Production of Oligosaccharides as Promising New Food Additive Generation

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Summary

Recent research in the area of carbohydrate food ingredients has shown the efficiency of oligosaccharides when they are used as prebiotics or biopreservatives. Considering the former, they have various origins and structures, whereas the latter are described mostly as oligochitosans or as low molecular mass chitosans. If new manufacturing biotechnologies have significantly increased the development of these functional food ingredients, the main drawback limiting their applications is the difficulty to engender specific glycosidic structures. The present review focuses on the knowledge in the area of food bioactive oligosaccharides and catalogues the processes employed to generate them.

Key words: oligosaccharides, prebiotics, food preservative

Introduction

In food industries, as chemical additives are becoming less and less welcome by consumers, there has been an increasing interest in the use of saccharidic natural substances known as prebiotic and biopreservative oligosaccharides.

Traditionally, oligosaccharides are defined as polymers of monosaccharides with degrees of polymerization (DP) between 2 and 10 (3 and 10 according to the IUB-IUPAC nomenclature) but DPs up to 20–25 are often assimilated with them. Prebiotic oligosaccharides are noncariogenic, nondigestible (NDO) and low calorific compounds stimulating the growth and development of gastrointestinal microflora described as probiotic bacteria. It is claimed that these bacteria belonging to *Bifidobacteria* and *Lactobacilli* have several health-promoting effects (*1,2*). Moreover, the recent development of commercial prebiotic oligosaccharides and probiotic bacteria has led naturally to a new concept, that of symbiotic one, combining probiotics and prebiotics (*3*). Paradoxically, other oligosaccharides and more specifically chitosan oligosaccharides (COS) or low molecular mass chitosans (LMMC) are described as food additives for their antimicrobial effects against pathogenic bacteria or fungi (*4–6*). Additionally, data suggest that specific COS or LMMC could also have beneficial effect on the growth of *Bifidobacteria* and *Lactobacilli* (*7,8*). Structural features of these oligomers appear as modulators for their biological activities.

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Abbreviations: COS: chitosan oligosaccharides; DP: degree of polymerization; FOS: fructooligosaccharides; GF: glucose-fructosyl unit; GOS: galactooligosaccharides; GRAS: generally recognized as safe; IMO: isomaltooligosaccharides; LMMC: low molecular mass chitosan; NDO: nondigestible oligosaccharides; OGAs: oligogalacturonides; PI: prebiotic index; SHIME: simulator of the human intestinal microbial ecosystem; SOS: soybean oligosaccharides; TAG: triacylglycerol; TGOS: *trans*-galactooligosaccharides; USFDA: US Food and Drug Administration; XOS: xylooligosaccharides; VLDL: very low density lipoproteins

In the current context of functional foods generating a global market of 33 billion US dollars (*9*), oligosaccharides could play a major role as functional ingredients compared to dietary fibers, sugar alcohols, peptides, probiotics, polyunsaturated fatty acids and antioxidants. Nonetheless, they will have a very large development in the future, depending on the viability of their large scale production. Oligosaccharides have currently two origins: they can be synthesized by chemical glycosylation and *de novo* using glycosidase and glycosyltransferase activities, or they can derive from chemical, physical or biological degradation of polysaccharides. As a consequence, this review focuses on the present uses of oligosaccharides as nutraceuticals, but also on the recent developments in the area of their production.

Oligosaccharides As Prebiotics

Presently, standard prebiotics are largely used depending on their putative positive action on the host's health. For these reasons, this new class of food ingredients has been added to human and domestic animals' foods. Concerning carbohydrates, the term prebiotic may be ambiguous because a lot of saccharidic compounds are present in feeding with or without prebiotic action (dietary fibers for example). In this context, Gibson *et al.* (*10*) established clear criteria for classifying a food ingredient as a prebiotic. Accordingly, a prebiotic oligosaccharide firstly needs to be resistant to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. Secondly, this oligomer has to be fermented by the intestinal microflora. Thirdly, it stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing such as *Bifidobacteria* and *Lactobacilli*.

We have noticed that the majority of studies focuses on the *in vitro* metabolism of prebiotic oligosaccharides, and the mechanisms operating *in vivo* need to be elucidated. Uses of simulators of the human intestinal microbial ecosystem (SHIME) could lead to the design of more effective forms of prebiotics in the future (*11*). Furthermore, quantification strategies of prebiotic effects are currently assayed *in vitro* on faecal batch cultures (*12*) and a prebiotic index (PI) has been created (*13*).

FOS and GOS prebiotics

If some NDOs with prebiotic activities occur naturally in human milk (*14*) and plants (*15*), most of them are synthesised or isolated from plant polysaccharides such as fructooligosaccharides (FOS), galactooligosaccharides (GOS) or *trans*-galactooligosaccharides (TGOS), isomaltooligosaccharides (IMO), xylooligosaccharides (XOS), plant cell wall derived polysaccharides and other. At this time, FOS and GOS are leaders on the world market. There is little information about the structure-function relationships of these oligosaccharides apart from the studies comparing the fermentation properties of commercial products.

The prebiotic effects of FOS or inulin (a mixture of FOS and polysaccharides) have been investigated by studying the metabolism of this mixture with DP from 3 up to 40–50 using *Bifidobacterium* sp. or *Lactobacillus* sp.

(*9,16*). We noted a high degree of variability in DP distribution depending on industrial preparations. All FOS consist of a glucose monomer α-(1,2) linked to two (GF_2) or more (GF_n) β-(2,1) fructosyl units. Generally, *in vitro* data support the selective stimulation of bacterial growth by FOS and inulin using pure bacterial or/and faecal batch culture. However, metabolism of pure oligomers with controlled DP by colonic microflora has not been studied much even if the presence of fermentable mono- or disaccharides is well known in commercial preparations obtained from natural sources (*e.g.* inulin) or naturally synthesized from sucrose. In this context, the use of pure FOS mixtures containing three FOS species (GF_2, GF_3 and GF_4) led to the identification of only 2 oligosaccharides (GF_2 and GF_3) consumed by *Lactobacillus* strains. None of the examined strains was able to metabolise the GF_4 species, which suggests an intracellular metabolism after the FOS transport (*17*). This transfer has been recognised as mediated by an ATP-dependent transport system having specificity for a narrow range of substrates (*18*). Nonetheless, another paradigm is the FOS degradation by probiotic cell-associated exoglycosidases and notably β-fructofuranosidases. With this mechanism, identified in a *Bifidobacterium infantis* strain, the monosaccharides generated are taken up by the bacteria (*19*).

Using animal models or volunteers fed with aliments containing inulin or FOS, *in vivo* experiments support the bifidogenic effect of FOS with large variations depending on the subjects, faecal microflora composition, doses and categories of FOS and/or inulin (*9,10*). Authors noted the end of the prebiotic effect and the decrease of colonic microflora when the addition of FOS in food was stopped (*20*).

The GOS and TGOS fermentations are also well documented. TGOS are GOS produced by transgalactosylation of lactose using a β-galactosidase. In the final product, different linkages between the galactose and the reducing terminal glucose have been identified [(1,2); (1,3) and (1,4)] and branched glucose residues occur. The galactan fragment is (1,4) or (1,6) linked as for GOS (*21, 22*). This high degree of variability in glycosidic linkage could implicate an incomplete resistance to gastric acidity and mammalian enzymes, as suggested by Tomomatsu (*23*). Generally, studies of the impact of TGOS on colic microflora species have shown that if many strains of enteric bacteria are unable to metabolise TGOS, *Bifidobacteria* and in a minor rate *Lactobacilli* will metabolise them (*24*). *Bifidobacterium adolescentis*, one of the predominant human faecal bacterium, can degrade and metabolise TGOS with DP 3 or higher, contrary to *Bifidobacterium infantis* and *Lactobacillus acidophilus*, which can use only TGOS with DP 3. This particularity is related to a β-galactosidase probably attached to the membrane (*25*). GOS metabolism was also investigated with fractionated GOS used as substrate for *Bacillus lactis* and for *Lactobacillus rhamnosus* by Gopal *et al.* (*26*), who noted that *B. lactis*, contrary to *L. rhamnosus*, was able to metabolise tri- and tetrasaccharidic fractions, suggesting a specific transport system. Data from *in vivo* experiments confirmed the increase of *Bifidobacteria* and *Lactobacilli* when TGOS were added in foods (*27,28*).

XOS, IMO and SOS prebiotics

Compared to FOS and GOS/TGOS, other prebiotic oligosaccharides are less documented, except for xylo-oligosaccharides (XOS), isomaltooligosaccharides (IMO), soybean oligosaccharides (SOS) and lactulose. However, even if the lactulose, resulting from the isomerisation of lactose to form galactosyl β-(1,4) fructose, is well known as a prebiotic oligosaccharide, its status in the oligosaccharide nomenclature (IUB-IUPAC) is not well established. Moreover, considering the possible lactose isomerisation during food engineering and notably during heat treatment of milk, this disaccharide may be naturally present in significant concentrations in food products. Nevertheless, *in vitro* comparative data showed that lactulose is one of the most efficient prebiotics in *Bifidobacteria* strains (*13*). Comparative results were found with *in vivo* experiments (*29–31*).

Considering XOS, IMO and SOS, their first commercial uses as prebiotics are presently being developed in Japan. Like the xylan, XOS are very resistant to acids and mammalian enzymes. They are manufactured by xylanase degradation of xylans and lead to an oligomeric mix where the xylobiose is the most representative compound (*32*). Data relating to XOS metabolism by intestinal microflora are ambiguous and Gibson *et al.* (*10*) concluded their recent review without the classification of XOS, as their fermentation does not seem to be selective (*33,34*).

Like XOS, IMO have a real positive effect, resulting in higher populations of *Bifidobacteria* (*10,11,13,35*). However, these α-(1,4)(1,6) oligoglucans produced from starch hydrolysis by α-amylases and pullulanases are potentially digestible by mammalians and can be metabolised by a wide range of bacteria. Consequently, their belonging to prebiotic oligosaccharides is not actually really defined (*10*).

The soybean oligosaccharides (raffinose and stachyose) are well known α-galactosyl sucrose derivatives extracted from soybeans (*11,12*). They are present in soy-germ powder, whose fermentation properties have been successfully tested on *Lactobacilli* in the SHIME with faecal bacteria inoculum (*11*). A comparative *in vitro* evaluation of SOS on predominant gut bacterial groups showed that SOS have comparable effects with other galactooligosaccharides (*12*). On pure cultures, similar results have been obtained with individual purified compounds or mixture of oligosaccharides (*36*).

New prebiotic oligosaccharides

At present, the advancement of knowledge about polysaccharides from plant cell wall and plant cell wall polysaccharide cleavage enzymes allows the development of novel prebiotics. Effectively, these polysaccharides are available in large amounts notably from food industry by-products. Therefore, the use of specific hydrolysis conditions leads to processes for oligosaccharide productions. These oligomers have a large variety of structures and could become an interesting way to increase the value of plant by-products in the future. We noted that some of these oligomers are naturally produced during processing of food where glycanases are used for technological benefit.

In this way, arabinogalactooligosaccharides, arabinoxylooligosaccharides, arabinooligosaccharides, galacturonan oligosaccharides, rhamnogalacturonan oligosaccharides and pectic oligosaccharides have been successfully experimented with (*25,37–39*). These oligomers have been fermented in pure cultures by intestinal bacteria such as *Bifidobacteria*, *Lactobacilli*, *Bacteroides* sp., *Clostridium* sp., *Escherichia coli* and *Klebsiella* sp.

In addition, recent literature has detailed numerous other oligosaccharidic structures as glucooligosaccharides and oligosaccharides from melibiose, mannan oligosaccharides, oligodextrans and gentiooligosaccharides with prebiotic activities (*13,38,40,41*). We also noted that some probiotic bacteria could produce by themselves polysaccharides (but no oligosaccharides) having prebiotic effects (*42*).

Use of prebiotics for additional beneficial effects

As classical prebiotic oligosaccharides added in food, human milk oligosaccharides stimulate the proliferation of bifidogenic microflora in breastfed children (*43*), but have also other important roles in the local intestine immune system (*44*). They play a role of additional defence mechanism as receptors (*45*) or block the progress of inflammatory responses (*46*). All these functions detected for sialylated and fucosylated oligosaccharides from human milk have not yet been tested with commercial FOS or GOS, but it is possible that these compounds have these effects as well.

Moreover, in addition to the increase of *Bifidobacteria* and *Lactobacilli*, prebiotic oligosaccharides have other identified effects that could enhance their use for therapeutic actions. One of them is the detection of short chain fatty acid (as propionate or butyrate) production as end fermentative products. These compounds have been recognised for their role in the prevention of colon cancer (*47*). It is also reported that FOS significantly increase the effects of different cytotoxic drugs used in human cancer treatment (*48*).

The proliferation of beneficial bacteria under the influence of prebiotic oligosaccharides has also a significant impact on the prevention of the proliferation of pathogenic bacteria. This has been attributed to the low pH environment created during the fermentation of FOS in the colon (*49*).

Other data described a role of FOS in mineral absorption (mainly magnesium and calcium) because of the pH decrease in colon during their fermentation (*50*). The role of FOS in the control of diabetes has also been suggested (*51*). However, the important rate of residual monosaccharides in commercial FOS limits their uses in diabetic food products. FOS have also been implicated in the lipid metabolism and a lot of data suggest that FOS in foods modify the hepatic metabolism of lipids (*52*), inhibit secretion of triacylglycerol (TAG)-rich very low density lipoproteins (VLDL) (*9*) and reduce blood levels of TAG (*53*). FOS are also known to decrease the cholesterol in insulin-independent diabetic patients (*54*).

Oligosaccharides As Natural Food Preservatives

The term biopreservative includes a wide range of natural products from both plants and microorganisms, able to extend shelf life of foods, reduce or eliminate survival of pathogenic bacteria and increase overall quality of food products (*55*). These natural occurring antimicrobials can be, for example, peptides such as bacteriocins (*56,57*) or lipophilic substances such as essential oils (*58*). Compared to these two kinds of antimicrobial molecules, sugar molecules seem to be less investigated as potential food preservatives.

In this context, one of the currently most studied polysaccharides is indisputably chitin. This linear homopolymer of β-(1,4)-linked-*N*-acetyl-D-glucosamine residues (Fig. 1) is one of the most abundant renewable natural polymers, second to cellulose. Chitin is commonly found in the exoskeletons or cuticles of many invertebrates like crustaceans and arthropods, in the cell walls of most fungi and is extracted commercially from shellfish wastes (*59*). As it is estimated to be synthesised in nature at a level of up to 10^9–10^{10} tonnes a year, the potential of chitin is evident in various industrial fields. Because of its limited solubility in aqueous solutions and organic solvents, many studies were realised on its low acetylated form, called chitosan (Fig. 1c). This biopolymer is easily obtained by alkali *N*-deacetylation of chitin. Polycationic at pH=6, biodegradable, nontoxic, soluble in acetic acid solutions, chitosan offers properties with great potential for many industrial applications. Accordingly, chitosan attracted considerable attention since it has been reported to exhibit interesting activities, notably to improve human health (*60*) and food quality with its antioxidative (*61*) and antimicrobial (*62,63*) properties. Moreover, concerning this last point and with respect to antimicrobial activity, chitosan seems superior to chitin since it contains amino groups which could interact with the negatively charged bacterial cell membranes and then inhibit the bacterial growth (*64–67*). Other mechanisms for antimicrobial activity of chitosan have also been suggested, as the blockage of RNA transcription by adsorption of penetrated chitosan to bacterial DNA (*68*) or the chelating action of chitosan with metal trace elements or essential nutrients, leading to microbial growth inhibition (*69*).

Use of chitosan as potential food preservative

Most commercial native chitosans have a degree of deacetylation greater than 70 % and a molecular mass ranging between 100 and 1200 kDa. The legislation about their uses as food additives varies according to the country. Chitosan is sold in the European market in the form of dietary capsules to assist mass loss; it is reportedly used in Japan as a preservative in many food products (*6,70*), whereas the United States Food and Drug Administration (USFDA) approved its use in 1983 only as a feed additive (*71*) and has recently recognized it as a GRAS (Generally Recognised As Safe) component (*72*).

Chitosan antioxidative activities

Several studies reported antioxidative activities exhibited by chitosan. As the use of molecules with such properties is one way to extend the shelf life of food products, this biopolymer was tested on muscle foods, such as meat or seafoods, which contain highly unsaturated fatty acids particularly sensitive to oxidative change during storage (*61*). St. Angelo (*73*) reported that iron bound to proteins such as myoglobin or haemoglobin can be released during postharvest storage and cooking and then activate oxygen and initiate lipid oxidation. The mechanism involved in chitosan antioxidative activity is thought to be related to chelation of free iron. Effectiveness of chitosan treatment on oxidative stability of beef was also studied by Darmadji and Izumimoto (*74*) who observed that the addition of chitosan at 1 % concentration decreased the 2-thiobarbituric acid value of meat for 70 % after three days of storage at 4 °C.

Chitosan antimicrobial activities

Antimicrobial activities of chitosan were also demonstrated against many different kinds of microorganisms. Accordingly, chitosan was shown to inhibit food spoilage microorganisms, such as *Candida* sp., *Escherichia coli* and *Staphylococcus aureus* (*74,75*). However, as the culture media employed poorly represent what really happens in complex food systems, this polysaccharide has also been tested in food products. Several studies were realized in fruit juices and emulsified sauces, but also in solid foods such as meat (*74,76*), mayonnaise (*66,77*), tofu (*78*), houmous and chilled salads (*75*). Finally, chitosan was also studied as an edible antimicrobial film to cover fresh fruits and vegetables (*79*), pizza (*80*) and meat (*81*).

Properties of chitosan oligosaccharides

If all the investigated studies recognize the antimicrobial activities of chitosan, those seem to depend on many factors, such as molecular mass, degree of acetylation, type of screened microorganisms and tested envi-
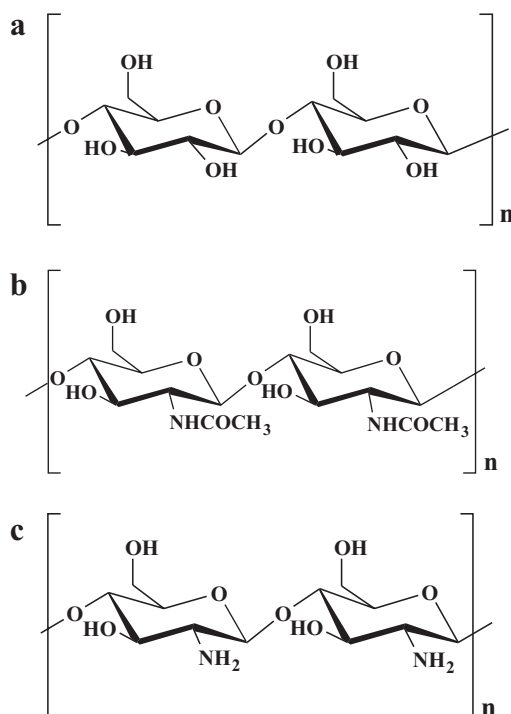
Fig. 1. Structures of (a) cellulose, (b) chitin and (c) chitosan

ronmental conditions (*82*). Accordingly, DP is one of the most investigated factors. Finally, chitosan oligomers have received considerable attention since they were reported to be able to exhibit biological activities as interesting as those of their corresponding polymers even if the results about it are still controversial. In this way, No *et al.* (*78*) examined the antibacterial activities against several spoilage and food-borne bacteria of six chitosans and chitosan oligomers with widely different molecular mass. Their results led them to conclude that chitosans have higher antibacterial activities and markedly inhibited growth of most tested bacteria at a 0.1 % concentration. On the other hand, Tsai *et al.* (*83*) evaluated the antibacterial activity of a mixture of chitooligosaccharides prepared by digestion of shrimp chitosan against food-borne bacteria in nutrient broth. They showed that the required chitooligosaccharide mixture concentration to reach bactericidal effect on the tested bacteria was lower than that required with native chitosan. As to Kittur *et al.* (*84*), they showed that the growth inhibitory effect of a chitooligosaccharide preparation with DP ranging from 2 to 6 was better than that of native chitosan.

For their part, Rhoades and Roller (*75*) investigated the antimicrobial actions in laboratory media of degraded and native chitosans against spoilage microorganisms. They proved that the inhibitory activity of chitosan against spoilage yeasts such as *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae* was enhanced with chitosan degradation products. Tsai *et al.* (*85*) showed that LMM chitosan (12 kDa) has much higher antimicrobial activity than the mixture of chitooligosaccharides (DP 1 to 8) against pathogenic microorganisms. In the same way Kittur *et al.* (*84*) demonstrated that the inhibition percentage of *Bacillus cereus* growth doubled from chitooligosaccharides trimer to hexamer at 10 % concentration. All these results were in agreement with those of Kendra and Hadwinger (*86*), who demonstrated that, if chitooligosaccharides can exhibit strong antibacterial effect, it is nevertheless greatly dependant on their DP and requires glucosamine oligomers with DP 7 or greater.

In addition, environmental conditions and the nature of food products seem to influence the antimicrobial activity of chitosan and chitosan oligomers. Accordingly, Savard *et al.* (*65*) reported different inhibition patterns in liquid and solid media for chitosan oligomers tested against yeasts. Unlike previous results, the inhibitory activity of chitosan oligomers in agar medium was lost with the increase of the DP. As in liquid medium, the intermediate molecular mass chitosans were more toxic than the low molecular mass ones; the authors concluded that medium composition influenced the inhibitory activity of chitosan oligomers. Rhoades and Roller (*75*) explained the differences observed by the nature of solid medium which could restrict the mass transfer of macromolecules. Then, the choice of the food product to preserve from spoilage with chitosan or chitosan oligomers has to be judicious in order to keep a maximal antimicrobial activity. In the case of food products that need the use of microorganisms, such as lactic or vegetable fermentation, the antimicrobial action of chitosan or chitosan oligosaccharides may not permit their uses. Nonetheless, it can be conceivable to add them before packaging, in order to fight against spoilage yeasts (*65*).

Concerning the pH effects on various molecular mass chitosans, it affected their antibacterial activity independently of their molecular mass (*78*). This result clearly indicates that, whatever their molecular mass, applications of chitosans or chitosan oligomers in acidic food products would enhance their effectiveness.

Oligosaccharide Engineering

Even if the potentialities of oligosaccharides are real, in most cases the lack of oligosaccharide production processes is the main drawback limiting their applications. That is why the development of oligosaccharide engineering strategies actually represents a challenge.

Bioactive oligosaccharides come from oligomer engineering with either (*i*) synthesis (using enzymatic or chemical engineering) or (*ii*) polysaccharide depolymerization (using physical, chemical or enzymatic methods).

Chemical and biochemical synthesis of oligosaccharides

The chemical or biochemical synthesis of oligosaccharidic structures is much more difficult than it was observed in the synthesis processes of other biopolymers such as peptides and nucleic acids because of the higher number of possibilities in monomeric unit combinations. So, the stereospecific introduction of glycosidic linkages appears as the challenge of oligosaccharide synthesis. Nevertheless, recent advances in enzymatic and chemical synthesis allow envisaging the preparation of a wide range of oligomers.

Chemical glycosylation in the production of oligosaccharides

Different strategies have been published for the chemical synthesis of oligosaccharides (*87,88*). Generally, the preparation of oligomers by chemical processes takes place as illustrated in Fig. 2. The glycosylation reaction is achieved by an inter-glycosidic condensation between a completely protected glycosyl donor (R-OH) that has an excellent leaving group (halogenides) at its anomeric position and a glycosyl acceptor that possesses only one free hydroxyl group. Among these glycosyl donors, the anomeric fluorides, trichloroacetimidates and thioglycosides (Fig. 3) are currently being employed (*87*). These compounds can be prepared under mild conditions and are sufficiently stable to be purified and stored for a considerable period of time. The preparation of specific glycosyl donor and acceptor implies many protection and deprotection steps to combine high yields with regio- and stereoselectivity. With these strategies oligosaccharides of interest such as oligogalacturonides (OGAs) have been synthetised (*89,90*). These compounds used as glycosylation intermediates should permit the production of higher oligomers of D-galacturonates. Likewise, other oligomers as for example, xylooligosaccharides (DP 4 up to 10) have also been generated by a complex blockwise synthesis approach (*91*). These syntheses show that the formation of oligosaccharides is only possible when each step in the assembly of the glycosyl (donor and acceptor) is high yielding.

Therefore, in spite of recent developments, synthesis of oligosaccharides by chemical glycosylations seems actually nonrealistic for industrial processes. Conversely,
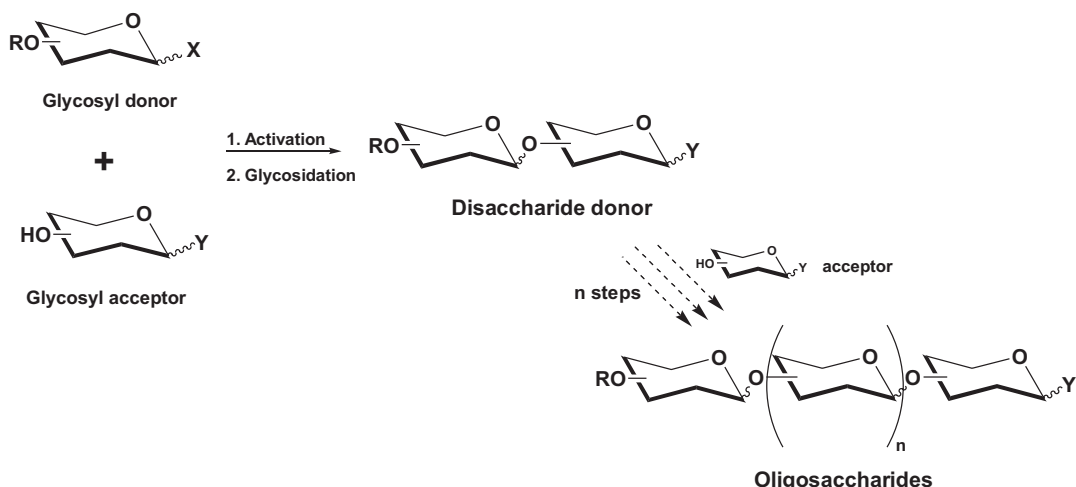
Glycosyl donor

+

Glycosyl acceptor

1. Activation
2. Glycosidation

Disaccharide donor

n steps

acceptor

Oligosaccharides

Fig. 2. Current strategy of the chemical synthesis of oligosaccharides (R: H or saccharides)

Glycosyl sulphoxyde

Glycosyl halide
(L: F, Cl, Br)

Trichloroacetamidate

Thioglycosides
(R: alkyl, aryl, pyridyl, cyanide)
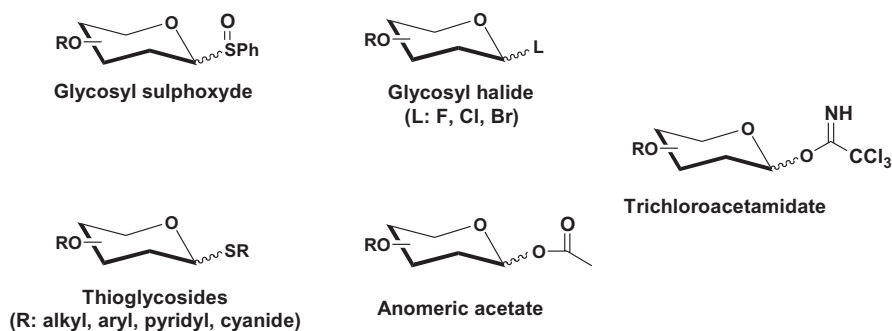
Anomeric acetate

Fig. 3. Currently used glycosyl donors in the chemical synthesis of oligosaccharides (R: H or saccharides)

other chemical alternative methods such as chemome-chanical synthesis of bifidogenic glucooligosaccharides have been successfully tested by extrusion process (*92*).

Enzymatic glycosylation in the production
of oligosaccharides

The large-scale productions of oligosaccharides thanks to enzymatic synthesis have largely been developed these last decades. Effectively, specific studies of biosynthesis pathways of oligosaccharides and the use of glycosida-

ses and glycosyltransferases permit to avoid the disadvantages of chemical methods, since the enzymes control both regio- and stereoselectivity of glycosylation.

Glycosyltransferases (E.C. 2.4.xy) catalyse the transfer of sugar moieties from activated donor molecules to specific acceptor molecules, forming glycosidic bonds (Fig. 4). These enzymes are highly regio- and stereoselective and have been obtained after purification or after cloning and overexpression.
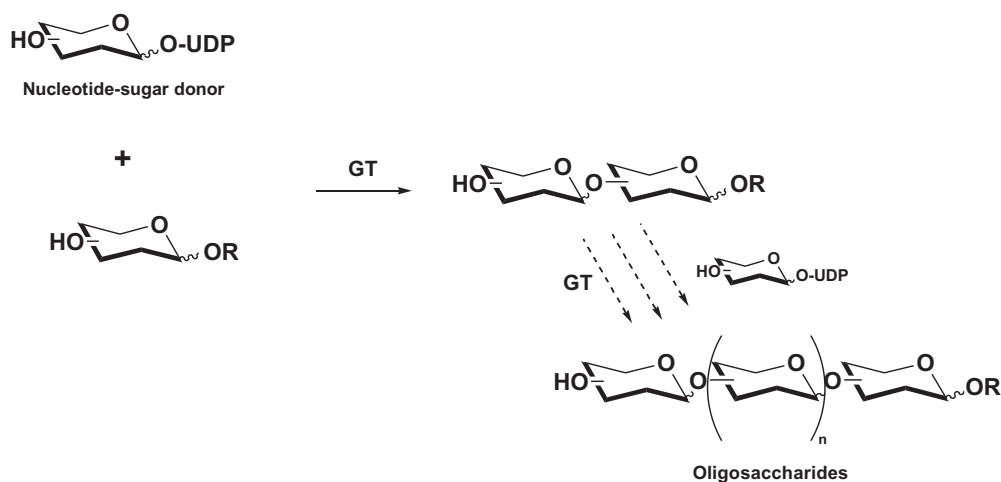
Nucleotide-sugar donor

+

GT

GT

Oligosaccharides

Fig. 4. Current strategy of the enzymatic synthesis of oligosaccharides using glycosyltransferase (GT) (R: H or saccharides)

Glycosylhydrolases (E.C. 3.2.1.-) are much more readily available than glycosyltransferases but are generally less stereoselective. These specific biocatalysts were traditionally used for the degradation of poly- and oligosaccharides. Nevertheless, the reverse hydrolytic activities under suitable reaction conditions and the utilizations of nucleotide-activated sugars permit the formation of glycosidic bond (Fig. 5a). Yields were generally lower with glycosidases except with mutated derivative named glycosynthase that operates more efficiently with appropriated activated glycosyl donors such as fluorides (Fig. 5b).

Many bioactive oligosaccharides are produced by the enzymatic engineering such as FOS from sucrose using fructosyltransferase (*9*), TOS or GOS from lactose using β-galactosidase (*93*) and gentiooligosaccharides from glucose by transglucosylation (*94*).

Finally, note the possibility to produce isomaltooligosaccharides, galactooligosaccharides and fructooligosaccharides using immobilized dextransucrase, galactosidase and fructosyltransferase, respectively (*95–97*).

Polysaccharide depolymerization

Except for chemical treatments with acid and radical hydrolysis (Fig. 6) or physical treatments using thermal, microwave, γ–irradiation and ultrasonication deg-
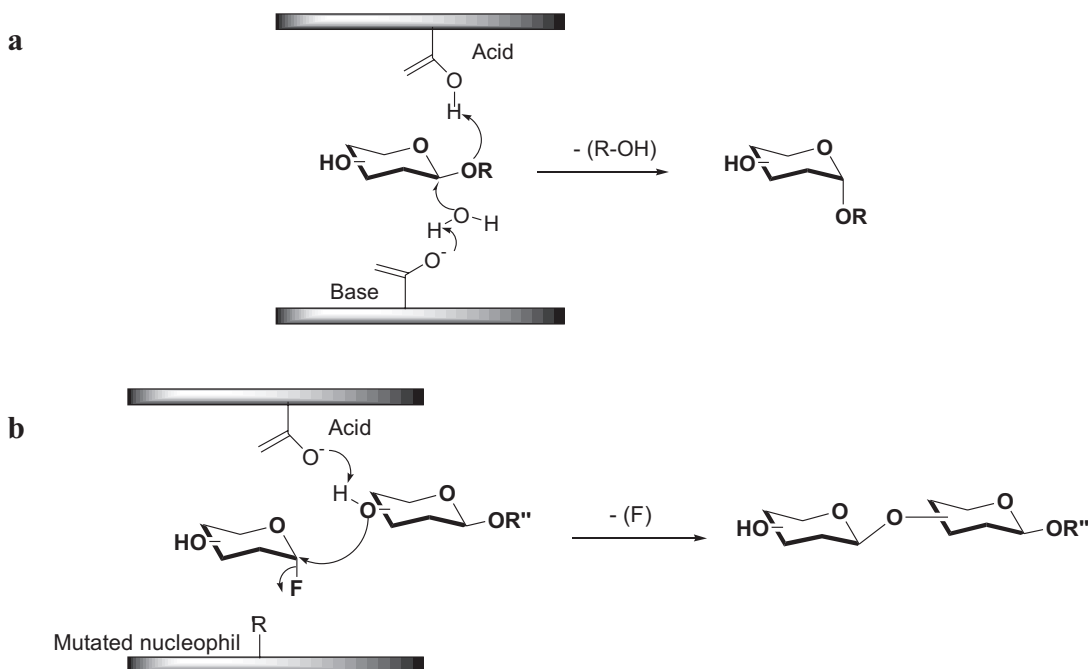

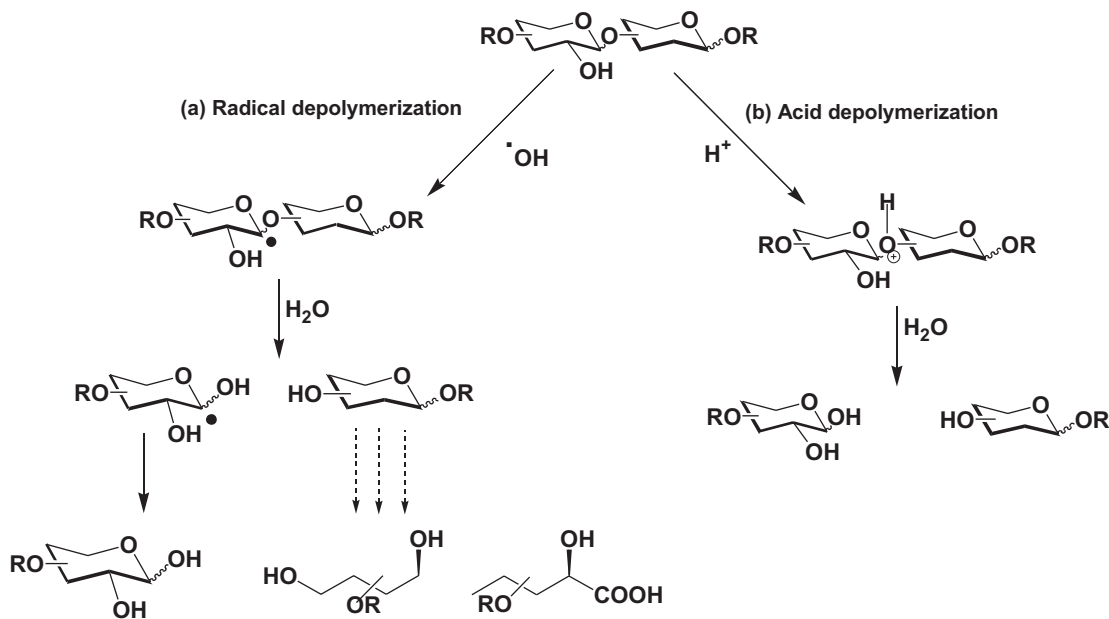**Fig. 5.** Reaction mechanism of (a) glycosidase and (b) glycosynthase (R, R'': saccharides)

Fig. 6. Reactional mechanism of (a) radical and (b) acid polysaccharide depolymerization (R: H or saccharide)

radations, the enzymatic depolymerization is the main approach to prepare large amounts of oligomers.

The largely found strategies are microbiotechnology procedures that degrade various polysaccharides using regio- and stereospecifically microbial enzymes such as polysaccharide hydrolases and polysaccharide lyases. The polysaccharide hydrolases (E.C. 3.2.1.-) catalyze the hydrolysis of the glycosidic bonds *via* a general acid catalysis requiring two critical residues: a proton donor and a nucleophile/base. This hydrolysis occurs *via* two mechanisms giving rise to either an overall retention or an inversion of anomeric configuration. Concerning polysaccharide lyases (E.C. 4.2.2.-), they constitute a specific group of enzymes which generate the cleavage of polysaccharide chains *via* a β-elimination resulting in the formation of a double bond at the newly formed non-reducing end (Fig. 7).
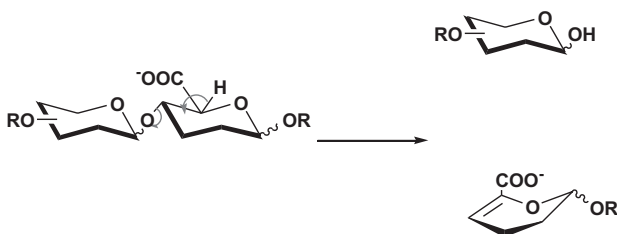

Fig. 7. Reactional mechanism of polysaccharide depolymerization by β-elimination (R: H or saccharide)

Actually, these selective biocatalysts are largely exploited and developed to prepare a majority of bioactive oligosaccharides. In this manner, several oligosaccharides with bifidogenic application were commercially prepared (*94*); for example, maltooligosaccharides from starch using α-amylases, isomaltooligosaccharides with the action of α,β-amylases and α-glucosidases, fructooligosaccharides from enzymatic hydrolysis of inulin, and xylooligosaccharides from xylan cleavage with β-(1,4) xylanases. In addition, pectinases, pectate lyases and other polygalacturonases (*98*) were used to generate oligogalacturonides from pectin cleavage.

Conclusion

The expansion of the knowledge of new oligosaccharidic structures, associated with the advances of glycobiotechnologies, could authorize a larger employment of these compounds as food ingredients in the future. The expansion of this market implies strategies for their low cost manufacturing. Even if the recent advances in polysaccharide cleavage enzymes and in the microorganisms are applied to generate some oligosaccharides in large scale, the improvement of glycosylation strategies (chemical or biochemical) seems to be much more promising and reliable in the manufacture of oligosaccharides and derivatives. Many di-, tri- and tetrasaccharides can be now synthesized routinely involving a minimum number of glycosylation steps and solid phase oligosaccharide synthesis is developed. It consequently opens the way to original oligomer preparations notably in regard to advances of automated systems. Nonetheless, as these

methods are limited, chemists and biochemists focus on the preparation of large amounts of more oligosaccharides using enzymatic polysaccharide depolymerization. In this way, the chitinous biooligomers offer a wide range of unique applications to prevent foods from microbial deterioration. As they are able to exhibit interesting antioxidative and antimicrobial activities, they may be of advantageous use in the field of natural food additives. Considering the prebiotic oligosaccharides, the understanding of structure-function relationships related to probiotic bacteria targets could move the research towards specific structures with a higher level of functionality. Additionally, the screening of new oligosaccharidic structures and notably those obtained after bacterial polysaccharide degradations could enhance the prebiotic oligosaccharide families. Effectively, bacterial polysaccharide as exopolysaccharides offer a large range of structures and are produced in controlled conditions (reactors). So their degradations by specific enzymes will offer, in the future, numerous oligosaccharides with potentialities as prebiotics. Moreover, the next generations of prebiotic oligosaccharides could be supplied by probiotics themselves. Effectively, a part of *Lactobacilli* are exopolysaccharide-producing strains and no data have been reported related to the prebiotic activity of oligomers that come from them even if the polymeric patterns as levans are known as having prebiotic effects.

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333

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