ISSN 1330-9862
(FTB-2540)

review

Production of Fructooligosaccharides from Inulin by Endoinulinases and Their Prebiotic Potential

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Received: March 24, 2010
Accepted: April 29, 2010

Summary

The production and applications of food-grade oligosaccharides are increasing rapidly. Amongst them, fructooligosaccharides represent one of their major classes in terms of production. They are relatively new functional food ingredients that have great potential as prebiotics, apart from having a number of desirable characteristics which are beneficial to the health of consumers. These are manufactured either by transfructosylation of sucrose using β-fructofuranosidases or hydrolysis of inulin by endoinulinases. Inulin, a polyfructan, occurs as a reserve carbohydrate in many plant families, representing more than 30 000 species. It is a potent substrate both for the production of inulinases and fructooligosaccharides. The review focuses on the recent developments in the production of fructooligosaccharides from inulin by endoinulinases, their prebiotic potential, functionalities and applications in food industry, and future perspectives.

Key words: endoinulinase, inulin, fructooligosaccharides, fructans, prebiotic, probiotic, bifidogenic effect, dietary fibre, food industry

Introduction

Inulinases have been characterized from inulin-storing tissue of plants and a wide variety of microorganisms (*1*). However, their quantity in plant material is not enough to be exploited for commercial use (*2*). Microorganisms are the best source of inulinases for commercial production because of their easy cultivation, rapid multiplication and high production yields (*3*). Microbial inulinases are an important class of industrial enzymes, which are usually inducible and extracellular. A number of fungal, yeast and bacterial strains have been reported for the production of inulinases (*3–6*). Amongst the filamentous fungi, *Aspergillus* sp. and *Penicillium* sp. are high inulinase producers, while among the yeasts this is *Kluyveromyces* sp. (*1*). From bacteria, *Bacillus* sp., *Pseudomonas* sp. and *Streptomyces* sp. have been reported as high-yielding inulinase strains (*1*). Inulinases have attracted researchers because of their usage both for the production of high fructose syrup (*1,3,7–9*) and fructooligosaccharides (*4,5*). Apart from these, inulinases have various other applications such as in the production of ethanol (*10,11*), acetone and butanol (*12*), pullulan (*13*), gluconic acid and sorbitol (*14*).

Inulinases can be produced by growing various microorganisms in an inulin-based medium (*6*). Inulin, a polyfructan, occurs as a reserve carbohydrate in many families of plants, representing more than 30 000 species (*15,16*). It consists of linear chains of β-2,1-linked fructosyl units terminating at the reducing end with a glucose residue attached through a sucrose-type linkage as illustrated in Fig. 1. Apart from its use for the production of inulinases, inulin has received a great interest as a renewable raw material both for the production of high fructose syrup (*1*) and fructooligosaccharides (*17*). Inulinases target the β-2,1 linkage of inulin and are both exo and endo acting. Exo-acting inulinases produce fructose as the main end product, whereas endo-acting ones produce mainly fructooligosaccharides and monosaccha-

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Special issue: Probiotics, Prebiotics and Synbiotics

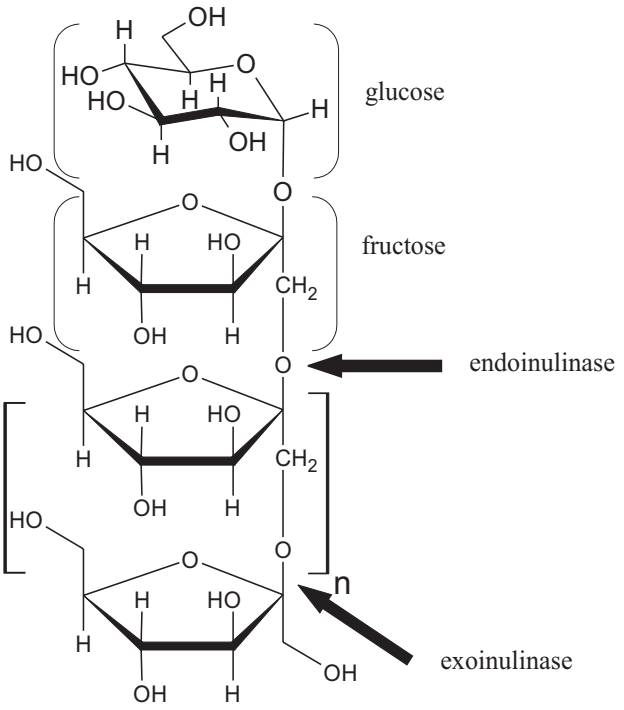

Fig. 1. Action pattern of inulinases on inulin

rides in a minor amount. Fructose has many superior technical and functional properties compared to sucrose, and also has a GRAS (Generally Recognized as Safe) status (*1,18*). Fructooligosaccharides constitute one of the most popular functional food components because of their bifidogenic and health-promoting properties (*17, 19–22*). Earlier, most of the commercially available fructooligosaccharides were either synthesized from sucrose (*19*) or extracted from edible parts of some plants (*22*). Nowadays, these are synthesized enzymatically either from sucrose by fructosyltransferases or from inulin by endoinulinases. This review focuses on the recent developments in the production of fructooligosaccharides from inulin by endoinulinases, their prebiotic potential, functionalities and applications in food industry, and future perspectives.

Action Pattern of Inulinases

It is clear from the literature (*1,3,4,6,18*) that inulinases are classified as exo and endo acting on the basis of cleavage of β-2,1 linkage in inulin (Fig. 1). Exoinulinases (EC 3.2.1.80) cleave β-2,1 linkages sequentially starting from the non-reducing end of inulin and split off terminal fructosyl units, releasing fructose with a molecule of glucose, whereas endoinulinases (EC 3.2.1.7) act randomly and hydrolyze internal linkages of inulin to yield fructooligosaccharides. Inulinases often show a certain activity towards sucrose (*1*). However, sucrose hydrolytic enzymes are called invertases (EC 3.2.1.26) and have specific characteristics. Because of the overlapping substrate specificity of invertases and inulinases, their distinction is difficult and controversial. Generally, inulinases are distinguished from invertases on the basis of I/S (inulinase activity/invertase activity) ratio (*1,18*). If the I/S ratio is $>10^{-2}$, the enzyme is referred to as an inulinases, and if I/S ratio is $<10^{-4}$, it is considered an invertase (*23*).

The property of having an exo or endo action depends upon the microbial origin of the enzyme. The mode of action of inulinases from different microbial sources has been described recently (*1,3*). Inulinases from fungi are generally exo acting (*4*), but some fungal strains like *Aspergillus ficuum* (*24*), *Chrysosporium pannorum* (*25*) and *Penicillium rugulosum* (*26*) produce both exo- and endoinulinases. Most of the strains of *Aspergillus niger* have been reported to produce only endoinulinases extracellularly (*27–29*), while surprisingly *A. niger* strain 12 has been reported to produce both exo- and endoinulinases intracellularly (*30*). Two forms of exo-acting (EI and EII) extracellular inulinases have been reported from *Penicillium* sp. (*31*). Interestingly, two intracellular (EI, EII) and one extracellular endoinulinase (Eexo) have been investigated from *Kluyveromyces* sp. Y-85 (*32*). In another interesting case, one invertase (Inv), five exoinulinases (Exo I, II, III, IV and V) and three endoinulinases (Endo I, II and III) were isolated from a commercial preparation derived from *Aspergillus ficuum* (*23*).

Inulin, A Potent Substrate

Inulin is a potent substrate for the production of inulinases (*3,4,6*), high fructose syrup (*1,15*) and fructooligosaccharides (*3,17*). Rose, a German scientist, in the early 1800s first isolated it as a carbohydrate substance from the roots of *Inula helenium* (*33*) and the substance was later named 'inulin' by Thomson (*34*). Inulin consists of linear chains of D-fructofuranose molecules linked by β--2,1-glycosidic bonds and terminated with a D-glucose moiety linked to fructose by α-1,2 bonds as in sucrose (Fig. 1). In nature, it is the second most abundant storage carbohydrate after starch (*16*). Most of the inulin--containing plants are dicotyledonous, belonging to the Asteraceae and Campanulaceae families, but a small amount is also found in some monocotyledonous plants from the Poaceae, Liliaceae and Amaryllidaceae families (*15*). Some inulin-containing plants commonly used in human nutrition are leek, onion, garlic, asparagus, Jerusalem artichoke, dahlia, chicory, yacon, *etc.* (*35*). Inulin content of some plants is given in Table 1 (*35–39*). The degree of polymerization (DP) is one of the important properties of inulin, because it influences the functionalities of the fructans (*16*). The DP of plant inulin is rather low (DP<200) and varies according to the plant species, weather conditions and physiological age of the plant (*16*).

Inulin and its hydrolyzed products are legally classified as food or food ingredients in all the countries where they are used (*21*). Inulin is also the most commonly used substrate for the production of inulinases (*6*). Apart from pure inulin, naturally occurring inulin--rich plant materials have been used for the production of inulinases (*3,4*). Inulin and its partially hydrolyzed products (fructooligosaccharides) may significantly improve organoleptic characteristics such as both taste and mouth-

Table 1. Inulin content of some plants

Botanical name	Common name	Plant part	Inulin/%*	Reference
Agave americana	agave	lobes	7–10	*36*
Allium ampeloprasum var. *porrum*	leek	bulb	3–10	*35*
Allium cepa	onion	bulb	2–6	*35*
Allium sativum	garlic	bulb	9–16	*35*
Arctium sp.	burdock	roots	3.5–4.0	*35*
Asparagus officinalis	shatwaar	root tubers	10–15	*37*
Asparagus racemosus	safed musli/shatwaar	root tubers	10–15	*37*
Camassia sp.	camas	bulb	12–22	*35*
Cichorium intybus	chicory	roots	15–20	*35,37*
Cynara cardunculus	artichoke	leaves/heart	3–10	*35*
Dahlia sp.	dahlia	root tubers	15–20	*37*
Helianthus tuberosus	Jerusalem artichoke	tubers	14–19	*35,37*
Hordeum vulgare	barley	grains	0.5–1.5	*35*
Microseris lanceolata	murnong	roots	8–13	*35*
Musa acuminate	banana	fruit	0.3–0.7	*35*
Secale cereale	rye	grains	0.5–1.0	*35*
Smallanthus sonchifolius	yacon	roots	3–19	*35*
Taraxacum officinale	dandelion	leaves	12–15	*35*
Tragopogon sp.	salsify	roots	15–20	*37*
Scorzonera hispanica	Spanish salsify	roots	8.15–10.75	*38*
Saussurea lappa	kuth	roots	18–20	*39*

*percentage of fresh mass

feel in a wide range of food applications (*21*). Apart from this, fructooligosaccharides produced from inulin by endoinulinases are used as potent prebiotics and dietary fibres and also have many more beneficial functionalities (*10,17*). Furthermore, their complete hydrolyzed product, fructose, produced by exoinulinase is emerging as a safe sweetener in food industry. Nowadays, inulin is a promising substrate for the enzymatic synthesis of fructooligosaccharides and high fructose syrup.

Microbial Production of Endoinulinases

Fungi, yeast and bacteria are all capable of producing inulinases and many of them have been successfully used for enzyme production (*1,3–6*). Inulinases are both extra- and intracellular, but some microorganisms have the ability to express this enzyme in both ways (*4,18*). Similarly, inulinases are both exo and endo acting but many microorganisms are known to produce mixed forms (*23–26,30,32,40*). The localization of an enzyme, its mode of action and yield depend upon the kind of microorganism and the substrate used during fermentation (*1*). Microorganisms expressing endoinulinases and their enzyme activities in submerged fermentation are listed in Table 2 (*5,24–30,32,41–60*). All the endoinulinase-expressing microorganisms are from bacteria and fungi except for two yeast strains of *Yarrowia lipolytica* (*60*) and *Kluyveromyces* sp. Y-85 (*32*). Furthermore, all the strains are reported to produce extracellular endoinulinases, except for *Aspergillus niger* strain 12 (*30*) and *Kluyveromyces* sp. Y-85 (*32*), which are known to produce endoinulinase intracellularly.

The raw material used for the production of inulinases includes a wide range of pure, mixed and natural substrates (*4*). Although inulin is the most commonly used substrate, a variety of other substrates (glucose, sucrose, lactose, maltose, fructan, fructosan, *etc*.) have also been used for different microorganisms (*4,61*). If the microorganism exhibits inulinase activity coupled with invertase activity, sucrose may serve as a better source for enzyme production, otherwise it may exhibit an inhibitory effect on inulinase production (*4,18,52,62*). Pure inulin from various sources and many naturally occurring inulin-rich substrates has been used for most of the endoinulinase-producing microorganisms (Table 3; *24,26–30, 41–51,55–60,63,64*). Jerusalem artichoke tubers, dahlia tubers and chicory roots are the most commonly used inulin-rich substrates for endoinulinase production. Complex N-sources such as yeast extract, beef extract, meat extract, corn steep liquor, peptone, urea, *etc*. and inorganic N-sources like $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, NH_4Cl, $NaNO_3$, KNO_3, *etc*. have been widely used in inulinase-producing media (*65*). The complex N-sources have been reported to be better than inorganic N-sources (*24,26,66–71*). Ammonium salts usually cause acidic conditions, because acid is liberated in the medium after the utilization of ammonium ions and highly acidic conditions may inhibit the growth and synthesis of inulinase (*18*). The positive influence of inorganic salts like Mn^{2+}, Ca^{2+} (*70–72*), Mg^{2+}, Fe^{2+} and K^+ (*73*) on inulinase synthesis has also been reported.

Table 2. Microbial sources producing extracellular endoinulinases in submerged fermentation

Source	Enzyme activity U/mL	Ref.
Bacteria		
Arthrobacter sp.	NS; 0.84 U/mg[c]	*41*
Bacillus smithii	135.2	*42*
Paenibacillus sp.	NS	*27*
Pseudomonas mucidolens	NS	*5*
Pseudomonas sp.	NS	*43,44*
	3	*45*
Streptomyces rochei	1	*46*
Xanthomonas campestris pv. *phaseoli* mutant KM 24	9.24	*47*
Xanthomonas oryzae	NS; 49 U/mg[c]	*48,49*
Xanthomonas sp.	11	*50*
	15	*45*
Fungi		
Aspergillus ficuum[a]	NS	*24*
	NS	*51*
	NS	*40*
Aspergillus fumigatus	NS	*5*
Aspergillus niger	55	*29*
	33.53	*28*
	NS	*27*
A. niger strain 12[a,b]	NS; 108 U/mg[c]	*30*
A. niger mutant strain	40	*52*
Chaetomium sp.	NS	*53*
Chrysosporium pannorum[a]	115	*25*
Penicillium purpurogenum var. *rubisclerotium*	3.74	*54*
Penicillium rugulosum[a]	NS; 22.6 U/mg[c]	*26*
	NS	*27*
Penicillium sp.	9.9	*55*
Rhizoctonia solani	NS; 0.25 U/mg[c]	*56*
Rhizopus sp.	NS; 1.4 U/mg[c]	*57*
Trichoderma harzianum	0.75	*58*
Trichoderma viride	94	*59*
Yeast		
Kluyveromyces sp. Y-85[a,b]	NS	*32*
Yarrowia lipolytica	62.85	*60*

[a]produces both exo- and endoinulinases

[b]intracellular enzyme localization

[c]specific activity (units/mg of protein) of enzyme in the crude extract

NS – not specified

Table 3. Inulin from different sources used for the production of endoinulinases

Microorganism	Inulin source	*w*/%	Ref.
Arthrobacter sp.	Jerusalem artichoke extract*	1.5	*41*
Aspergillus ficuum	chicory roots	2.0	*24*
	Jerusalem artichoke extract*	2.0	*51*
Aspergillus niger	dandelion tap root extract*	40	*29*
	NS	1.0	*28*
	dahlia tubers	1.0	*52*
	NS	0.5	*27*
	dahlia tubers	1.0	*30*
Bacillus smithii	NS	2.0	*42*
Chrysosporium pannorum	NS	1.0	*63*
Paenibacillus sp.	NS	0.5	*27*
Penicillium purpurogenum	NS	2.0	*64*
Penicillium rugulosum	dahlia tubers	3.0	*26*
	NS	0.5	*27*
Penicillium sp.	NS	1.0	*55*
Pseudomonas sp.	dahlia tubers	1.0	*43,44*
	chicory root powder*	2.0	*45*
Rhizoctonia solani	Jerusalem artichoke powder*	3.0	*56*
Rhizopus sp.	dahlia tubers	1.0	*57*
Streptomyces rochei	chicory roots	1.0	*46*
Trichoderma harzianum	Jerusalem artichoke powder*	3.0	*58*
Trichoderma viride	Jerusalem artichoke powder*	3.0	*59*
Xanthomonas campestris pv. *phaseoli*	chicory roots	2.0	*47*
Xanthomonas oryzae	chicory root extract*	2.0	*48,49*
Xanthomonas sp.	dahlia tubers	2.0	*50*
	chicory root powder	2.0	*45*
Yarrowia lipolytica	NS	4.0	*60*

*natural substrates were used while in others commercial inulin was used for the production of endoinulinase

NS – not specified

Inulinase production is also influenced by many other factors like pH of the medium, aeration and temperature of fermentation. Generally, inulinase from fungal strains shows pH optima between 4.5 and 7.0, from yeast strains between 4.4 and 6.5, and from bacterial strains between 4.8 and 7.0 (*65*). Fungal and bacterial inulinases show temperature optima in the mesophilic and thermophilic range, while yeasts have the optima in the mesophilic range (*65*). Agitation, aeration and shear stress are the key factors influencing inulinase yield in bioreactors. Agitation not only affects the O_2 availability, but it also exerts influence on the availability of other nutrients in the medium. The higher agitation in bioreactors causes shear stress on the producer organism as well on the enzyme structure (*70*). Shear stress in a bioreactor is related to rheological properties of broth and shear rate. Rheological properties are defined for broth viscosity during the fermentation process, whereas the shear rate is a function of impeller geometry and impeller rotational speed (*74,75*). The rate of oxygen transfer also plays an important role in the overall microbial metabolism and different microorganisms behave differently under different conditions of oxygen supply. The

supply of oxygen to microorganisms poses many problems specific to it like foam formation, *etc*. The two mechanisms, namely oxygen supply and shear stress, generally act in antagonism. The former contributes to biomass increase and the latter acts against better enzyme yield (*76*). Most of the research reports on inulinase production are on submerged fermentation, however, attempts have also been made to use solid-state fermentation (Table 4; *77–86*). Recently, a hybrid neural network approach has been described to model inulinase production in a batch bioreactor using agroindustrial residues as a substrate (*87*). The mathematical framework developed has been proved a useful tool for simulation of this process.

Table 4. Inulinase production in solid-state fermentation

Microorganism	Substrate	Enzyme activity U/gds*	Ref.
Cryptococcus aureus	wheat bran, rice husk	436	*77*
Kluyveromyces marxianus	sugarcane bagasse	391.9	*78*
	sugarcane bagasse, corn steep liquor, soybean bran	250	*79*
	sugarcane bagasse, corn steep liquor, soybean bran	199	*80*
	sugarcane bagasse, corn steep liquor, soybean bran	436.7	*81*
	sugarcane bagasse, corn steep liquor, soybean bran	463	*82*
	sugarcane bagasse, corn steep liquor, sugar cane molasses	445	*83*
	wheat bran, rice bran, coconut oil cake, corn flour	122.88	*84*
Kluyveromyces sp.	wheat bran	409.8	*85*
Staphylococcus sp.	wheat bran, rice bran, coconut oil cake, corn flour	107.64	*84*
Xanthomonas campestris pv. *phaseoli*	onion peel,	117	*86*
	garlic peel	101	

*units/gram of dry substrate

To make the process economically feasible, it is imperative to improve the genetic characteristics of the producer organism. An endoinulinase-encoding gene *INU2* from *Aspergillus ficuum* has been cloned and sequenced (*88*). Alignment of amino acid sequence revealed 73.9 % similarity between *A. ficuum* and *Penicillum purpurogenum* endoinulinases. The endoinulinase *INU2* gene has been successfully expressed in *Saccharomyces cerevisiae* (*89*). Furthermore, the enhanced production of *A. ficuum* endoinulinase in *Saccharomyces cerevisiae* was achieved by *SUC2* deletion mutation (*90*).

Characteristics of Endoinulinases Purified from Various Microbial Sources

Most of the reports on purification of extracellular endoinulinases from various microorganisms deal with the conventional methods of centrifugation, salt/organic precipitation or ultrafiltration followed by ion-exchange and gel filtration chromatography (Table 5; *23,30,32,40–42, 49,52,53,55,57,63,64,91,92*). In some cases fast protein liquid chromatography (*23*), hydrophobic interaction chromatography (*41,49,92*) and preparative electrophoresis (*40,91*) have also been used. However, endoinulinases localized intracellularly need cell disruption first and then followed by similar techniques. Endoinulinases purified from most of the fungal and bacterial strains are extracellular. Their intracellular nature has also been reported only from *Aspergillus niger* strain 12 (*30*) and *Kluyveromyces* sp. Y-85 (*32*).

Most of the reports on purification of endoinulinases are from fungi (Table 5). Inulinases purified from all the bacterial strains are either exo- or endoinulinase, while from some fungal strains both forms are purified (*24–26,30,40*). Two forms (P-1A and P-1B) of endoinulinases have been purified from *Aspergillus niger* mutant 817 (*52*). Their specific activities were 3.5-fold higher and apparent K_m for inulin was much lower than for the wild-type endoinulinase (III). From a commercial inulinase preparation from *Aspergillus ficuum*, five exoinulinases (Exo I, II, III, IV and V), three endoinulinases (Endo I, II and III) and one invertase (Inv) were purified by conventional techniques (*23*). All the exoinulinases showed the same molecular mass (74 kDa), while endoinulinases were of 64 kDa. Recently, three exoinulinases (Exo-I, Exo-II and Exo-III) and two endoinulinases (Endo-I and Endo-II) have been purified from the culture broth of *A. ficuum* JNSP5-06 (*40*). All the inulinases were stable below 50 °C with optimum activity at 45 °C. Their pH optima were 4.5 and 5.0 for exoinulinase and endoinulinase, respectively. Two intracellular (EI and EII) and one extracellular endoinulinase (Exo) were purified from *Kluyveromyces* Y-85 (*32*). All of them were glycoproteins.

The characteristics of some purified endoinulinases from different microorganisms are also summarized in Table 5. There is not much difference in pH and temperature optima, and molecular mass of endoinulinases purified from different strains. Fungal strains show pH and temperature optima of 4.5–7.0 and 45–55 °C, respectively, while for bacterial strains they are between 4.5 and 7.5, and 50 and 70 °C, respectively. Generally, bacterial strains show higher temperature optima. The molecular mass ranged from 31 to 75 kDa for all the purified endoinulinases except *Xanthomonas oryzae*, which has 139 kDa. Endoinulinases from *Aspergillus niger* (*52,92*) were strongly inactivated by *p*-chloromercuribenzoate (*p*-CMB). This indicates that the active site of inulinases contains the sulfhydryl (SH) groups (*93*). *p*-CMB reacts with thiol groups forming mercaptides (a reversible process) and serves as a specific reagent for SH groups (*94*). Several metal ions have a stimulatory or inhibitory effect on endoinulinase activity. The stimulatory effect of Mn^{2+} (*30,52,55,57*) and Ca^{2+} (*57,91*) on endoinulinases from different microorganisms is well established. Conversely, some endoinulinases are reported to be inactivated by

Table 5. Characteristics of some purified endoinulinases from microbial sources

Source	Purification technique	Purification fold	Specific activity U/mg	K_m	v_{max}	M_r kD	Optimum pH	Optimum Temp. °C	Stability pH	Stability Temp. °C	Ref.
Arthrobacter sp.	$(NH)_2SO_4$ PPT, IEC, HIC	63	53	1.7 mM	NS	75	7.5	50	5–10.5	30–40	*41*
Aspergillius ficuum	IEC-PREP PAGE	NS	348	8.1 mM	773 U/mg	66	5.0	50	4.8–5.2	<60	*91*
A. ficuum											
Endo I	$(NH)_2SO_4$ PPT, IEC, GFC, FPLC	12.1	61.6	NS	NS	64	NS	NS	NS	NS	23
Endo II		64.5	328	NS	NS	64	NS	NS	NS	NS	
Endo III		9.9	50.3	NS	NS	64	NS	NS	NS	NS	
A. ficuum											
Endo I	$(NH)_2SO_4$ PPT, dialysis, IEC, GFC, PREP PAGE	NS	NS	14.8 mg/mL	40.8 mg/(mL·min)	34	5.0	45	4–8	<50	*40*
Endo II		NS	NS	25.6 mg/mL	53.8 mg/(mL·min)	31	5.0	45	4–8	<50	
Aspergillus niger	$(NH)_2SO_4$ PPT, ethanol PPT, IEC, GFC	146	0.74	0.80 mM	NS	56	5.3	45	5–7	<60	*30*
A. niger	isopropanol PPT, IEC, HIC, GFC	22.54	1158.3	6.7 g/L	0.0476 mg/(mL·min)	69	5.0	55	4–7	<55	*92*
A. niger mutant 817	dialysis, UF, IEC										*52*
form P-1A		NS	352	0.48 mM	109 μmol/(min·mg)	70	5.3	50	5–7	<50	
form P-1B		NS	338	0.50 mM	139 μmol/(min·mg)	68	5.3	50–55	3.5–9	<50	
Bacillus smithii	$(NH)_2SO_4$ PPT, IEC, GFC	31.4	1105.4	4.17 mM	833.3 IU/mg	47	4.5	70	4–7	<70	*42*
Chaetomium sp.	$(NH)_2SO_4$ PPT, IEC, GFC, HIC	30.8	NS	0.199 mmol/L	115 μmol/(min·mg)	66	6.0	55	NS	NS	*53*
Chrysosporium pannorum	$(NH)_2SO_4$ PPT, IEC, GFC	NS	106.2	NS	NS	58	6.0–7.0	50	4.5–8.5	<45	*63*
Kluyveromyces sp.											
EI	$(NH)_2SO_4$ PPT, IEC, GFC	NS	NS	NS	NS	42	4.6	52	NS	NS	*32*
EII		NS	NS	NS	NS	65	4.5	52	NS	NS	
Eexo		NS	NS	NS	NS	57	4.6	55	NS	NS	
Penicillium purpurogenum	IEC, GFC	394	82.8	0.21 mM	NS	64	5.1	55	5–7.5	<55	*64*
Penicillium sp.	dialysis, UF, IEC	45	105	0.20 mM	106 μmol/(min·mg)	68	5.2	50	5–7	<40	*55*
Rhizopus sp.	UF, IEC, GFC	12	17	9.0 mM	NS	83	6.0	40	5–8	<30	*57*
Xanthomonas oryzae	$(NH)_2SO_4$ PPT, IEC, HIC	28.7	1407	16.7 g/L	12.1 g/(L·h)	139	7.5	50	6–9	<45	*49*

IEC – ion exchange chromatography, FPLC – fast protein liquid chromatography, GFC – gel filtration chromatography, HIC – hydrophobic interaction chromatography, PPT – precipitation, PREP PAGE – preparative polyacrylamide gel electrophoresis, UF – ultrafiltration, NS – not specified

both Mn^{2+} (*40,91,92*) and Ca^{2+} (*40*). Inhibitory effects of Ag^+, Hg^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+} and Al^{3+} (*23,30, 40,41,52,55,57,91,92*) on endoinulinases from various sources are also well known.

Enzymatic Production of Food-Grade Oligosaccharides

Oligosaccharides are attracting increasing interest as prebiotic and functional food ingredients. They are important primarily because of their functional properties rather than sweetness. They can be extracted from some biological materials or synthesized enzymatically from a variety of substrates (*95*). This makes it more important to develop efficient synthesis reactions for the production of oligosaccharides. At this level, the outstanding stereo- and regioselectivity of enzyme catalysis can be considered as a complementary tool to the chemical synthesis which necessitates the complex successive protection and deprotection steps (*96*). As a consequence, it has become more and more important to develop efficient synthesis routes that are easy to scale-up. The synthesis

of novel fructooligosaccharides can also be carried out by substrate and enzyme engineering (*97*). The enzymatic synthesis of various oligosaccharides has been extensively reviewed (*19,20,95,98*). Various enzymatic routes currently used for the commercial production of oligosaccharides are summarized in Table 6. Unlike other oligosaccharides, soybean oligosaccharides are extracted directly from the soybean whey and do not require enzymatic manufacturing process. Lactulose is unusual among prebiotics as it is the only one manufactured by chemical synthesis. A survey of commercial oligosaccharide manufacturers reveals that galacto-, fructo-, malto-, isomaltooligosaccharides and lectulose are the major classes of food-grade oligosaccharides (Fig. 2).

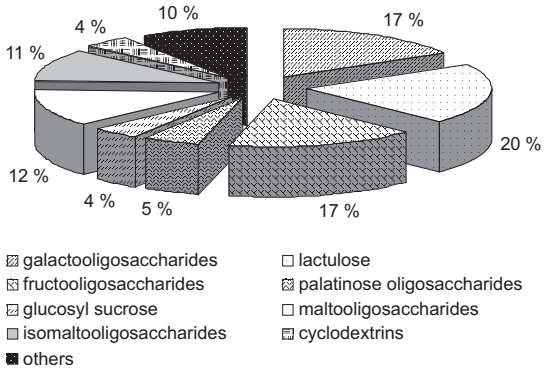

Fig. 2. Current status of the production of food-grade oligosaccharides. Data obtained by surveying the major manufacturers of food-grade oligosaccharides

Table 6. Enzymatic synthesis of food-grade oligosaccharides

Class of oligosaccharides	Production process
Galactooligosaccharides	transgalactosylation of lactose by β-galactosidase
Fructooligosaccharides	transfructosylation of sucrose by β-fructofuranosidase or hydrolysis of inulin by endoinulinase
Isomaltooligosaccharides	debranching of starch by isoamylase or pullulanase, and then hydrolysis by specific oligosaccharide forming α-amylase
Maltooligosaccharides	hydrolysis of starch into maltose by α-amylase and β-amylase, and transglucosylation of maltose by α-glucosidase
Glucooligosaccharides	transglycosylation of sucrose by dextransucrase/alternansucrose
Palatinose (isomaltulose) oligosaccharides	synthesis of platinose by palatinose synthetase, followed by intermolecular dehydration of palatinose
Xylooligosaccharides	hydrolysis of xylan by endoacting 1,4-β-xylanase
Gentiooligosaccharides	enzymatic transglucosylation of glucose syrup
Soybean oligosaccharides*	extracted directly from soybean whey by removal of proteins and salts
Cyclodextrins	debranching of starch by pullulanase and α-amylase, and then transglucosylation by cyclomaltodextrin glucanotransferase
Lactosucrose	transfructosylation of mixture of lactose and sucrose by β-fructofuranosidase
Lactulose**	alkali-isomerization of lactose

*manufactured by physicochemical process
**manufactured by chemical synthesis

Production of fructooligosaccharides (FOSs)

FOSs represent one of the major classes of bifidogenic oligosaccharides (oligosaccharides selectively stimulate the growth and/or activity of bifidobacteria and lactobacilli in the colon). The developments in industrial enzymology enabled the large scale production of FOSs by enzymatic synthesis. On industrial scale, they are manufactured enzymatically by two different processes which produce slightly different end products (Fig. 3). The companies that commercially manufacture FOSs from sucrose or inulin and their trade names are listed in Table 7.

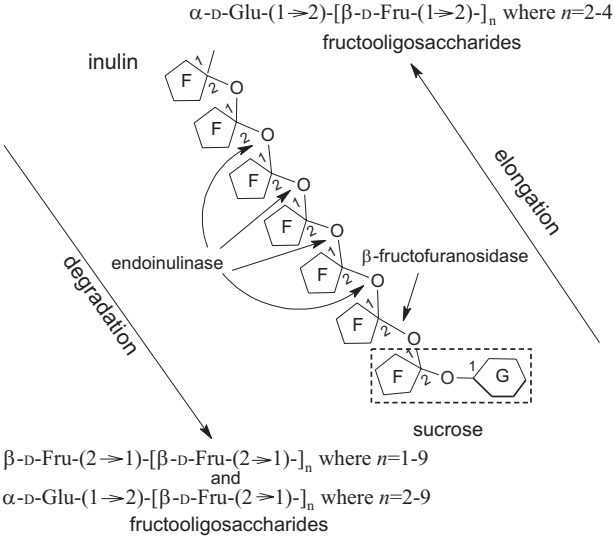

Fig. 3. Schematic presentation of the production of fructooligosaccharides from inulin by endoinulinase and sucrose by β-fructofuranosidase

Table 7. Commercially produced food-grade fructooligosaccharides*

Substrate	Manufacturer	Trade name
Sucrose	Beghin-Meiji Industries, France	Actilight
	Cheil Foods and Chemicals Inc., Korea	Oligo-Sugar
	GTC Nutrition, USA	NutraFlora
	Meiji Seika Kaisha Ltd., Japan	Meioligo
	Victory Biology Engineering Co., Ltd., China	Prebiovis scFOS
Inulin	Orafti Active Food Ingredients, USA	Raftilose
	Beneo-Orafti, Belgium	Orafti
	Cosucra Groupe Warcoing, Belgium	Fibrulose
	Jarrow Formulas, USA	Inulin FOS

*data obtained by surveying major manufacturers of food-grade fructooligosaccharides

Transfructosylation of sucrose by β-fructofuranosidases

FOSs can be manufactured from sucrose by glycosyl transfer reactions (*17,19,20*). Fructosyl tranferases (FTases) are the enzymes for the microbial production of FOSs. In this case, sucrose plays the dual role of fructose donor and fructose acceptor (*95*). High concentration of starting material is required for efficient transfructosylation (*99–101*). FOSs produced by this process contain 2–4 fructosyl units linked by β-1,2-glycosidic bonds and terminated with α-D-glucose residue. The composition of products from sucrose by transfructosylation is given in Fig. 4. The first reaction of β-fructofuranosidase on two sucrose molecules leads to 1-ketose and glucose. The

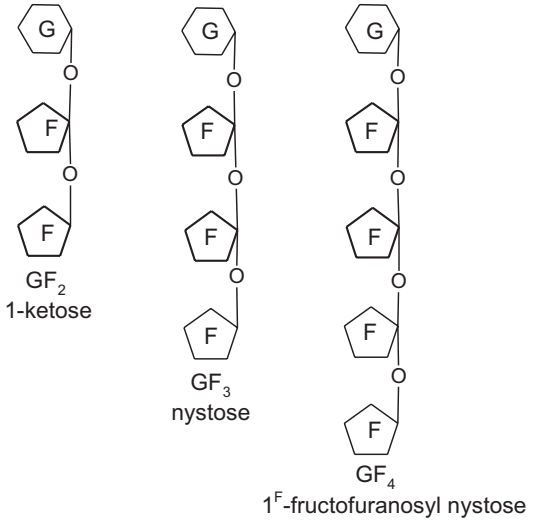

Fig. 4. Chemical structure of fructooligosaccharides produced from sucrose by β-fructofuranosidase

action of β-fructofuranose on 1-ketose produces nystose, and on nystose, it produces 1^F-fructofuranosyl nystose. FOSs produced from sucrose have a lower DP range (2–4) than inulin-derived FOSs and are frequently described as short-chain FOSs (*22*). After the completion of the reaction, FOSs can be purified by chromatographic or membrane processes to remove glucose and a small amount of fructose formed as a by-product as well as unreacted sucrose (*95*). Therefore, high content of FOSs can be produced by these techniques. The enzymatic synthesis route to FOSs using FTase from *Aspergillus niger* was first developed by Meiji Seika Kaisha Ltd., Japan to launch the commercial product Meioligo®. Then, this company also established a joint venture with Beghin-Meiji Industries, France to produce FOSs marketed as Actilight®, and also with GTC Nutrition, USA to make FOSs under the trade name NutraFlora®.

Bacterial strains producing FTase are rare and only a few reports on *Bacillus macerans* (*102*) and *Lactobacillus reuteri* (*103*) are available in literature. On the contrary, several fungal strains, especially from *Aspergillus* sp. are known to produce extracellular or intracellular FTases. *Aspergillus niger* (*104*), *A. japonicus* (*105*), *A. oryzae* (*106*), *A. phoenicis* (*17*), *Penicillium citrinum* (*107*), *P. frequentens*, *P. rugulosum*, *Fusarium oxysporum* (*17*) and *Aureobasidium pullulans* (*108*) have been reported to produce FOSs from sucrose. The use of mixed enzyme system of fructosyltransferase and glucose oxidase for the production of high content of FOSs has been investigated and highly concentrated (90–98 %) FOSs were obtained (*109,110*). The whole cells of *Aspergillus* sp. having both hydrolytic and transfructosylating activity were used for the production of FOSs and high yield was obtained (*111*). A forced flow membrane reactor system by immobilizing β-fructofuranosidase from *A. niger* on ceramic membranes of varied pore sizes has been developed for transfructosylation of sucrose (*112*). The developed system showed a long operational stability and half life of the immobilized enzyme was estimated to be 35 days. A complex biocatalyst system with a bioreactor equipped with a microfiltration (MF) module to produce high-content FOSs in a countinous system initiated by a batch process has also been reported (*113*). FTases have a great potential to manufacture prebiotics and also offer some process enhancements using thermophilic enzymes (*114*). Recombinant FTases also have interesting biocatalytic properties (*115*).

Hydrolysis of inulin by endoinulinases

The second approach used for the production of FOSs is the controlled hydrolysis of inulin by endoinulinases. Endoinulinases act randomly and cleave β-2,1 linkages of inulin to yield FOSs containing a mixture of β-D-Fru (2→1)-[β-D-Fru-(2→1)-]$_n$, where n=1–9 and α-D-Glu-(1→2)-[β-D-Fru-(2→1)-]$_n$, where n=2–9. FOSs produced from inulin closely resemble the mixture of FOSs obtained by transfructosylation of sucrose by FTases (*17*). However, not all the β-2,1-linked fructosyl chains end with a terminal glucose moiety. Furthermore, the FOS mixture produced from inulin hydrolysis contains longer (DP2-9) chains compared to that (DP2-4) obtained from sucrose by transfructosylation process (*95*).

Endoinulinases purified from different microorganisms have been used for the production of FOSs (*43,44, 46–48,50,51*). The hydrolytic conditions and the yield of FOSs from inulin by various endoinulinases are listed in Table 8 (*43,44,46–48,50,51*). Most of the microorganisms used for the production of FOSs are bacteria except for one fungus, *Aspergillus ficuum* (*51*). Both pure inulin and naturally occurring inulin-rich substrates have been used for the production of FOSs. Temperature range of 37–55 °C and pH optima of 6.0–7.0 have been used for the hydrolysis of inulin. In all the cases, FOS yield ranged from 60 to 86 % under optimal hydrolysis conditions.

Batch production of FOSs from inulin has been compared using soluble and immobilized endoinulinases from *Pseudomonas* sp. (*44*). Inulin was completely hydrolyzed to FOSs with DP ranging from 2 to 7. The maximum yield of fructooligosaccharides using soluble and immobilized endoinulinase was 72 and 83 %, respectively, under optimal conditions. The composition of the product was considerably affected by inulin concentration and enzyme form. Continuous production of FOSs from chicory juice was also carried out using the polystyrene-immobilized endoinulinase from *Pseudomonas* sp. (*116*). The enzyme reactor was successfully run for 28 days at 55 °C achieving a yield of 82 % without any significant loss of enzyme activity. Furthermore, no marked difference has been reported in operational stability between

Table 8. Hydrolysis conditions and yield of fructooligosaccharides (FOSs) from inulin by various endoinulinases

Enzyme source	Enzyme dosage U/g[a]	*w*(inulin) %	Inulin source	pH	Temp. °C	Time h	FOS yield %	Ref.
Aspergillus ficuum	10	5[b]	Jerusalem artichoke tubers	6.0	45	72	70.37	*51*
Pseudomonas sp.	15	5	dahlia tubers	NS	55	48	75.60	*43,44*
Streptomyces sp.	20	5	NS	6.2	40	24	71.00	*46*
Xanthomonas campestris	NS	3[b]	chicory roots	NS	37	24	60.00	*47*
Xanthomonas oryzae	840	5[b]	chicory roots	7.0	45	4	74.90	*48*
Xanthomonas sp.	NS	5	dahlia tubers	6.0	45	60	86.00	*50*

[a]units/gram of substrate
[b]inulin-rich natural substrates were used
NS – not specified

the two reactors fed with pure inulin solution and chicory juice as a substrate. There are a few reports on the use of recombinant endoinulinases for the production of FOSs from inulin (*117,118*). Endoinulinase gene (*inu1*) of *Pseudomonas* sp. was successfully cloned in *E. coli* and used for the production of FOSs in a batch and continuous system (*117*). In batch system, 79 % yield of FOSs was obtained under optimal conditions of 55 °C, pH=7.5, substrate concentration 10 % and enzyme dosage of 20 U/g of substrate. Continuous production of FOSs was also carried out at 50 °C using a bioreactor packed with recombinant cells immobilized on alginate and the system was successfully operated for 15 days without significant loss of initial activity. The whole cells of recombinant *E. coli* containing *inu1* gene from *Pseudomonas* were also used as a biocatalyst at 50 °C for FOS production from inulin in a batch and continuous system and a high yield of 78 % was obtained (*118*). Continuous production of FOSs under optimal conditions was achieved with a productivity of 150 g/(L·h) for 17 days with significant loss of enzyme activity of immobilized biocatalyst.

Inulin and inulin-derived products are marketed under different trade names. The companies manufacturing FOSs from inulin and their trade names are listed in Table 7. Orafti Active Food Ingredients, USA makes the inulin and inulin-derived products from chicory roots under the trade names Raftiline® and Raftilose®. The inulin extracted from chicory contains some FOSs in addition to polysaccharides. However, Raftiline® HP contains mainly inulin (995 g/kg) and is devoid of fructans with lower DP. Its DP ranges between 10 and 60 (average DP of 25). Raftilose® is an inulin-derived product produced by partial enzymatic hydrolysis of chicory inulin. It contains mainly FOSs (950 g/kg) and is a mixture of β(2→1) fructans with a DP ranging from 3 to 7 (average DP of 4). Similar inulin-hydrolyzed products on the market are Fibrulose® and Inulin FOS®.

Functionalities and Applications of FOSs

FOSs have received considerable attention due to their functional properties. Their nutritional and health benefits have been well proven (*17,19,20,95,119–123*). Their best known nutritional effect is the stimulation of growth of bifidobacteria in the intestine. Because of the large number of health promoting functions, FOSs have a wide range of food applications.

FOSs as potent prebiotics

Prebiotic FOSs are gaining increasing recognition as agents to modulate the colonic microbiota in humans and animals. These so-called prebiotics were first defined in 1995 as 'nondigestible food ingredient(s) that beneficially affect host's health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon' (*124*). Consequently, the attempts have been made to redefine prebiotics on the basis of new outcomes on prebiotic interventions. At a meeting of International Scientific Association of Probiotics and Prebiotics (ISAPP) in London in November, 2008 a prebiotic was defined as 'a fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host's health' (*95*). This new definition reaffirms that prebiotics act by inducing specific changes but allows changes in wording such that the term could be used for extra-intestinal applications.

Edible parts of many plants containing inulin and oligofructose are used in daily diet of many of the world's populations (*35*). Both inulin and oligofructose have been termed as 'prebiotics' (*125*), because they are nondigestible food ingredients that selectively stimulate growth and/or activity of a number of potentially-stimulating bacteria. Due to the β-configuration of the anomeric C_2 in their fructose monomers, these fructans are resistant to hydrolysis by human digestive enzymes (α-glucosidase, maltase, isomaltase, sucrase, *etc.*), which is why they are classified as nondigestible fructans (*123*). The most convincing studies were done with the use of an ileostomy model which provides a valuable alternative to the study of digestive physiology in man (*126*). In the ileostomy effluent 86–88 % of inulin and oligofructose were recovered, which supports the idea that these fructans are indigestible in the small intestine of man. The small loss during the passage through the small intestine may be due to fermentation by the microbiota colonizing the ileum. Another explanation was acid and/or enzymatic hydrolysis of the low-molecular-mass fructans which are more sensitive to stomach and/or small intestinal hydrolysis than the high-molecular-mass components.

The bifidogenic effect of inulin and oligofructose has been well established (*95,119,123,127–129*). Although inulin and FOSs are present in significant amount in several edible fruits and vegetables, many companies are manufacturing them from inulin by enzymatic synthesis. Their enzymatic synthesis from inulin produces a mixture of fructans containing F_n and GF_n types of oligofructose. It has been reported that F_n type of fructans had a similar prebiotic effect in humans as GF_n type of fructans (*130*). FOSs from inulin are long-chain fructans (DP<9) as compared to the fructans (DP<4) from sucrose (*95*). The bifidogenic effect of inulin and its long-chain FOSs in humans are well established (*123*). It has been established that short-chain FOSs are fermented in the proximal colon, thereby leaving the long-chain prebiotics for more distal colonic activity (*128*). A number of *in vitro* and *in vivo* studies have confirmed that inulin long-chain FOSs are fermented into lactic and short-chain carboxylic acids (*123,127*). Furthermore, *in vivo* studies in humans have demonstrated that this fermentation leads to the selective stimulation of growth of bifidobacteria, making long-chain fructans as prototype prebiotic (*125,131–133*). Functional activities of some commercial prebiotics like NutraFlora® P-95 (DP=2–4), Raftilose® P95 (DP=2–7), Inulin®-S (DP=2–60), Raftiline® HP (DP>23) and GOS® (DP=2–4) were assessed in *in vitro* studies (*134*). From the biomass data, a prebiotic activity score was calculated for different strains of lactobacilli and bifidobacteria. It has been concluded that the scores were dependent on both the probiotic bacterial strains and the type of prebiotic utilized. The highest score was obtained for *Lactobacillus paracasei* on inulin. The influence of oligofructose and long-chain inulin on the gut microbial ecology was investigated *in vivo* in rats (*135*). It has been established that fructan-containing diets increase the number of a bacterial population (*Clostridium coccoides–Eubacterium rectale* cluster) other than bifidobacteria or lactobacilli. The bifidogenic effect of inulin and oligofructose and its consequences for gut health have been reviewed recently (*123*). In a number of studies, both *in vitro* and *in vivo*, it has been well demonstrated that inulin and FOS selectively stimulate the growth of bifidobacteria or lactobacilli, both of which are considered to be beneficial to the host (*125,128,130,136–142*). These bacteria are also known for creating conditions unfavourable for the growth of potentially pathogenic organisms such as enterobacteria and certain clostridia (*143,144*).

Inulin and FOSs are often used in combination with 'probiotics', which are added to the host's diet to promote health. The combinations of pre- and probiotics have synergistic effect referred to as 'synbiotics'. Fructans promote the growth of existing microflora in the colon and also act to improve the survival, implantation and growth of newly added probiotic strains (*119*). The synbiotic health concept using FOSs is being used in many dairy products in European countries (*119*).

Other functionalities of FOSs

Apart from their bifidogenic effect, FOSs also have many other health-promoting functions (*15,17,19–21,95*). One of the important nutritional attributes of inulin and FOSs is their action as dietary fibre. FOSs resist digestion and absorption in the stomach and small intestine of humans (*145*). They influence intestinal function by increasing stool frequency, particularly in constipated patients (*146*) and/or lead to softer stools (*147,148*). Inulin and FOSs are known to increase daily stool output (*125*). The increase in stool output could be ascribed to a significant increase in faecal bacterial mass and to a significant increase in water content (*149*).

It is well established that FOSs, besides their effect on the gastrointestinal tract, are also able to exert systemic effect by modifying the hepatic metabolism of lipids in many animal models (*150*). Colonic fermentation of FOSs results in the synthesis of short-chain fatty acids, which influence the lipid metabolism in humans (*151*). It has been reported in an animal study on obese Zucker fa/fa rats that dietary enrichment with FOSs can reduce both the fat mass development and the occurrence of hepatic steatosis (*152*). In a comparative study on the effects of FOSs on lipid metabolism in man and in animal models, it has been shown that FOSs have triacylglycerol- (TAG) and cholesterol-lowering effects in rodents (*153*). They may also have a protective effect on the accumulation of TAG in the liver and the development of steatosis in animals. The results in man were more conflicting. Out of nine studies reviewed on the response of blood lipids to inulin and FOSs (*151*), three have shown no effects on blood levels of cholesterol or TAG, three have shown significant reductions in TAG, while four have shown modest reductions in total and LDL cholesterol. Animal studies provide strong evidence that FOSs inhibit secretion of TAG-rich very low density lipoprotein (VLDL) particles *via* inhibition of *de novo* fatty acid synthesis (*152*). Short-chain fructans have been shown to lower serum total and LDL-cholesterol in non-insulin dependent diabetic patients, but not in healthy subjects (*154*).

There has been a lot of interest in the potential of prebiotics to increase mineral absorption from the gut. Fermentation of FOSs to short-chain fatty acids reduces pH in the colon and this facilitates the absorption of mineral ions from the intestine, mainly calcium and magnesium (*95*). Most of the animal studies show that fructan consumption increases calcium absorption (*155–158*). Although the mechanism of the effect of fructans on calcium is not known with any certainty, several hypotheses have been put forward (*159*). In an animal study, cellulose/FOS feeding enhanced the apparent absorption and apparent retention of Ca, Mg, Zn and Fe in rats (*160*).

FOSs are known to prevent colonization of human gut by pathogenic microorganisms. Bifidobacteria and lactobacilli are known to produce a range of antimicrobial agents from short-chain fatty acids to peptides (*161–163*). That is why there has been much interest in the potential of using prebiotics to reduce the risk of acute infections. FOSs have been found effective in inhibiting colonization by *Clostridium difficile* (*164*), *Listeria monocytogenes* and *Salmonella typhimurium* (*165*). Fructans have also been evaluated for their ability to act synergistically with probiotics to inhibit pathogens (*166,167*).

The role of FOSs in the control of diabetes is also the subject of study by many workers (*17*). It has been reported that the daily consumption of 20 g of FOSs decreases basal hepatic glucose production in healthy sub-

jects without any effect on insulin-stimulated glucose metabolism (*168*). The effect of chronic ingestion of FOS on plasma lipid, glucose concentration, hepatic glucose production and inulin resistance was evaluated in type 2 diabetic subjects, and it was found that FOSs did not modify fasting plasma glucose and insulin concentration or basal hepatic glucose production. The inulin type fructans may be helpful for non-insulin-dependent diabetes patients (*169*). A significant reduction of cholesterolemia in diabetic subjects receiving a diet supplemented with fructans has been reported (*170*).

The studies with inulin and FOSs have shown reduction of chemically induced aberrant crypts and prevention of colon cancer. The effect of inulin-type fructans on the reduction of colon cancer in experimental animals and humans has been extensively reviewed (*171*).

Applications of FOSs in food industry

FOSs have many interesting nutritional and functional properties which enhance shelf-life and taste profile of various food products (*172*). The use of inulin or inulin-derived fructans is not possible in most of the soft drinks and fruit jams. In such acidic foods with long shelf-life, both are strongly hydrolyzed into fructose. That is why FOSs are used as a sugar substitute mainly in dairy and bakery products (*21*). Furthermore, being low-calorie sweetener, they are often used in combination with high intensity sweeteners to replace sugars and provide a well-balanced sweetener profile and mask the bitter aftertaste of aspartame or acesulfame K (*173*). However, FOSs can be used as the sole sweetening agent in light jam products, which gives 34 % calorie reduction compared to sucrose (*17*). The organoleptic characteristics of such products are acceptable. FOSs with inulin can be used in ice creams to replace all the sugar and reduce the fat content, and it also gives excellent mouthfeel characteristics (*17*). Since the freezing point depression of such products is lower with fructans than with sugar, their texture can be harder. Confectionery items like hard candies, gums, and marshmallows can be made while achieving significantly reduced energy values (*174*). FOSs were first introduced into the market as foodstuffs by Meiji Seika Co., Japan in 1984. Since then they have been widely used in confectionery and dairy industry. FOSs are also included in probiotic yoghurt and dairy drinks to produce 'synbiotic' products. Some of such commercial products are Aktifit (Emmi, Switzerland), Probioplus (Migros, Switzerland), Symbalance (Tonilait, Switzerland), Proghurt (Ja! Natürlich Naturprodukte, Austria), Fysiq (Mona, the Netherlands), Vifit (Sudmilch/Stassano, Belgium, Germany, UK), Fyos (Nutricia, Belgium), *etc*. (*119*). The current applications of FOSs include desserts such as jellies and ice creams; bakery products including biscuits, breads and pastries; spreads such as jams and marmalades; and infant milk formulations (*20*). The use of oligosaccharides in infant food formulations has been extensively reviewed (*175*). Inulin and FOSs are widely used as functional foods throughout the world for their health-promoting and technical properties. They are ingredients of the future that meet the needs of the food industry today, and are on the leading edge of the emerging trend towards functional foods.

Future Perspectives

Today's consumers hold high standards for the foods they consume. They demand foods that taste great, are fat- and/or calorie-reduced, and are also interested in such foods that provide added health benefits. Of course, it is also expected that these foods should be convenient and affordable. Inulin and FOSs are the novel dietary fibres which fulfill these considerations. The market for oligosaccharides is already substantial and continues to expand rapidly. The development of functional foods is a unique opportunity to contribute to the improvement of the quality of consumer's health and well-being. FOSs, due to their recognized bifidogenic action, their safety and beneficial effects on health, represent one of the most widely produced oligosaccharide groups and those most used in food industry (*17,19,20,95*).

The substantial market of FOSs as food ingredients supports a wide scope of isolation of novel FTase- and endoinulinase-producing strains. More emphasis should also be given to elaborative characterization of FOSs using sophisticated analytical techniques (*17*). Novel production techniques for FOSs using native/recombinant enzymes, highly efficient purification systems and/or new substrates should be explored.

Most of the feeding trials on FOSs are short-term, so consequences of their long-term consumption should be investigated (*95*). Currently, a sound understanding of the structure-function effects of the FOS prebiotics or the mechanisms behind their specific metabolism are not known (*95*). With this knowledge and enzyme engineering, tailor-made prebiotics can be produced in future. Novel FOSs can also be produced by substrate and enzyme engineering (*97*). Which microbiological outcomes a prebiotic should target, may be one of the aspects of future research on prebiotics. FOSs are the ingredients of the future that meet the needs of the food industry today and are on the leading edge of the emerging trend towards functional foods.

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