phthalocyanine. After impregnation, the solids were dried again under supercritical CO_2 conditions. The textural properties are maintained and surface areas were greater than 140 $m^2\ g^{-1}$ (Table 4).

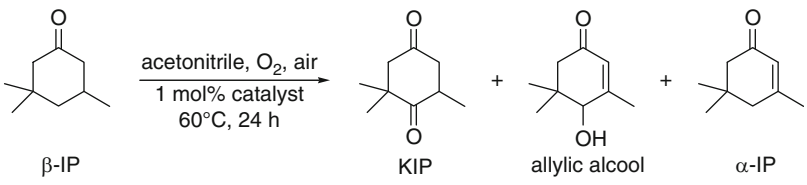


Fig. 14 Air oxidation of β-isophorone

Table 4 Phthalocyanine chitosan-based catalysts characterization

Solid	wt% M (Fe or Co)	S_{BET} m^2 g^{-1}
Chitosan	0	188 ± 5
FePcS@chitosan	0.18	223 ± 5
CoPcS@chitosan	0.18	174 ± 5
CoPcS@chitosan	0.48	142 ± 5

The diffuse reflectance UV–Vis spectra of the MPcS@chitosan showed a large Q band, indicating the presence of the monomeric species along with a dimeric species (shoulder at 600–620 nm) [141–143]. Interestingly, while FePcS was supported onto polysaccharides in a dimeric form, the monomeric and dimeric species content was comparable in the case of CoPcS, indicating a lower tendency of CoPcS to form aggregated species. The amount of the CoPcS dimeric species increased when increasing the loading of phthalocyanine complex. The catalysts were evaluated in the reaction of β-isophorone oxidation (catalyst/IP=100).

The experiments performed with FePcS@alginate and FePcS@chitosan showed 48% conversions of β-isophorone in both cases. However, while FePcS@alginate catalyzed the reaction of isomerization of β-IP to α-IP (39% yield) along with only 3% KIP yield, FePcS@chitosan provided more selective oxidation of β-IP to KIP. This finding indicated that combination of the FePcS catalyst with base sites of chitosan gave a material with improved catalytic properties. Although the conversion was still low, the KIP selectivity (up to 40%) was superior to that previously published for heterogeneous oxidation [138]. The Co-based catalyst is more efficient with a conversion of 62% and selectivity close to 48%. Activity and selectivity are maintained among successive reuse of the catalysts (three runs).

These materials are thus promising heterogeneous catalysts for the oxidation of β-isophorone to ketoisophorone. This novel method corresponds to several criteria of green chemistry and sustainable development: (1) support from inexpensive renewables, (2) dioxygen as an oxidant, (3) combination of basic and oxidation sites in one solid material to avoid an addition of external base or other additives resulting in no wastes, and (4) easy separation of catalyst from reaction mixture and possibility of recycling.

5.2.4 Functionalized Chitosan as Support for Catalysts

Chitosan functionalization relies on the nucleophilicity of the amine group although a few take place on the hydroxyls. It readily reacts with electrophilic reagents such as acid anhydrides and epoxides [144], acid chlorides [145], and aldehydes [146]. The latter is the most straightforward route to functionalize primary amine containing materials through the formation of an imine (Schiff base formation) [147, 148]. Chitosan was functionalized with the proper ligand for copper complexation. This property was usefully exploited to synthesize heterogeneous catalyst for "Click chemistry" (Huisgen reaction) [149].

5.2.5 Polysaccharide Aerogels as Supports for Metal Particles

Different palladium/polysaccharide heterogeneous catalysts are described in the literature such as Pd/chitosan [150–152], Pd/starch [153] and Pd/arabinogalactan [154]. Unfortunately, these systems present various limitations, e.g., (1) the low surface area of the supports which decreases the adsorption of the reactants, the dispersion of metallic sites, and their accessibility and (2) palladium immobilization which induces a dramatic decrease in catalytic activity as compared to the homogenous version. The poor stability of these catalysts and the metal leaching constitute major drawbacks, both for selectivity and recycling of the catalyst [147, 155]. For catalytic applications, one of the most important parameter is the texture of the support. Porosity (both meso and macro) can facilitate adsorption of the reactants and desorption of the products, making the catalytic process faster than the nonporous materials. In this context, many works are devoted to the preparation of porous materials and a new generation of catalysts with highly catalytic performance was recently described [156–158].

The aerogel formulation of ionotropic gel of alginate is particularly adapted for this purpose. Indeed, calcium cations in a Ca-alginate gel can be exchanged by palladium cations. Palladium is easily reduced by ethanol, and in situ formation of palladium particles supported on Ca-alginate occurs during the process, leading to the aerogel. The process affords a double homogeneity in bead size and metal distribution inside the bead. A narrow size in particles size distribution is obtained (Fig. 15). Palladium loading is controlled by the exchange solution, and particle size is related to the palladium loading [159].

This strategy appears very simple, fulfills some of the principles of green chemistry, and can be applied to other transition metal gels.

The materials are highly porous with surface areas ranging from 390 $m^2\ g^{-1}$ to 600 $m^2\ g^{-1}$ and mesoporous volumes ranging from 1.8 $cm^3\ g^{-1}$ to 4.8 $cm^3\ g^{-1}$. These materials show high activity for the Suzuki carbon–carbon coupling reactions

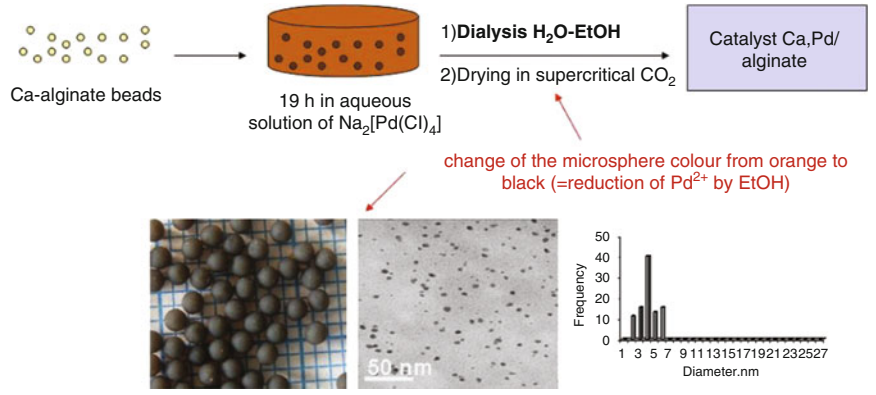


Fig. 15 In situ formation of supported palladium particles on Ca-alginate aerogels

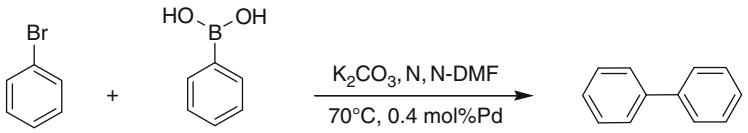
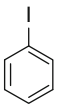
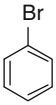
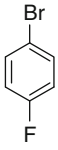
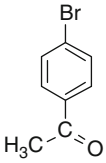
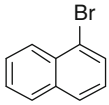


Fig. 16 Suzuki cross-coupling reaction between bromobenzene and phenylboronic acid

Table 5 Suzuki cross-coupling reaction with phenylboronic acid (conditions of Fig.16)

Substrate					
Conv.%	70	95.4	92.3	97.2	94.2

(Fig. 16) between halogeno-aryl and phenyl boronic acid with a strong relation between activity and particle size. Thus, the higher catalytic activity is obtained for catalyst with 2.5–3.5-nm particles. The increase of size of nanoparticles dramatically decreases the catalytic activity.

The reaction was extended to different substrates with the best catalyst, Ca,Pd/alginate (2.5 wt% Pd). The conversions reached are reported in Table 5. The results obtained for the bromo-substituted substrates are similar and quantitative conversions are achieved. Interestingly, no homocoupling reaction occurs, thereby showing the high selectivity of this catalyst.

The stability of the catalyst allows several reuses with only slight loss of activity. A similar nanoparticles size distribution was observed before and after catalysis.

6 Conclusion

The attractiveness of the applications of polysaccharides as materials stems both from their availability from renewable resources and their intrinsic properties. As far as the availability is concerned, most gelling polysaccharides are obtained from biomass wastes or from purposely grown biomass not in competition with food resources. Algal polysaccharides, for the economics of their growth and their ease of extraction, are classified among the less energy consuming alternatives to fossil fuels [160, 161]. Moreover, several gelling polysaccharides naturally bear functional groups that have to be inserted by energy-consuming operations in oil-derived polymers. The variety of functional groups renders polysaccharides especially appealing as polymers with the surface reactivity needed for specific catalysis processes as well as for adsorption, separation, and remediation processes. The interaction between functional groups and their glucidic backbone confers on them

their viscoelastic mechanical properties which, coupled with their easy formation in different shapes and sizes, are among their more important assets. Their biodegradability made them especially adapted for environment-friendly and consumer-friendly applications as well as for extended use in the biomedical or pharmaceutical fields. The reproducibility of their preparation and the reliability of their sources have reached high enough levels to allow the exploitation of biomass-derived functional polysaccharides in large-scale industrial productions.

References

1. Pierre AC, Pajonk GM (2002) Chem Rev 102:4243
2. Pekala RW (1989) J Mater Sci 24:3221
3. Ouverx C, Velings N, Mestdagh MM, Axelos MAV (1998) Polym Gels Networks 6:393
4. Guibal E (2005) Prog Polym Sci 30:71
5. Macquarrie DJ, Hardy JJE (2005) Ind Eng Chem Res 44:8499
6. Percival E, McDowell RH (1967) Chemistry and enzymology of marine algal polysaccharides. Academic Press, London, p 23
7. Smidsrød O (1974) Faraday Discuss Chem Soc 57:263
8. Velings N, Mestdagh MM (1995) Polym Gels Networks 3:311
9. Grant GT, Morris ER, Rees DA, Smith PJC, Thom D (1973) FEBS Lett 32:195
10. Morris ER, Rees DA, Thom D, Boyd J (1978) J Carbohydr Res 66:145
11. Braccini I, Pérez S (2001) Biomacromolecules 2:1089
12. Li L, Fang Y, Vreeker R, Appelqvist I (2007) Biomacromolecules 8:464
13. Draget KI, Skjak-Braek G, Smidsrϕd O (1994) Carbohyd Polym 25:31
14. Wang X, Spencer HG (1998) Polymer 39:2759
15. Siew CK, Williams PA, Young NWG (2005) Biomacromolecules 6:963
16. Lin TY, Hassid WZ (1966) J Biol Chem 241:5284
17. Nyvall P, Corre E, Boisset C, Barbeyron T, Rousvoal S, Scornet D, Kloareg B, Boyen C (2003) Plant Physiol 133:726
18. Campa C, Holtan S, Nilsen N, Bjerkan TM, Stoke BT, Skjak-Bræk G (2004) Biochem J 381:155
19. Furth AJ (1980) Lipids and polysaccharides in biology. Edwards Arnold, London, 28
20. Mongar IL, Wassermann A (1952) J Chem Soc 492
21. Draget KI (2001) Ann Trans Nordic Rheol Soc 8:149
22. Draget KI, Stokke BT, Yuguchi Y, Urakawa H, Kajiwara K (2003) Biomacromolecules 4:1661
23. Tønnesen HH, Karlsen J (2002) Drug Dev Ind Pharm 28:621
24. Dhoot NO, Wheatley MA (2003) J Pharm Sci 92:679, and references therein
25. Teng WL, Khor E, Tan TK, Lim LY, Tan SC (2001) Carbohydr Res 332:305
26. Roberts GAF (1992) Chitin chemistry. Macmillan Press Ltd, London
27. Saito Y, Putaux J-L, Okano T, Gaill F, Chanzy H (1997) Macromolecules 30:3867
28. Lamarque G, Viton C, Domard A (2004) Biomacromolecules 5:992
29. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R (2004) Eur J Pharm Biopharm 57:19
30. Berger J, Reist M, Mayer JM, Felt O, Gurny R (2004) Eur J Pharm Biopharm 57:35
31. Maraca R, Suder BJ, Wightman JP (1982) J Appl Polym Sci 27:4827
32. Shi HQ, Tsai WB, Garrison MD, Ferrari S, Ratner BD (1999) Nature 398:593
33. Shu XZ, Zhu KJ (2001) J Microencapsul 18:237
34. Koyama Y, Taniguchi A (1986) J Appl Polym Sci 31:1951

35. Amiji MM (1997) Carbohyd Polym 32:193
36. Zeng X, Ruckenstein E (1998) J Memb Sci 148:195
37. Muzzarelli RAA (1973) Natural chelating polymers. Oxford, Pergamon Press, pp 83–176
38. Roberts GAF (1992) Chitin chemistry. London, Macmillan, pp 206–229
39. Gerentesa P, Vachoud L, Dourya J, Domard A (2002) Biomaterials 23:1295
40. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, Dhawan S (2004) Int J Pharm 274:1
41. Winterowd PA, Sandford JG (1995) In: Stephen AM (ed) Food polysaccharides and their applications. Marcel Dekker, New York, p 441
42. Barbotin JN, Nava Saucedo JE (1998) In: Dumitriu S (ed) Polysaccharides. Marcel Dekker, New York, p 749
43. Slootmaekers D, Mandel M (1990) Int J Biol Macromol 13:17
44. Rochas C, Rinaudo M (1980) Biopolymers 19:1675
45. Borgstrom J, Piculell L, Viebke C, Talmon Y (1996) Int J Biol Macromol 18:223
46. Walsh PK, Isdell FV, Noone SM, O'Donovan MG, Malone DM (1996) Enzyme Microb Tech 18:366
47. Sipahigil O, Dortunc B (2001) Int J Pharm 228:119
48. Denkbas EB, Kiliçay E, Birliksevenand C, Oztürk E (2002) React Funct Polym 50:225
49. Denkbas EB, Odabasi M (2000) J Appl Polym Sci 76:1637
50. Rorrer GL, Hsien T-Y, Way JD (1993) Ind Eng Chem Res 32:2170
51. Fundueanu G, Nastruzzi C, Carpov A, Desbrieres J, Rinaudo M (1999) Biomaterials 20:1427
52. Potter K, McFarland EW (1996) Solid State Nucl Magn Reson 6:323
53. Konishi Y, Shimaoka JI, Asai S (1998) React Funct Polym 36:197
54. Khairou KS (2002) J Therm Anal Calorim 69:583
55. Khairou KS, Al-Gethami WM, Hassan RM (2002) J Membr Sci 209:445
56. Khairou KS, Al-Gethami WM, Hassan RM (2002) Bull Pol Acad Sci Chem 50:299
57. Dambies L, Vincent T, Domard A, Guibal E (2001) Biomacromolecules 2:1198
58. Draget KI, Vårum KM, Moen E, Gynnild H, Smidsrød O (1992) Biomaterials 13:635
59. Dambies L, Vincent T, Guibal E (2002) Water Res 36:3699
60. Kurokawa Y, Hanaya K (1995) Carbohydr Polym 27:313
61. Yoshida H, Okamoto A, Kataoka T (1993) Chem Eng Sci 48:2267
62. Vincent T, Guibal E (2001) Ind Eng Chem Res 40:1406
63. Vincent T, Guibal E (2000) Solvent Extr Ion Exch 18:1241
64. Guibal E, Vincent T (2004) Environ Sci Technol 38:4233
65. Guibal E, Vincent T, Spinelli S (2005) Sep Sci Technol 40:633
66. Peirano F, Vincent T, Quignard F, Robitzer M, Guibal E (2009) J Memb Sci 329:30
67. Wang X, Spencer HG (1996) J Appl Polym Sci 61:827
68. Yeom CK, Lee K-H (1998) J Appl Polym Sci 67:209
69. Mi F-L, Shyu S-S, Wu Y-B, Lee S-T, Shyong J-Y, Huang R-N (2001) Biomaterials 22:165
70. Krajewska B (2001) React Funct Polym 47:37
71. Wang X-P, Shen Z-Q (2000) Polym Int 49:1426
72. Arvanitoyannis IS, Nakayama A, Aiba S-I (1998) Carbohydr Polym 37:371
73. Kubota N (1997) J Appl Polym Sci 64:819
74. Zheng X, Ruckenstein E (1996) Ind Eng Chem Res 35:4169
75. Valentin R, Molvinger K, Quignard F, DiRenzo F (2005) Macromol Symp 222:93
76. Di Renzo F, Valentin R, Boissière M, Tourrette A, Sparapano G, Molvinger K, Devoisselle JM, Gérardin C, Quignard F (2005) Chem Mater 17:4693
77. Robitzer M, David L, Rochas C, Di Renzo F, Quignard F (2008) Langmuir 24:12547
78. Rouquerol F, Rouquerol J, Sing KSW (1999) Adsorption by powders and porous solids. Academic Press, San Diego
79. Sing KSW, Everett DH, Haul RAW, Moscou L, Pierotti RA, Rouquerol J, Siemieniewska T (1985) Pure Appl Chem 57:603
80. Broekhoff JCP, de Boer JH (1968) J Catal 10:377

81. Atkins EDT, Nieduszynski IA, Mackie W, Parker KD, Smolko EE (1973) Biopolymers 12:1865
82. Atkins EDT, Nieduszynski IA, Mackie W, Parker KD, Smolko EE (1973) Biopolymers 12:1879
83. Mackie W, Pérez S, Rizzo R, Taravel F, Vignon M (1983) Int J Biomacromol 5:329
84. Ogawa T, Okuyama K (2004) Int J Biol Macromol 34:1
85. Tourrette A, Vachoud L, Tourné-Péteilh C, Bégu S, Devoisselle JM, Quignard F (2006) Adv Chitin Sci 9:284
86. Clayer A, Vachoud L, Viton C, Domard A (2003) Macromol Symp 200:1
87. Capitani D, De Angelis AA, Crescenzi V, Masci G, Segre AL (2001) Carbohydr Polym 45:245
88. Valentin R, Horga R, Bonelli B, Garrone E, Di Renzo F, Quignard F (2005) Macromol Symp 230:71
89. Valentin R, Horga R, Bonelli B, Garrone E, Di Renzo F, Quignard F (2006) Biomacromolecules 7:877
90. Valentin R, Bonelli B, Garrone E, Di Renzo F, Quignard F (2007) Biomacromolecules 8:3646
91. Rodriguez Escudero R, Robitzer M, Di Renzo F, Quignard F (2009) Carbohydr Polym 75:52
92. Valentin R, Molvinger K, Quignard F, Brunel D (2002) New J Chem 27:1690
93. Pearson RG (1963) J Am Chem Soc 85:3533
94. DeRamos CM, Irwin AE, Nauss JL, Stout BE (1997) Inorg Chim Acta 256:69
95. Varma AJ, Deshpande SV, Kennedy JF (2004) Carbohydr Polym 55:77
96. Guibal E (2004) Sep Purif Technol 38:43
97. Ibanez JP, Umetsu Y (2004) Hydrometallurgy 72:327
98. Papageorgiou SK, Katsaros FK, Kouvelos EP, Kanellopoulos NK (2009) J Hazard Mater 162:1347
99. Dewangan T, Tiwari A, Bajpai AK (2009) J Dispers Sci Technol 30:56
100. Ngah WSW, Fatinathan S (2008) Chem Eng J 143:62
101. Maxim K, Valeri K, Yuri K (2008) J Environ Sci – China 20:827
102. Lai YL, Annadurai G, Huang FC, Lee JF (2008) Bioresour Technol 99:6480
103. Pandey A, Bera D, Shukla A, Ray L (2007) Chem Spec Bioavail 19:17
104. Sorlier P, Denuzière A, Viton C, Domard A (2001) Biomacromolecules 2:765
105. Erosa MSD, Medina TIS, Mendoza RN, Rodriguez MA, Guibal E (2001) Hydrometallurgy 61:157
106. Ruiz M, Sastre AM, Guibal E (2000) React Funct Polym 45:155
107. Chassary P, Vincent T, Marcano JS, Macaskie LE, Guibal E (2005) Hydrometallurgy 76:131
108. Guibal E, Sweeney NV, Vincent T, Tobin JM (2002) React Funct Polym 50:149
109. Kuncoro EP, Roussy J, Guibal E (2005) Sep Sci Technol 40:659
110. Maureira A, Rivas BL (2009) Eur Polym J 45:573
111. Guibal E, Touraud E, Roussy J (2005) World J Microbiol Biotechnol 21:913
112. Piron E, Accominotti M, Domard A (1997) Langmuir 13:1653
113. Guibal E, Milot C, Tobin JM (1998) Ind Eng Chem Res 37:1454
114. Quignard F, Valentin R, Di Renzo F (2008) New J Chem 32:1300
115. Ruiz M, Sastre A, Guibal E (2002) Sep Sci Technol 37:2143
116. Guzman J, Saucedo I, Revilla J, Navarro R, Guibal E (2003) Int J Biol Macromol 33:57
117. Guibal E, Milot C, Roussy J (2000) Sep Sci Technol 35:1021
118. Guzman J, Saucedo I, Navarro R, Revilla J, Guibal E (2002) Langmuir 18:1567
119. Min JH, Hering JG (1998) Water Res 32:1544
120. Yoshizuka K, Lou Z, Inoue K (2000) React Funct Polym 44:47
121. Ahmed SR, Kelly AB, Barbari TA (2006) J Membr Sci 280:553
122. Wang X, Du Y, Liu H (2004) Carbohydr Polym 56:21
123. Cha J, Lee WB, Park CR, Cho YW, Ahn CH, Kwon IC (2006) Macromol Res 14:573
124. Saha TK, Ichikawa H, Fukumori Y (2006) Carbohydr Res 342:2835

125. Liu FY, Carlos LD, Ferreira RAS, Rocha J, Gaudino MC, Robitzer M, Quignard F (2008) Biomacromolecules 9:1945
126. Cauvel A, Renard G, Brunel D (1997) J Org Chem 62:749
127. Molvinger K, Quignard F, Brunel D, Boissiere M, Devoisselle JM (2004) Chem Mater 16:3367
128. El Kadib A, Molvinger K, Guimon C, Quignard F, Brunel D (2008) Chem Mater 20:2198
129. Caron M, Sharpless K (1985) J Org Chem 50:1560
130. Burgos CE, Ayer DE, Johnson RA (1987) J Org Chem 52:4973
131. Arhancet JP, Davis ME, Merola JS, Hanson BE (1989) Nature 339:454
132. Valentin R, Molvinger K, Viton C, Domard A, Quignard F (2005) Biomacromolecules 6:2785
133. Quignard F, Choplin A, Domard A (2000) Langmuir 16:9106
134. Buisson P, Quignard F (2002) Aust J Chem 55:73
135. Murphy EF, Mallat T, Baiker A (2000) Catalysis Today 57:115
136. Constantini M, Dromard A, Jouffret M, Brossard B, Varagnat J (1980) J Mol Catal 7:89
137. Itoh N, Etoh T, Hagiwara H, Kato M (1997) Synthesis 2:153
138. Murphy EF, Baiker A (2002) J Mol Catal 179:233
139. Sorokin AB, Tuel A (2000) Catal Today 57:45
140. Sorokin AB, Quignard F, Valentin R, Mangematin S (2006) Appl Catal A 30:162
141. Abel EW, Pratt JM, Wherlan R (1976) J Chem Soc Dalton Trans 509
142. Skorobogaty A, Smith TD (1982) J Mol Catal 16:131
143. Yang Y-C, Ward JR, Seiders RP (1985) Inorg Chem 24:1765
144. Zeng X, Ruckenstein E (1998) Ind Eng Chem Res 37:159
145. Wei YC, Hudson SM, Mayer JM, Kaplan DL (1992) J Polym Sci A 30:2187
146. Roberts GAF, Moore GK (1989) Makromol Chem 190:951
147. Hardy JJE, Hubert S, Macquarrie DJ, Wilson AJ (2004) Green Chem 6:53
148. Justi K, Laranjeira MCM, Neves A, Mangrich AS, Favere VF (2004) Polymer 45:6285
149. Chtchigrovsky M, Primo A, Gonzalez P, Molvinger K, Robitzer M, Quignard F, Taran F (2009) Angew Chem Int Ed 48:5916
150. Vincent T, Guibal E (2003) Langmuir 19:8475
151. Tong J, Li Z, Xia C (2005) J Mol Catal A: Chem 231:197
152. Sun Y, Guo Y, Lu Q, Meng X, Xiaohua W, Guo Y, Wang Y, Liu X, Zhang Z (2005) Catal Lett 100:213
153. Budarin VL, Clark JH, Luque R, Macquarrie DJ, White RJ (2008) Green Chem 10:382
154. Mucalo MR, Bullen CR, Manley-Harris M (2002) J Mater Sci 37:493
155. Xu X, Liu P, Li S, Zhang P, Wang X (2006) React Kinet Catal Lett 88:217
156. White RJ, Budarin VL, Clark JH (2008) Chemsuschem 1:408
157. Budarin VL, Clark JH, Luque R, Macquarrie DJ, Koutinas A, Webb C (2007) Green Chem 9:992
158. Clark JH, Budarin V, Dugmore T, Luque R, Macquarrie DJ, Strelko V (2008) Catal Commun 9:1709
159. Primo A, Liebel M, Quignard F (2009) Chem Mater 21:621
160. McHugh DJ (2003) A guide to the seaweed industry. FAO, Rome
161. Towler JP, Oroskar AR, Amity SE (2004) Environ Prog 23:334

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