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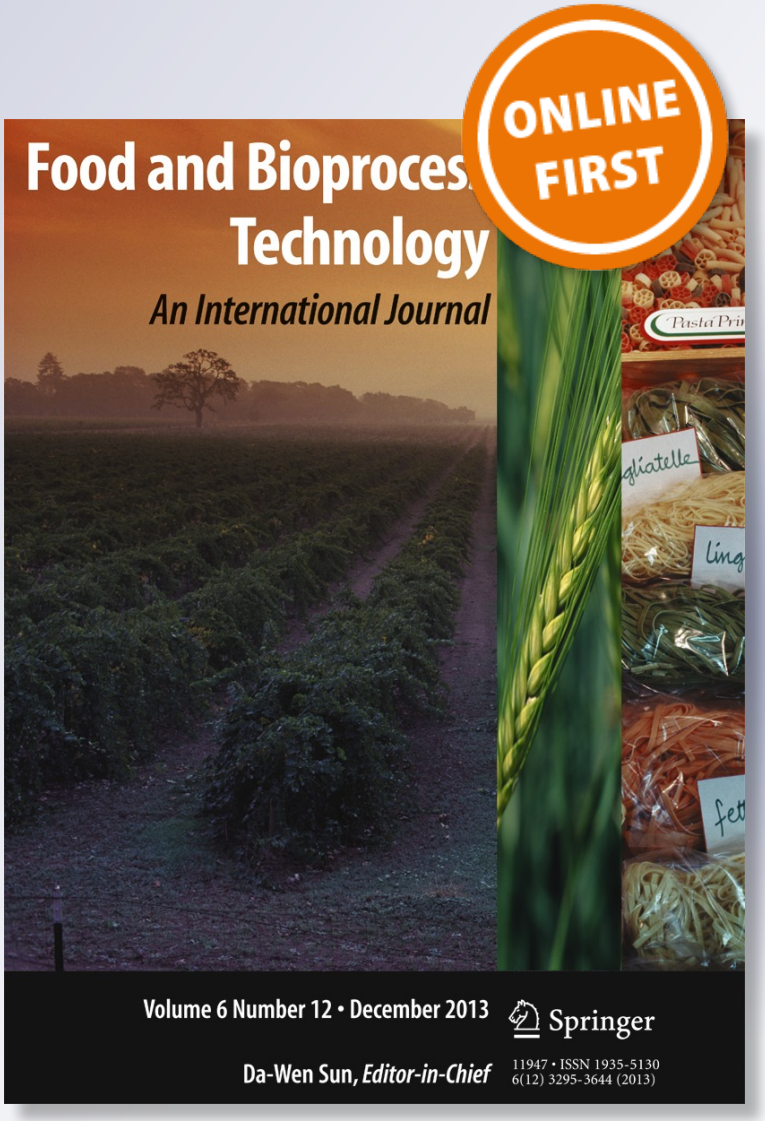

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REVIEW

An Overview of the Recent Developments on Fructooligosaccharide Production and Applications

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Abstract Over the past years, many researchers have suggested that deficiencies in the diet can lead to disease states and that some diseases can be avoided through an adequate intake of relevant dietary components. Recently, a great interest in dietary modulation of the human gut has been registered. Prebiotics, such as fructooligosaccharides (FOS), play a key role in the improvement of gut microbiota balance and in individual health. FOS are generally used as components of functional foods, are generally regarded as safe (generally recognized as safe status—from the Food and Drug Administration, USA), and worth about 150€ per kilogram. Due to their nutrition- and health-relevant properties, such as moderate sweetness, low carcinogenicity, low calorimetric value, and low glycemic index, FOS have been increasingly used by the food industry. Conventionally, FOS are produced through a two-stage process that requires an enzyme production and purification step in order to proceed with the chemical reaction itself. Several studies have been conducted on the production of FOS, aiming its optimization toward the development of more efficient production processes and their potential as food ingredients. The improvement of FOS yield and productivity can be achieved by the use of different fermentative methods and different microbial sources of FOS-producing enzymes and the optimization of nutritional and culture parameter; therefore, this review focuses on the latest progresses in FOS research such as its production, functional properties, and market data.

Keywords Fructooligosaccharides · Prebiotics · Transfructosylation · Production yield · Fructosyltransferase · Fructofuranosidase

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Introduction

While food has long been used to improve health, knowledge of health is now being used to improve food. The first systematic exploration of the positive aspects of food functionality was undertaken in Japan where research programs funded by the Japanese government during the 1980s focused on the ability of some foods to influence physiological functions (Clydesdale 2004; Howlett 2008).

A narrow analysis of the topic suggests that all food is functional since it provides basic energy and nutrients essential for surviving. Yet, the term "functional food" in use nowadays implies that such food carries broader health benefits than just basic nutrition. Food science has evolved from solving problems related to nutritional deficiencies to designing foods that have the potential to induce health benefits and reduce the risk of disease (Clydesdale 2004).

Building a path to an optimum nutrition, which is an aspiring long-term goal, functional food is a newsworthy and stimulating concept, as much as it is supported by consensual scientific data generated by the development of functional food science aimed at the improvement of dietary guidelines that integrate new information on the food elements and body function interactions (Roberfroid 2000a).

The gut is a body organ with major influence in several body functions; thus, it became a clear target for the evolution of functional food, and over the past decade, we have witnessed an increase of new information concerning the

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interface between the diet and its possible role on gut microbiota and, consequently, in health and disease. This has led to great efforts in the development of strategies aimed at dietary modulation of the microbiota composition and activity, achieved by the use of prebiotics, probiotics, and a combination of both (synbiotics; Howlett 2008; Szajewska 2010; De Preter et al. 2011).

Various non-digestible carbohydrates have been tested for their effect on the microbial community. Among them, the functional oligosaccharides, which are intermediate in nature between simple sugars and polysaccharides, present important physicochemical and physiological properties beneficial to the consumer's health, behaving as dietary fibers and prebiotics, and therefore their use as food ingredients has increased rapidly (Simmering and Blaut 2001; Kunz and Rudloff 2006; Mussatto and Mancilha 2007).

FOS, galactooligosaccharides (GOS), isomaltooligosaccharides (IMO), soybean oligosaccharides, xylooligosaccharides (XOS), and maltitol are among the commonly used prebiotics, FOS being the most studied and the best implemented oligosaccharides in the European market (Chen et al. 2000; Qiang et al. 2009; Nobre et al. 2013).

Prebiotics

According to the FAO, "a prebiotic is a non-viable food component that confers a health benefit to the host associated with the modulation of the microbiota" (Gibson et al. 2004; Piñeiro et al. 2008).

Prebiotics can be used as a strategy to improve the balance of intestinal bacteria differing from the probiotic approach in which exogenous strains are included in food and ultimately reach the individual's intestinal tract. These compounds selectively stimulate the proliferation and/or activity of beneficial groups of bacteria that exist in the intestinal microbiota. As a consequence, they can constitute a more practical and efficient way to manipulate the gut microbiota compared to probiotics. However, if for any reason, such as disease, aging, antibiotic, or drug therapy, the appropriate health-promoting species are not present in the bowel, the prebiotic is unlikely to be effective (Playne and Crittenden 2004; Macfarlane et al. 2008).

The gut microbiota ferments a range of substances, mainly provided by the diet, which cannot be digested by the host in the small intestine and are available for fermentation by the colonic microbiota. These include resistant starch, non-starch polysaccharides (dietary fiber), oligosaccharides, proteins, and amino acids, among others. In a typical adult, about 100 g of food ingested each day reaches the large intestine and therefore is susceptible to fermentation by the gut microbiota. Key criteria for a food ingredient to be classified as a prebiotic are (1) it must not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract so that it reaches the colon in significant amounts and (2) it must be a selective substrate for one or more beneficial bacteria that are stimulated to grow. Furthermore, prebiotics may also induce local (in the colon) or systemic effects through bacterial fermentation products that are beneficial to host health (Manning and Gibson 2004; Howlett 2008; De Preter et al. 2011).

These ingredients are normally restricted to certain carbohydrates, i.e., non-digestible oligosaccharides (NDO) that are distinguished from other carbohydrates for being resistant to digestion in the stomach and small intestine (Playne and Crittenden 2004).

Most prebiotics and prebiotic candidates that have been identified are NDO and are obtained either by extraction from plants (e.g., chicory inulin), possibly followed by an enzymatic hydrolysis (e.g., oligofructose from inulin), or by synthesis (by transglycosylation reactions) from mono- or disaccharides such as sucrose (FOS) or lactose (GOS; Crittenden and Playne 1996). Nevertheless, other NDO including XOS, IMO, and soybean oligosaccharides have also been evaluated for their prebiotic effect (Gibson and Roberfroid 1995; Simmering and Blaut 2001).

Apart from their potential to modify the gut microbiota and its metabolic activities in a beneficial way, many other helpful and useful effects of prebiotics are being investigated. These include their ability to modulate gut function and transit time; to activate the immune system; to increase the production of butyric and other short-chain fatty acids; to increase the absorption of minerals, such as calcium and magnesium; and to inhibit lesions that are precursors of adenomas and carcinomas. Thus, prebiotics can potentially reduce some of the risk factors involved in the causes of colorectal diseases and reduce the risk of diseases such as cardiovascular disease, colon cancer, and obesity. Strategies for developing prebiotic products aim to provide specific fermentable substrates for beneficial bacteria (bifidobacteria and lactobacilli). These may provide adequate amounts and proportions of fermentation products, especially in the lower part of the colon where the effects are believed to be most favorable. Although the association with past outbreaks will remain conjectural, it is reasonable to suggest that prebiotic administration may possibly exert very important prophylactic effects against gut pathogens. Even as part of a more complex story, the potential of prebiotics is too large to be ignored (Ziemer and Gibson 1998; Howlett 2008; Qiang et al. 2009; Shimizu and Hachimura 2011).

Fructooligosaccharides as Prebiotics

FOS is a common name for fructose oligomers, and these are usually understood as inulin-type oligosaccharides. They

constitute a series of homologous oligosaccharides derived from sucrose usually represented by the formula GF_n, which are mainly composed of 1-kestose (GF2), nystose (GF3), and 1^F-β- fructofuranosyl nystose (GF4), in which two, three, and four fructosyl units are bound at the β-2,1 position of glucose, respectively, as shown in Fig. 1 (Yun 1996; Lee et al. 1999).

FOS Functional Properties

FOS have a low sweetness intensity since they are only about one third as sweet as sucrose, supplying small amounts of energy, approximately 0–3 kcal/g of sugar substitute. This property is quite useful in the various kinds of foods in which the use of sucrose is restricted by its high sweetness. FOS are also calorie-free since they are scarcely hydrolyzed by the digestive enzymes and not utilized as an energy source in the body; thus, they are safe for diabetics. Also, prebiotics are known to prevent the colonization of human gut by pathogenic microorganisms because they encourage the growth of beneficial bacteria (Roberfroid 2000b; Mishra and Mishra 2013). As FOS cannot be digested by the enzymes of the small intestine, they are fermented in the large intestine to selectively stimulate the growth of probiotic-like bacteria that are part of the commensal gut microbiota and then produce short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate (Yun 1996; Yu Wang et al. 2010). It is also importance to notice that long-chain FOS may exert a prebiotic effect in more distal colonic regions compared with the lower-molecular-weight FOS, which may be more quickly fermented in the saccharolytic proximal bowel (Manning and Gibson 2004).

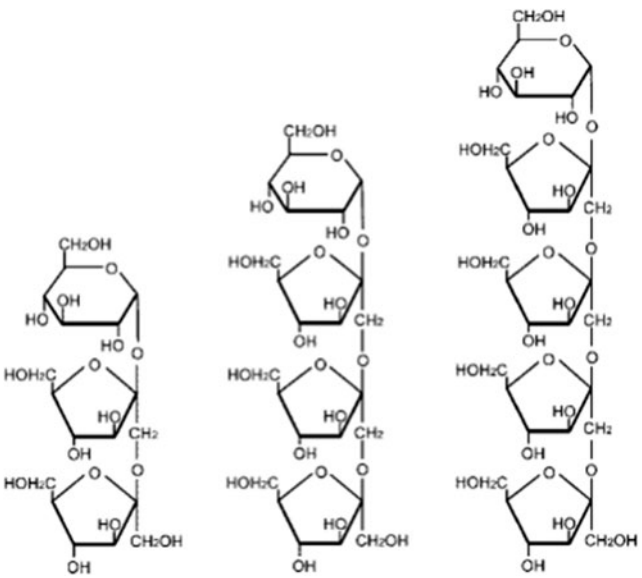

Fig. 1 Structures of 1-kestose (GF2, *left*), nystose (GF3, *center*), and fructofuranosyl nystose (GF4, *right*)

As previously mentioned, these compounds have the capacity of promoting a good balance of intestinal microbiota and decrease gastrointestinal infections. The beneficial physiologic functions that have been reported for FOS are briefly summarized below.

Protection Against Colon Cancer

Prebiotics have been postulated to be protective against the development of colon cancer, which is the second most prevalent cancer in humans. It is known that several species of bacteria commonly found in the colon produce carcinogens and tumor promoters from the metabolism of food components. There have been several studies on the use of prebiotics in cancer prevention mainly focusing on animal models, but the "dietary fiber hypothesis" for protection against colorectal cancer was advanced by Burkitt (1969) based on epidemiological evidence supporting a relationship between diet and colon health. These findings showed lower rates of colorectal cancer in Africa compared to industrialized Western countries, where traditional diets consisted of high amounts of unrefined fiber and high intakes of refined carbohydrates, respectively (Manning and Gibson 2004; Lim et al. 2005a).

The two main types of fermentation that are carried out in the gut are saccharolytic and proteolytic. The saccharolytic activity is more favorable than the proteolytic one due to the type of metabolic end products that are formed, such as SCFAs, acetate, propionate, and butyrate. Butyrate is an important source of energy and is thought to have antitumor properties. Many studies were performed to demonstrate such activity, and according to Kim et al. (1982), butyrate is a protective agent against colon cancer by promoting cell differentiation. Bugaut and Bentéjac (1993) reported that butyrate is used by the epithelial cells of the colon mucosa as an energy source, being also a growth factor, and recent preclinical studies demonstrated that it might be chemopreventive in carcinogenesis (Scheppach and Weiler 2004). In addition to butyrate, propionate can have anti-inflammatory effects on colon cancer cells (Munjal et al. 2009).

Immunomodulatory Effect

Functional foods are reported to enhance the immunity of the consumers. Indeed, the dietary components and their fermentation metabolites are in close contact with the gut-associated lymphoid tissue (GALT), which is the biggest tissue in the immune system comprising 60 % of all lymphocytes in the body (Delgado et al. 2011; Saad et al. 2013). It has been estimated that about 10–60 g/day of dietary carbohydrate reaches the colon acting like a growth factor to particular commensal bacteria, which inhibit the adherence and invasion of pathogens in the colonic epithelia by competing for the same glycoconjugates present on the surface of epithelial

cells, altering the colonic pH, favoring the barrier function, improving the mucus production, producing SCFAs, and inducing cytokine production (Korzenik and Podolskyl 2006).

Many researchers recognized the effect of prebiotics oral administration. In 1997, Pierre and co-workers (Pierre et al. 1997) reported the development of GALT associated with inulin and FOS intake. Furthermore, Hosono et al. (2003) stated that inulin and FOS were able to modulate various parameters of the immune system. Schley and Field (2002) have reviewed some evidences of the immune-enhancing effects of dietary fibers and prebiotic and identified for the first time the gene markers associated with the physiological effects of a particular FOS on a host animal, thus making it possible to clarify the mechanisms behind the immunomodulatory effects of FOS.

Obesity and Diabetes

Fiber intakes are associated with increased satiety and lower body weight. Beyond glycemic control, the fiber's fermentation in the intestine may also influence satiety. However, these mechanisms are not completely understood. One hypothesis is that the production of SCFAs influences postprandial glucose response by reducing fat competition for glucose disposal. On the other hand, another hypothesis relies on the idea that fermentation of SCFAs influences gut hormones and gastric motility (Brighenti et al. 2006; Nilsson et al. 2008). Also, other scientific data suggest that the decrease in food intake associated with prebiotics feeding in animals might be linked to the modulation of gastrointestinal peptides involved in the regulation of food intake (Druce et al. 2004; Chaudhri et al. 2008).

Obesity and obesity-related type II diabetes are typical diseases of the modern Western society. Current recommendations for the management of type II diabetes and obesity include an increase in dietary fiber intake. Dietary fiber's viscose and fibrous structure can control the release of glucose with time in the blood, thus helping in the proper control and management of diabetes mellitus and obesity (Bennett et al. 2006).

Improving Mineral Adsorption

Another interesting feature of FOS is their positive effect on mineral absorption. Mineral deficiencies remain an important nutritional issue in the World. Calcium (Ca) intake in many underdeveloped countries is below the recommended daily allowance, which leads to progressive bone loss. Ca is critical in achieving optimal peak bone mass and modulating the rate of bone loss associated with aging. Then, if Ca intake is not enough to offset obligatory losses, acquired skeletal mass cannot be maintained, leading to osteoporosis, a major public health problem. Magnesium is the second most abundant intracellular cation in vertebrates, and its deficiency has been implicated as a risk factor for osteoporosis. Another mineral with a low intake is iron (Fe), which is a major cause of anemia. The importance of zinc (Zn) nurture for growth and development has been widely documented. Marginal Zn deficiency is suspected to be widespread in populations heavily dependent on cereal-based diets containing few animal products (Baba et al. 1996; Roberfroid et al. 2010; Yu Wang et al. 2010).

Several mechanisms have been proposed to elucidate the possible roles of FOS in improving mineral absorption. Even though mineral absorption generally occurs in the upper part of the intestine, it seems that, after consumption of FOS, the major site of action leading to improved bioavailability of minerals is the large intestine. The main action of FOS is mainly associated with their fermentation by resident microbiota. SCFAs decrease luminal pH and thus create an acidic environment more favorable for mineral solubility (Gudiel-Urbano and Goñi 2002; Yu Wang et al. 2010). Another way to contribute to the enhanced mineral absorption is the trophic effect of prebiotics on the gut (cell growth and functional enhancement of the absorptive area). It has been suggested that this is mediated by an increased production of butyrate and/or certain polyamines (Roberfroid 1993; Raschka and Daniel 2005).

Effects on Serum Lipid and Cholesterol Concentrations

Since cardiovascular risk is the major public health concern in many countries and accounts for more deaths than any other disease or group of diseases, the food industry is increasingly interested in the development of functional foods that modulate blood lipids, such as cholesterol and triglycerides (Manning and Gibson 2004; Qiang et al. 2009).

Many attempts have been made to control serum triacylglycerol (TAG) concentrations through the modification of dietary habits. The hypotriglyceridemic effect of non-digestible but fermentable carbohydrates, including resistant starch or FOS, has been described by Glore et al. (1994) and Jackson et al. (1999) in humans and by Tokunaga et al. (1986) and Yamamoto et al. (1999) in animals.

Ingredients showing a prebiotic effect are able to modulate hepatic lipid metabolism in rats or hamsters, resulting in changes in either TAG accumulation in the liver (steatosis) or serum lipids. The decrease in TAG synthesis and the accumulation of dietary prebiotics compounds could be linked to several events. First, a decrease in glycemia could be part of the process since glucose (together with insulin) is a driver of lipogenesis. Secondly, the SCFAs produced through the fermentation process could play a role in the regulation of lipid metabolism (Delzenne and Kok 2001; Delzenne et al. 2002).

Among several studies, Levrat et al. (1994) and Fiordaliso et al. (1995) reported a decrease in total serum cholesterol after

dietary supplementation with inulin (10 %) in mice or rats. This is accompanied by a significant decrease in the hepatic cholesterol content. The authors suggested that the decrease in serum cholesterol could reflect a decrease in TAG-rich lipoproteins.

FOS Occurrence

FOS are found in trace amounts as natural components in fruits, vegetables, and honey. As a series of fructose oligomers and polymers derived from sucrose, they occur in many higher plants as reserve carbohydrates. Asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, and rye are well-known sources of FOS (Yun 1996; Sangeetha et al. 2005a; Mussatto and Mancilha 2007).

For most of these sources, FOS concentrations range between 0.3 and 6 %; for chicory, these values are between 5 and 10 %, while in Jerusalem artichoke they can reach 20 %. Based on the usual consumption of these natural sources, an average daily consumption of FOS of approximately 13.7 mg/$kg_{body\ weight}$ per day or 806 mg/day has been estimated (Voragen 1998).

FOS Production

FOS represent one of the major classes of bifidogenic oligosaccharides in terms of their production volume. They can be produced by the hydrolysis of inulin or by the transfructosylation of sucrose. The FOS mixture obtained by the enzymatic hydrolysis of inulin closely resembles the mixture produced by the transfructosylation process, although slightly different end products are obtained as not all of the β(1→2)-linked fructosyl chains end with a terminal glucose. Additionally, the oligosaccharide mixture produced from inulin hydrolysis contains longer fructo-oligomer chains than that produced by the sucrose transfructosylation process. In this process, FOS are produced from the disaccharide sucrose using enzymes with transfructosylation activity. These enzymes are invertase (β-fructofuranosidase fructohydrolase, FFase, EC 3.2.1.26) and sucrase (sucrose fructosyltransferase, FTase, EC 2.4.1.9), which are mainly derived from fungal and bacterial sources. A high concentration of the starting substrate is required for efficient transfructosylation. In this reaction, FOS are formed, namely, kestose, nystose, and fructofuranosyl nystose (Fig. 1), but also some by-products such as glucose and small amounts of fructose (Brenda 2005; Crittenden and Playne 1996; Singh and Singh 2010; Saad et al. 2013).

FOS Microbial Production—Transfructosylation Enzymes

The commercially available FOS are produced through the enzymatic synthesis from sucrose by microbial enzymes with transfructosylation activity. Some bacterial strains have been reported to produce such enzymes. On the other hand, several fungal strains, such as *Aspergillus* sp., *Aureobasidium* sp., and *Penicillium* sp., are known to produce extracellular and/or intracellular enzymes with transfructosylation activity (Table 1).

Many authors have reported the purification and characterization of FOS-producing enzymes from various sources and by different microorganisms (bacteria and fungi; Hayashi et al. 1992; L'Hocine et al. 2000; Park et al. 2001; Nguyen et al. 2005; Jedrzejczak-Krzepkowska et al. 2011; Risso et al. 2012). Although these proteins differ in their subunit structure, molecular weight, degree of glycosylation, chemical susceptibility, and substrate specificity, they all display both hydrolytic and transfer activities. The same microorganism can even produce several enzymes with transfructosylation activity holding different characteristics (Yoshikawa et al. 2007).

There is still a dispute in the scientific community regarding the nomenclature of FOS-producing enzymes. Some researchers use the term β-fructofuranosidase (FFase, EC. 3.2.1.26; Nguyen et al. 1999; Sheu et al. 2001), whereas others designate it as fructosyltransferase (FTase, EC.2.4.1.9; Yun et al. 1997; Sangeetha et al. 2004; Vandáková et al. 2004). The latter denomination is probably due to the fact that FTase activity was originally found from the side action in the course of FFase preparation when acting on a high concentration of sucrose (Straathof et al. 1986; L'Hocine et al. 2000).

Transfructosylating activity acts on sucrose by cleaving the β(1,2) linkage and transferring the fructosyl group to an acceptor molecule such as sucrose, releasing glucose. This reaction yields FOS, i.e., fructose oligomers, in which fructosyl units are bound at the β(2,1) position of sucrose (Belghith et al. 2012). Although FFase is generally known as an enzyme catalyzing the hydrolysis of sucrose, some FFases have much higher transfructosylating activity (U_t) than hydrolyzing activity (U_h). A wide variety of fungi produce FFases, but the activity ratios (U_t/U_h) of their enzymes vary significantly (Sangeetha et al. 2005a; Yoshikawa et al. 2006). Fructosyl-transferring enzymes have been purified and characterized from higher plants, such as asparagus (Shiomi 1982), onion (Fujishima et al. 2005), Jerusalem artichoke (Koops and Jonker 1994), and from different microorganisms (fungi and bacteria) such as *Aspergillus niger* (L'Hocine et al. 2000), *Aspergillus japonicus* (Hayashi et al. 1992), *Aureobasidium pullulans* (Yoshikawa et al. 2006), *Bacillus macerans* (Park et al. 2001), and *Candida utilis* (Chávez et al. 1997). Although these proteins differ in their subunit structure, molecular weight, degree of glycosylation, chemical

Table 1 Microbial sources of enzymes with transfructosylation activity: FOS-producing enzymes

Source	Microorganism	Enzyme	Reference
Fungal transfructosylation enzyme	*Aureobasidium pullulans*	Intra- and extracellular FTase	Yun et al. (1997)
		Intra- and extracellular FTase	Sangeetha et al. (2004a)
		Intra- and extracellular FTase	Vandáková et al. (2004)
		Intracellular FTase	Lateef et al. (2007)
		Periplasmic FFase	Yoshikawa et al. (2006)
		Extracellular FTase	Dominguez et al. (2012)
	Aspergillus aculeatus	FTase from commercial enzyme preparation—Pectinex Ultra SP-L	Nemukula et al. (2009)
			Ghazi et al. (2005)
			Ghazi et al. (2007)
	Aspergillus flavus	Extracellular FTase	Ganaie et al. (2013)
	Aspergillus japonicus	Intra- and/or extracellular FFase	Chen and Liu (1996)
		Intra- and/or extracellular FFase	Wang and Zhou (2006)
		Extracellular FFase	Mussatto et al. (2009)
		Extracellular FFase	Mussatto and Teixeira (2010)
	Aspergillus niger	Extracellular FFase	Wallis et al. (1997)
		Intra- and extracellular FFase	Nguyen et al. (1999)
		Intra- and/or extracellular FTase	L'Hocine et al. (2000)
		Extracellular FTase	Ganaie et al. (2013)
	Aspergillus oryzae	Extracellular FTase	Sangeetha et al. (2004a)
		Extracellular FTase	Sangettha et al. (2005a)
	Aspergillus terreus	Extracellular FTase	Ganaie et al. (2013)
	Penicillium citrinum	Intra- and/or extracellular enzyme	Lim et al. (2005b)
	Penicillium islandicum	Extracellular FTase	Ganaie et al. (2013)
Bacterial transfructosylation enzyme	*Bacillus macerans*	Extracellular transfructosylation enzyme	Park et al. (2001)
	Lactobacillus reuteri	Intra and/or extracellular FTase	van Hijum et al. (2002)
	Zymomonas mobilis	Extracellular levansucrase	Bekers et al. (2002)

susceptibility, and substrate specificity, they all display both hydrolytic and transfer activities (Ghazi et al. 2007). Fungal FTases have molecular masses ranging between 180,000 and 600,000 and are homopolymers with two to six monomer units. Some studies stated the optimum temperature and pH for these enzyme activities between 50 and 60 °C and from 4.5 to 6.5, respectively (Madlová et al. 2000; Maiorano et al. 2008).

There are several studies reporting the characteristics of transfructosylation enzymes produced by fungi, mainly *Aureobasidium* sp. and *Aspergillus* sp. Antošová et al. (2002) reported U_t in *A. pullulans* CCY 27-1-1194 in the extracellular medium and in the whole cells. In their study, a maximum FTase U_t of 131,000 U dm^{-3} (131 U mL^{-1}) released into the cultivation medium, after four cultivation days, was reported. They also reported that with an initial sucrose concentration of 200 g L^{-1}, an FTase U_t of 80,000 U dm^{-3} (80 U mL^{-1}) is achieved. In 2008, Antošová et al. (2008) reported the chromatographic separation of an intracellular FTase from *A. pullulans*. The isolated enzyme exhibited both U_h and U_t activities, the U_t being higher in the sucrose concentration range tested (10–600 g L^{-1}) and completely dominating at higher sucrose concentrations. Yoshikawa et al. (2006) have reported five FFase extracted from the cell wall of *A. pullulans* with high U_t. This study also suggested that the expression of FFase I was not repressed by glucose, but those of FFases II–V were strongly inhibited in the presence of glucose, considering that FFase I played a key role in FOS production by this fungus, whereas FFase IV may function as a FOS-degrading enzyme with its strong hydrolyzing activity.

Hayashi et al. (1992) identified a FFase produced by *A. japonicus* and optimized the cultural condition for the production of the enzyme and the enzymatic reaction conditions for the industrial utilization of the strain to produce FOS. Later on, FFase activity in *A. japonicus* was also reported by Chen (1995); a maximum enzyme production of 910 U mL^{-1} was achieved. Wang and Zhou (2006) isolated an *A. japonicus* strain from soil and investigated the optimal conditions for FFase, reaching an activity of 55.42 U mL^{-1} at pH 5.5 and 30 °C. Wallis et al. (1997) identified two FFase secreted by *A. niger*. L'Hocine et al. purified and partially characterized a FTase and a FFase from the crude extract of *A. niger*. This strain showed very high enzyme productivity and high FTase

activity; however, the enzyme used in the crude state also showed hydrolytic activity. Balasubramaniem et al. (2001) reached a maximum FFase activity of 149 U L^{-1} h^{-1}, this enzyme being produced by *A. niger*. Nguyen et al. (2005) purified an intracellular FFase produced by *A. niger* and estimated its molecular mass to be in the range from 120 to 130 kDa. Extracellular FTase activity in *Aspergillus oryzae* was identified by Sangeetha et al. (2004b), with a molecular mass of 116.3 kDa. Later on, the same authors reported an FFase activity in that same strain in the range of 15± 2 U mL^{-1} min^{-1} (Sangeetha et al. 2005a).

There are mainly two methods that can be used to produce transfructosylation enzymes and FOS by fermentation, namely, by submerged fermentation (SmF) or by solid state fermentation (SSF). These two methods will be further discussed in the following sections.

FOS Production by SmF

The most common and studied method to produce FOS is by the transfructosylation of sucrose in a two-stage process, schematically represented in Fig. 2, the enzyme(s) being produced by SmF in the first stage. This is also the usual process used to produce the FOS that are currently available on the market (Singh and Singh 2010).

Most studies on the experimental conditions for enzyme production are mainly based on shake-flask experiments, and the work carried out in bioreactors is unusual. The variables generally studied to define the best operational conditions for enzyme production are the carbon and nitrogen sources, their concentrations, time of cultivation, agitation, and aeration rates. Other important factors are related with the addition of different mineral salts, small amounts of amino acids, polymers, and surfactants (Maiorano et al. 2008).

Chen and Liu (1996) evaluated the effect of various carbon and nitrogen sources in enzyme production and found that sucrose at 25 % (*w*/*v*) and yeast extract were the best ones, respectively. Later on, Antošová et al. (2002) obtained the highest FTase activity using sucrose at 350 g L^{-1} and found that biomass production was not influenced by sucrose concentration in the range from 50 to 350 g L^{-1}. Vandáková et al. (2004) also studied the effect of sucrose on FTase production by *Aerobasidium pullulans* and reported that sucrose content did not influence the total production of FTase. However, high sucrose concentrations resulted in high specific activities of the cells.

The effects of inorganic salts were reported by various authors. Chen and Liu (1996) studied the effect of inorganic salts and found that the addition of $MgSO_4 \cdot 7H_2O$ and K_2HPO_4 shifted the morphology of the fungal growth from filamentous to pellet form without affecting FFase production. The morphology of fungal growth can range from dispersed mycelia to compact pellets. Control of mycelia morphology in fermentations is often a prerequisite for industrial applications. Although

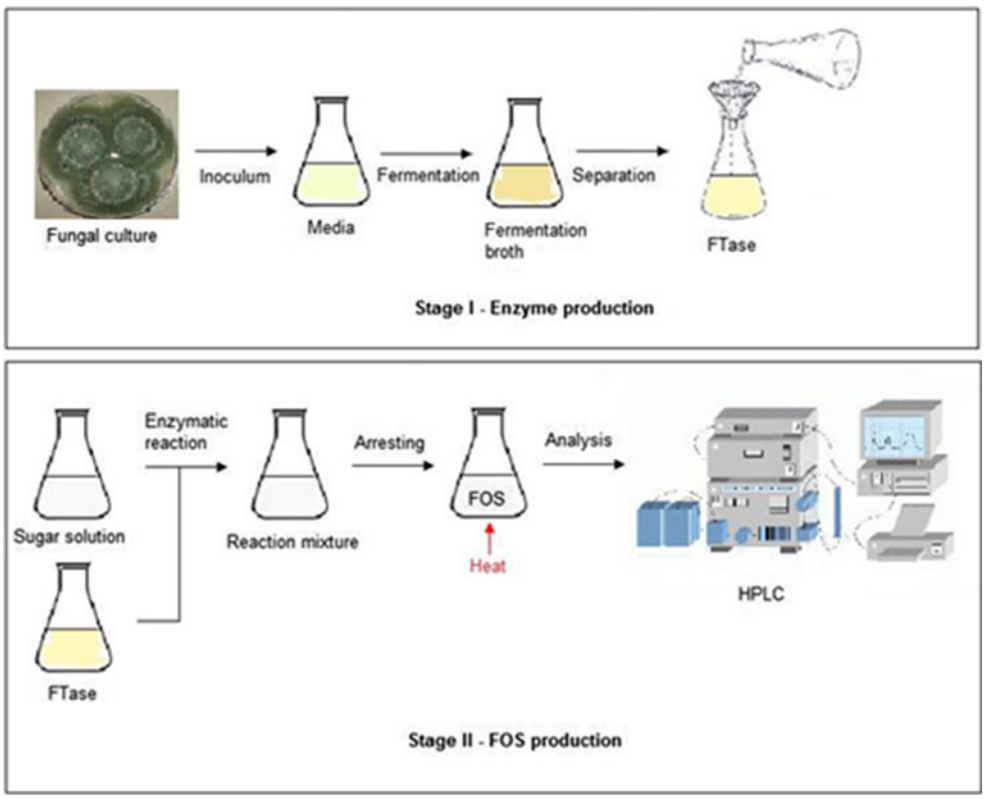

Fig. 2 Schematic representation of the two-stage FOS production process by SmF (adapted from Sangeetha et al. 2005c)

fungus, grown in the form of mycelia, may have a high specific growth rate, the growth of fungus in the form of pellets is more attractive to many industrial fermentation processes from the perspective of reducing the power input needed for adequate gas dispersion and mixing (Metz and Kossen 1977; Chen and Liu 1996). K_2HPO_4 is described as a microelement source for cell growth, as well as a buffering reagent. Its optimal concentration ranges between 4g L^{-1} (Sangeetha et al. 2005a, b) and 5 g L^{-1} (Vandáková et al. 2004; Shin et al. 2004a; Lim et al. 2005b). Mg^{2+} affects the permeability of the cell wall for *A. pullulans* (Vandáková et al. 2004), and its optimal concentration varies from 0.3 g L^{-1} (Sangeetha et al. 2005a, b) to 2 g L^{-1} (Balasubramaniem et al. 2001). $NaNO_3$ is the most common source of inorganic nitrogen in transfructosylation enzymes production by fungi and can be employed in concentrations from 2 g L^{-1} (Lim et al. 2005b) to 25 g L^{-1} (Dhake and Patil 2007). Other ions, such as $FeSO_4$ and $CuSO_4$, can also be added to the medium with positive effects, such as increasing FFase activity and FOS production (Balasubramaniem et al. 2001; Lim et al. 2005b; Wang and Zhou 2006).

The effect of the medium pH in FTase production and microorganism growth has been reported. pH of 5.5 was found to be the best initial value for FTase production by *A. oryzae* CFR202 (Sangeetha et al. 2005a, b), *A. japonicus* JN19 (Wang and Zhou 2006), and *Penicillium purpurogenum* (Dhake and Patil 2007).

The FOS production yield by *A. pullulans* in a two-stage process can vary between 44.0 % ($g_{FOS}/g_{sucrose}$; Shin et al. 2004b) and 62.0 % ($g_{FOS}/g_{sucrose}$; Yoshikawa et al. 2008). For *A. japonicas*, these values can vary between 55.8 % ($g_{FOS}/g_{sucrose}$; Wang and Zhou 2006) and 61.0 % ($g_{FOS}/g_{sucrose}$; Chiang et al. 1997), whereas for *A. niger*, values ranging between 24.0 % ($g_{FOS}/g_{sucrose}$; Nguyen et al. 1999) and 70.0 % ($g_{FOS}/g_{sucrose}$; Maldová et al. 1999) can be expected. On the other hand, Sangeetha et al. (2005a, b) reported FOS production yield by *A. oryzae* of 53.0 % ($g_{FOS}/g_{sucrose}$) to 57.4 % ($g_{FOS}/g_{sucrose}$).

FOS Production bySSF

Despite most industrial enzymes being produced in SmF, SSF presents an interesting potential for small-scale units. Some advantages of this process are the simplicity of operation, high volumetric productivity, product concentration, and low capital cost and energy consumption. Moreover, the risk of contamination is reduced. SSF requires low water volume and thus has a large impact on the economy of the process due to smaller fermenter size, reduced downstream processing, reduced stirring, and lower sterilization costs (Sangeetha et al. 2005c; Mussatto and Teixeira 2010). However, there are also some disadvantages associated with the use of SSF, which have discouraged the use of this technique for industrial enzyme production. The main drawbacks are due to the buildup of gradients—of temperature, pH, moisture, substrate concentration, or dissolved oxygen—during cultivation, which are difficult to control under limited water availability, leading to scale-up engineering problems (Pandey 2003; Hölker et al. 2004).

The use of low-cost agricultural and agro-industrial residues as substrates contributes also to the lower capital and operating costs of SSF as compared to SmF. Several agricultural by-products like cereal bran, corn products, sugarcane bagasse, and by-products of coffee and tea processing industries were used as substrates to produce FTase in SSF by *A. oryzae* CFR 202 (Sangeetha et al. 2004b). In this study, rice bran and wheat bran were found to be the substrates that maximize FTase production, with optimum activity at 60 °C and pH 6.0. The maximum FOS production (52.0 %, $g_{FOS}/g_{sucrose}$) was obtained after 8 h of reaction. Mussatto and Teixeira (2010) reported the increase of FOS yield and productivity by SSF with *A. japonicus* using agro-industrial residues as immobilization supports and nutrient sources. Corn cobs, coffee silverskin, and cork oak were used as support and nutrient sources. Coffee silverskin was found to be the one that provided the higher production yield and FFase activity, reaching 70.0 % ($g_{FOS}/g_{sucrose}$) after 16 h of reaction. These authors conducted a similar study with *Penicillum expansum* in which the use of low-cost materials including synthetic fiber, polyurethane foam, stainless steel sponge, loofah sponge, and cork oak were tested as carriers for fungus immobilization (Mussatto et al. 2012). Additionally, production of FOS and FFase by repeated batch fermentation of *P. expansum* immobilized on synthetic fiber was studied. Production yields of 87 % ($g_{FOS}/g_{sucrose}$), 72 % ($g_{FOS}/g_{sucrose}$), and 44 % ($g_{FOS}/g_{sucrose}$) in the three initial cycles (36, 48, and 60 h), respectively, were obtained.

Generally, the most efficient culture media and strain to produce enzymes in SSF are not the same as those in SmF, and vice versa (Maiorano et al. 2008). Optimization of the fermentation medium for β-fructofuranosidase production by *A. niger* NRRL 330 in SSF and SmF was carried out using a fractional factorial design (Balasubramaniem et al. 2001). The results showed different compositions of optimized media for SmF and SSF. After the optimization procedures, the medium composition for SSF required twice more sucrose and yeast extract than the medium for SmF, and the productivity in SSF was about twice that in SmF.

Table 2 presents a comparison between the FOS production yields reported in the literature, obtained in various production studies by either SmF or SSF.

Production of High-Content FOS

Industrial production of FOS carried out with microbial transfructosylation enzymes was found to give a maximum

Table 2 FOS yields obtained using FTase and/or FFase from various fungal strains

Microorganism	Production process	Reaction time (fungus cultivation+enzymatic reaction)	Production yield (%, $g_{FOS}/g_{sucrose}$)	Reference
Aureobasidium pullulans	Two-stage process using isolated intracellular enzyme produced by SmF	96+25 h	58.0	Yun and Song (1993)
	Two-stage process using extracellular enzyme produced by SmF	48+24 h	55.9	Sangeetha et al. (2004a)
	Two-stage process using culture broth homogenate produced by SmF	96+12 h	56.6	Sangeetha et al. (2004a)
	Two-stage process using isolated intracellular enzyme produced by SmF	72+24 h	53.0	Shin et al. (2004a)
	Two-stage process with immobilized cell (SmF)	72+24 h	44.0	Shin et al. (2004b)
	Two-stage process with isolated intracellular FTase produced by SmF	24+9 h	59.0	Lateef et al. (2007)
	Two-stage process using crude FFase preparation produced by SmF	24+24 h	62.0	Yoshikawa et al. (2008)
	One-stage process: present study—SmF	48 h	64.1	Dominguez et al. (2012)
Aspergillus flavus	Two-stage process using culture broth filtrate produced by SmF	120+24 h	63.4	Ganaie et al. (2013)
Aspergillus ibericus	One-stage process—SmF	24 h	61.0–64.0	Gomes (2009)
Aspergillus japonicus	Two-stage process using immobilized FFase produced by SmF	28+17 h	61.0	Chiang et al. (1997)
	Two-stage process with isolated intracellular FFase produced by SmF	72+24 h	55.8	Wang and Zhou (2006)
	One-stage process using corn cobs as the solid support—SSF	21 h	66.0	Mussatto et al. (2009)
	One-stage process—SmF	24 h	61.0	Mussatto et al. (2009)
	One-stage process using coffee silverskin as the solid support—SSF	16 h	70.0	Mussatto and Teixeira (2010)
Aspergillus niger	Two-stage process with isolated intracellular and extracellular FFase produced by SmF	24+72 h	24.0–26.0	Nguyen et al. (1999)
	Two-stage process using washed cells produced by SmF	92+8 h	70.0	Madlová et al. (1999)
	Two-stage process using culture broth filtrate produced by SmF	72+24 h	55.8	Ganaie et al. (2013)
Aspergillus oryzae	Two-stage process using extracellular enzyme produced by SmF	120+12 h	54.1	Sangeetha et al. (2004a)
	Two-stage process using culture broth homogenate produced by SmF	96+12 h	53.2	Sangeetha et al. (2004a)
	Two-stage process FTase produced by SSF using corn germ as the solid support	120+8 h	60.0	Sangeetha et al. (2004b)
	Two-stage process using extracellular enzyme produced by SmF	24+18 h	57.4	Sangeetha et al. (2005a)
	Two-stage process using whole cells produced by SmF	24+48 h	53.0	Sangeetha et al. (2005c)
Penicillium expansum	One-stage process—SmF	36 h	58.0	Prata et al. (2010)
	One-stage process using synthetic fiber as the solid support: SFF—repeated batch fermentation	60 h, three cycles	First: 87.0 (36 h); second: 72.0 (48 h); third: 44.0 (60 h)	Mussatto et al. (2012)
Penicillium chrysogenum	Two-stage process using culture broth filtrate produced by SmF	48+24 h	42.5	Ganaie et al. (2013)

theoretical yield of 55–60 % based on the initial sucrose concentration (Sangeetha et al. 2005c). This yield cannot be further increased due to the high amounts of glucose co-produced during the fermentation, which acts as an inhibitor (Yun 1996).

Therefore, in order to obtain higher fermentation yields, many authors have studied the impact of the continuous removal of glucose and residual sucrose from the medium during the FOS conversion. Consequently, by increasing the fermentation yields, a purer final product could be obtained (Nobre et al. 2013).

High FOS production yields adding glucose oxidase to the reaction media have been reported, and many authors manage to control this effect and increase FOS production yield (up to 90–98 %, $g_{FOS}/g_{sucrose}$) by adding glucose oxidase to the reaction media, which leads to the formation of gluconic acid, hence reducing the glucose content in the media (Yun and Song 1993; Sheu et al. 2001; Lin and Lee 2008).

Other systems aiming glucose removal from the media have also been studied. For example, Sheu et al. (2002) reported a complex biocatalyst system with a bioreactor equipped with a microfiltration module. The system used mycelia of *A. japonicus* CCRC 93007 or *A. pullulans* ATCC 9348 with FFase activity and *Gluconobacter oxydans* ATCC 23771 with glucose dehydrogenase activity. Calcium carbonate slurry was used to control the pH to 5.5, and gluconic acid in the reaction mixture was precipitated as calcium gluconate. Sucrose solution with an optimum concentration of 30 % (*w*/*v*) was employed as feed for the complex cell system, and high-content FOS was discharged continuously from a microfiltration module. The complex system produced more than 80 % ($g_{FOS}/g_{sucrose}$) FOS, with the remaining being 5–7 % glucose and 8–10 % sucrose on a dry weight basis, plus a small amount of calcium gluconate.

Nishizawa et al. (2001) achieved higher FOS yields with a simultaneous removal of glucose using a membrane reactor system with a nanofiltration membrane, through which glucose permeated, but not sucrose and FOS. FOS percentage of the reaction product was increased to above 90 % ($g_{FOS}/g_{sucrose}$), which was much higher than that of the batch reaction product (55–60 %, $g_{FOS}/g_{sucrose}$). Nonetheless, although these systems can be more efficient in terms of FOS production yield, it is important to notice that they are also more expensive.

With the same goal, Crittenden and Playne (2002) used a different approach. In their study, immobilized cells of the bacterium *Zymomonas mobilis* were used to remove glucose, fructose, and sucrose from food-grade oligosaccharide mixtures, which were completely fermented within 12 h. The fermentation end products were ethanol and carbon dioxide, a minimal amount of sorbitol also being formed. Similarly to *Z. mobilis*, *Saccharomyces cerevisiae* also showed the ability to ferment some common mono- and disaccharides (fructose, glucose, galactose, and sucrose) from a mixture of sugars, while oligosaccharides with four or more monosaccharide units were not fermented (Yoon et al. 2003; Goulas et al. 2007; Hernández et al. 2009). The microbial treatment seems to be a good alternative for increasing the percentage of FOS in a mixture through the removal of mono- and disaccharides, this process being adequate to be used during the enzymatic synthesis of FOS. However, the use of microbial treatments implies a further step of purification for the removal of biomass and metabolic products formed during the fermentation in order to obtain a FOS product with few contaminants, thus increasing the production cost. Also, the use of yeast treatment was found to modify the oligosaccharide composition (Sanz et al. 2005; Nobre et al. 2013).

Zuccaro et al. (2008) reported that recombinant FTases also have interesting biocatalytic properties. The combination of sucrose analogues, as novel substrates (substrate engineering), and highly active recombinant β-fructofuranosidase from *A. niger* (genetic engineering) provided a new powerful tool for the efficient preparative synthesis of tailor-made saccharides 1-kestose and 1-nystose type.

FOS Market Data

In general, the total cost of developing a conventional new food product is estimated to be up to 0.75–1.5 million Euros, while the development and marketing costs of functional food products may exceed this level by far. Presently, the European market of functional food is dominated by gut health-targeted products. Germany, France, UK, and the Netherlands account for around two thirds of all sales of functional dairy products in Europe. Functional dairy products have shown an impressive growth, bringing, for example the market volume in Germany, from around 4 million Euros in 1995 to 317 million Euros in 2000, of which 228 million Euros account for probiotic, prebiotic, and other functional yoghurts and around 89 million Euros for functional drinks. The World demand for prebiotics is estimated to be around 167,000 tons and 390 million Euros. Among them, FOS, inulin, isomalto-oligosaccharides, polydextrose, lactulose, and resistant starch are considered the most popular. In 1995, the global market for FOS from sucrose was estimated to be 20,000 tons (Menrad 2003; Siró et al. 2008).

As previously mentioned, at an industrial scale, FOS are produced enzymatically by two different processes

Table 3 Commercially available food-grade FOS

Substrate	Manufacturer	Trade name
Sucrose	Beghin-Meiji Industries, France	Actilight®
		Profeed®
	Cheil Foods and Chemicals Inc., Korea	Oligo-Sugar
	GTC Nutrition, USA	NutraFlora®
	Meiji Seika Kaisha Ltd., Japan	Meioligo®
	Victory Biology Engineering Co., Ltd., China	Beneshine™ P-type
Inulin	Beneo-Orafti, Belgium	Orafti®
	Cosucra Groupe Warcoing, Belgium	Fibrulose®
	Sensus, the Netherlands	Frutalose®
	Nutriagaves de Mexico S.A. de C.V., Mexico	OLIFRUCTINE-SP®

(transfructosylation of sucrose or hydrolysis of inulin) which yield slightly different end products. The companies that commercially produce FOS from sucrose or inulin and their trade names are listed in Table 3. These FOS products are provided with a purity level above 95 % (Singh and Singh 2010; Nobre et al. 2013).

The enzymatic synthesis route to FOS using FTase from *A. niger* was first developed by Meiji Seika Kaisha Ltd., Japan, resulting in the launch of the commercial product Meioligo®. Then, this company also established a joint venture with Beghin-Meiji Industries, France, to produce FOS marketed as Actilight®, and also with GTC Nutrition, USA, to produce FOS under the trade name NutraFlora® (Singh and Singh 2010). According to the Meiji Holdings Co., Ltd. Annual Report (2011), they presented total net sales of almost 21 million Euros and operating incomes of 0.5 million Euros.

Additionally, individual FOS molecules with purities varying between 80 and 99 % are only available for analytical purposes. The main companies supplying 1-kestose (GF2), nystose (GF3), and 1F-β- fructofuranosyl nystose (GF4) are Sigma Aldrich, Megazyme, and Wako Chemicals GmbH (Nobre et al. 2013).

Conclusions

Modern consumers are increasingly interested in their personal health and expect the foods they eat to be—beyond tasty and attractive—also safe and healthy. Non-digestible carbohydrates such as dietary fibers, oligosaccharides, and resistant starch have various physiologic functions, and the effects of many non-digestible carbohydrates on well-being, better health, and reduction of the risk of diseases have been well evaluated. Among functional oligosaccharides, FOS present important physicochemical and physiological properties beneficial to the health of consumers. For this reason, their use as food ingredients has increased rapidly, presenting a significant growth on the functional food market all over the world. Europe nutraceutics market is expected to have a share of over 20% until 2017. In view of the great demand for FOS as food ingredients, the opportunity exists for the screening and identification of novel strains capable of producing enzymes with transfructosylation activity and for developing improved and less expensive production methods. In this review, the beneficial effects of FOS and how they can play a key role in the food market have been discussed, bearing in mind that more effective less costly production methods can be a main advantage in the food industry.

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References

Antošová, M., Polakovič, M., Slovinská, M., Madlová, A., Illeová, V., & Báleš, V. (2002). Effect of sucrose concentration and cultivation time on batch production of fructosyltransferase by *Aureobasidium pullulans* CCY 27-1-1194. *Chemical Papers, 56*, 394–399.

Antošová, M., Illeová, V., Vandáková, M., Družkovská, A., & Polakovič, M. (2008). Chromatographic separation and kinetic properties of fructosyltransferase from *Aureobasidium pullulans*. *Journal of Biotechnology, 135*, 58–63.

Baba, S., Ohta, A., Ohtsuki, M., Takizawa, T., Adachi, T., & Hara, H. (1996). Fructooligosaccharides stimulate the absorption of magnesium from the hindgut in rats. *Nutrition Research, 16*, 657–666.

Balasubramaniem, A. K., Nagarajan, K. V., & Paramasamy, G. (2001). Optimization of media for β-fructofuranosidase production by *Aspergillus niger* in submerged and solid state fermentation. *Process Biochemistry, 36*, 1241–1247.

Bekers, M., Laukevics, J., Upite, D., Kaminska, E., Vigants, A., Viesturs, U., et al. (2002). Fructooligosaccharide and levan producing activity of *Zymomonas mobilis* extracellular levansucrase. *Process Biochemistry, 38*, 701–706.

Belghith, K. S., Dahecha, I., Belghith, H., & Mejdouba, H. (2012). Microbial production of levansucrase for synthesis of fructooligosaccharides and levan. *International Journal of Biological Macromolecules, 50*, 451–458.

Bennett, N., Greco, D. S., Peterson, M. E., Kirk, C., Mathes, M., & Fettman, M. J. (2006). Comparison of a low carbohydrate-low fiber diet and a moderate carbohydrate high fiber diet in the management of feline diabetes mellitus. *Journal of Feline Medicine & Surgery, 8*, 73–84.

Brenda—The Comprehensive Enzyme Information System. (2005). Cologne University BioInformatics Center, Germany. Retrieved 3 May 2005 from http://www.brenda.uni-koeln.de/.

Brighenti, F., Benini, L., Del Rio, D., Casiraghi, C., Pellegrini, N., Scazzina, F., et al. (2006). Colonic fermentation of indigestible carbohydrates contributes to the second-meal effect. *American Journal of Clinical Nutrition, 83*, 817–822.

Bugaut, M., & Bentéjac, M. (1993). Biological effects of short-chain fatty acids in nonruminant mammals. *Annual Review of Nutrition, 13*, 217–241.

Burkitt, D. P. (1969). Related disease—related cause? *The Lancet, 2*, 1229–1231.

Chaudhri, O. B., Salem, V., Murphy, K. G., & Bloom, S. R. (2008). Gastrointestinal satiety signals. *Annual Review of Physiology, 70*, 239–255.

Chávez, F. P., Rodriguez, L., Díaz, J., Delgado, J. M., & Cremata, J. A. (1997). Purification and characterization of an invertase from *Candida utilis*: comparison with natural and recombinant yeast invertases. *Journal of Biotechnology, 53*, 67–74.

Chen, W. C. (1995). Production of β-fructofuranosidase by *Aspergillus japonicus* in batch and fed-batch cultures. *Biotechnology Letters, 17*, 1291–1294.

Chen, W.-C., & Liu, C.-H. (1996). Production of β-fructofuranosidase by *Aspergillus japonicus*. *Enzyme and Microbial Technology, 18*, 153–160.

Chen, H.-L., Lu, Y.-H., Lin, J., & Ko, L.-Y. (2000). Effects of fructooligosaccharide on bowel function and indicators of nutritional status in constipated elderly men. *Nutrition Research, 20*, 1725–1733.

Chiang, C. J., Lee, W. C., Sheu, D. C., & Duan, K. J. (1997). Immobilization of β-fructofuranosidases from *Aspergillus* on methacrylamide-based polymeric beads for production of fructooligosaccharides. *Biotechnology Progress, 13*, 577–582.

Clydesdale, F. (2004). Functional foods: opportunities & challenges. *Food Technology, 58*, 35–40.

Crittenden, R. G., & Playne, M. J. (1996). Production, properties and applications of food grade oligosaccharides. *Trends in Food Science & Technology, 7*, 353–361.

Crittenden, R. G., & Playne, M. J. (2002). Purification of food-grade oligosaccharides using immobilised cells of *Zymomonas mobilis*. *Applied Microbiology and Biotechnology, 58*, 297–302.

De Preter, V., Hamer, H. M., Windey, K., & Verbeke, K. (2011). The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health? *Molecular Nutrition & Food Research, 55*, 46–57.

Delgado, G. T. C., Tamashiro, W. M. S. C., Junior, M. R. M., Moreno, Y. M. F., & Pastore, G. M. (2011). The putative effects of prebiotics as immunomodulatory agents. *Food Research International, 44*, 3167–3173.

Delzenne, N. M., & Kok, N. (2001). Effects of fructans-type prebiotics on lipid metabolism. *The American Journal of Clinical Nutrition, 73*(Suppl), 456S–458S.

Delzenne, N. M., Daubioul, C., Neyrinck, A., Lasa, M., & Taper, H. S. (2002). Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *British Journal of Nutrition, 87*(Suppl), S255–S259.

Dhake, A. B., & Patil, M. B. (2007). Effect of substrate feeding on production of fructosyltransferase by *Penicillium purpurogenum*. *Brazilian Journal of Microbiology, 38*, 194–199.

Dominguez, A., Nobre, C., Rodrigues, L. R., Peres, A. M., Torres, D., Rocha, I., et al. (2012). New improved method for fructooligosaccharides production by *Aureobasidium pullulans*. *Carbohydrate Polymers, 89*, 1174–1179.

Druce, M. R., Small, C. J., & Bloom, S. R. (2004). Minireview: gut peptides regulating satiety. *Endocrinology, 145*, 2660–2665.

Fiordaliso, M., Kok, N., Desager, J. P., Goethals, F., Deboyser, D., Roberfroid, M., et al. (1995). Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids, 30*, 163–167.

Fujishima, M., Sakai, H., Ueno, K., Takahashi, N., Onodera, S., Benkeblia, N., et al. (2005). Purification and characterization of a fructosyltransferase from onion bulbs and its key role in the synthesis of fructo-oligosaccharides in vivo. *New Phytologist, 165*, 513–524.

Ganaie, M. A., Gupta, U. S., & Kango, N. (2013). Screening of biocatalysts for transformation of sucrose to fructooligosaccharides. *Journal of Molecular Catalysis B: Enzymatic, 97*, 12–17.

Ghazi, I., De Segura, A. G., Fernández-Arrojo, L., Alcalde, M., Yates, M., Rojas-Cervantes, M. L., et al. (2005). Immobilisation of fructosyltransferase from *Aspergillus aculeatus* on epoxy-activated Sepabeads EC for the synthesis of fructo-oligosaccharides. *Journal of Molecular Catalysis B: Enzymatic, 35*, 19–27.

Ghazi, I., Fernández-Arrojo, L., Garcia-Arellano, H., Ferrer, M., Ballesteros, A., & Plou, F. J. (2007). Purification and kinetic characterization of a fructosyltransferase from *Aspergillus aculeatus*. *Journal of Biotechnology, 128*, 204–211.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition, 125*, 1401–1412.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews, 17*, 259–275.

Glore, S. R., Van Treeck, D., Knehans, A. W., & Guild, M. (1994). Soluble fiber and serum lipids: a literature review. *Journal of the American Dietetic Association, 94*, 425–436.

Gomes, A. J. P. (2009). Optimização da Produção de Frutooligossacáridos por Aspergillus. Unpublished master's thesis. University of Minho, Braga, Portugal.

Goulas, A., Tzortzis, G., & Gibson, G. R. (2007). Development of a process for the production and purification of α- and β-galactooligosaccharides from *Bifobacterium bifidum* NCIMB 41171. *International Dairy Journal, 17*, 648–656.

Gudiel-Urbano, M., & Goñi, I. (2002). Effect of fructooligosaccharides on nutritional parameters and mineral bioavailability in rats. *Journal of the Science of Food and Agriculture, 82*, 913–917.

Hayashi, S., Nonoguchi, M., Takasaki, Y., Ueno, H., & Imada, K. (1992). Purification and properties of β-fructofuranosidase from *Aureobasidium* sp. ATCC 20524. *Journal of Industrial Microbiology, 7*, 251–256.

Hernández, O., Ruiz-Matute, A. I., Olano, A., Moreno, F. J., & Sanz, M. L. (2009). Comparison of fractionation techniques to obtain prebiotic galactooligosaccharides. *International Dairy Journal, 19*, 531–536.

Hölker, U., Höfer, M., & Lenz, J. (2004). Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. *Applied Microbiology and Biotechnology, 64*, 175–186.

Hosono, A., Ozawa, A., Kato, R., Ohnishi, Y., Nakanishi, Y., Kimura, T., et al. (2003). Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer's patch cells. *Bioscience, Biotechnology, and Biochemistry, 67*, 758–764.

Howlett J (Ed.) (2008) Functional foods—from science to health and claims. ILSI Europe—Concise Monograph Series, Brussels, Belgium.

Jackson, K. G., Taylor, G. R. L., Clohessy, A. M., & Williams, C. M. (1999). The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. *British Journal of Nutrition, 82*, 23–30.

Jedrzejczak-Krzepkowska, M., Tkaczuk, K. L., & Bielecki, S. (2011). Biosynthesis, purification and characterization of β-fructofuranosidase from *Bifidobacterium longum* KN29.1. *Process Biochemistry, 46*, 1963–1972.

Kim, Y. S., Tsa, O. D., Morita, A., & Bella, A. (1982). Effect of sodium butyrate and three human colorectal adenocarcinoma cell lines in culture. *Falk Symposium, 31*, 317–323.

Koops, A. J., & Jonker, H. H. (1994). Purification and characterization of the enzymes of fructan biosynthesis in tubers of *Helianthus tuberosus* "Columbia": I.Fructan: fructan fructosyl transferase. *Journal of Experimental Botany, 45*, 1623–1631.

Korzenik, J. R., & Podolsky, D. K. (2006). Evolving knowledge and therapy of inflammatory bowel disease. *Nature Reviews Drug Discovery, 5*, 197–209.

Kunz, C., & Rudloff, S. (2006). Health promoting aspects of milk oligosaccharides. *International Dairy Journal, 16*, 1341–1346.

L'Hocine, L., Wang, Z., Jiang, B., & Xu, S. (2000). Purification and partial characterization of fructosyltransferase and invertase from *Aspergillus niger* AS0023. *Journal of Biotechnology, 81*, 73–84.

Lateef, A., Oloke, J. K., & Prapulla, S. G. (2007). Purification and partial characterization of intracellular fructosyltransferase from a novel strain of *Aureobasidium pullulans*. *Turkish Journal of Biology, 31*, 147–154.

Lee, W.-C., Chiang, C.-J., & Tsai, P.-Y. (1999). Kinetic modeling of fructo-oligosaccharide production catalyzed by immobilized β-fructofuranosidase. *Industrial & Engineering Chemistry Research, 38*, 2564–2570.

Levrat, M. A., Favier, M. L., Moundras, C., Rémésy, C., Demigné, C., & Morand, C. (1994). Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats. *Journal of Nutrition, 124*, 531–538.

Lim, C. C., Ferguson, L. R., & Tannock, G. W. (2005a). Dietary fibres as "prebiotics": implications for colorectal cancer. *Molecular Nutrition & Food Research, 49*, 609–619.

Lim, J. S., Park, M. C., Lee, J. H., Park, S. W., & Kim, S. W. (2005b). Optimization of culture medium and conditions for Neo-fructooligosaccharides production by *Penicillium citrinum*. *European Food Research and Technology, 221*, 639–644.

Lin, T.-J., & Lee, Y.-C. (2008). High-content fructooligosaccharides production using two immobilized microorganisms in an internal-loop airlift bioreactor. *Journal of the Chinese Institute of Chemical Engineers, 39*, 211–217.

Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology, 104*, 305–344.

Madlová, A., Antošová, M., Baráthová, M., Polakovič, M., Stefuca, V., & Báles, V. (1999). Screening of microorganisms for transfructosylating activity and optimization of biotransformation of sucrose to fructooligosaccharides. *Chemical Papers, 53*, 366–369.

Madlová, A., Antošová, M., Polakovič, M., & Báles, V. (2000). Thermal stability of fructosyltransferase from *Aureobasidium pullulans*. *Chemical Papers, 54*, 339–344.

Maiorano, A. E., Piccoli, R. M., Silva, E. S., & Rodrigues, M. F. A. (2008). Microbial production of fructosyltransferases for synthesis of pre-biotics. *Biotechnology Letters, 30*, 1867–1877.

Manning, T. S., & Gibson, G. R. (2004). Prebiotics. *Best Practice & Research Clinical Gastroenterology, 18*, 287–298.

Menrad, K. (2003). Market and marketing of functional food in Europe. *Journal of Food Engineering, 56*, 181–188.

Metz, B., & Kossen, N. W. F. (1977). Biotechnology review: the growth of the molds in the form of pellets, a literature. *Biotechnology and Bioengineering, 19*, 781–799.

Mishra, S., & Mishra, H. N. (2013). Effect of synbiotic interaction of fructooligosaccharide and probiotics on the acidification profile, textural and rheological characteristics of fermented soy milk. *Food and Bioprocess Technology, 6*, 3166–3176.

Munjal, U., Glei, M., Pool-Zobel, B. L., & Scharlau, D. (2009). Fermentation products of inulin-type fructans reduce proliferation and induce apoptosis in human colon tumour cells of different stages of carcinogenesis. *British Journal of Nutrition, 27*, 1–9.

Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: a review. *Carbohydrate Polymers, 68*, 587–597.

Mussatto, S. I., & Teixeira, J. A. (2010). Increase in the fructo-oligosaccharides yield and productivity by solid-state fermentation with *Aspergillus japonicus* using agro-industrial residues as support and nutrient source. *Biochemical Engineering Journal, 53*, 154–157.

Mussatto, S. I., Aguilar, C. N., Rodrigues, L. R., & Teixeira, J. A. (2009). Colonization of *Aspergillus japonicus* on synthetic materials and application to the production of fructooligosaccharides. *Carbohydrate Research, 344*, 795–800.

Mussatto, S. I., Prata, M. B., Rodrigues, L. R., & Teixeira, J. A. (2012). Production of fructooligosaccharides and β-fructofuranosidase by batch and repeated batch fermentation with immobilized cells of *Penicillium expansum*. *European Food Research and Technology, 235*, 13–22.

Nemukula, A., Mutanda, T., Wilhelmi, B. S., & Whiteley, C. G. (2009). Response surface methodology: synthesis of short chain fructooligosaccharides with a fructosyltransferase from *Aspergillus aculeatus*. *Bioresource Technology, 100*, 2040–2045.

Nguyen, Q. D., Mattes, F., Hoschke, Á., Rezessy-Szabó, J., & Bhat, M. K. (1999). Production, purification and identification of fructooligosaccharides produced by β-fructofuranosidase from *Aspergillus niger* IMI 303386. *Biotechnology Letters, 21*, 183–186.

Nguyen, Q. D., Rezessy-Szabó, J. M., Bhat, M. K., & Hoschke, Á. (2005). Purification and some properties of β-fructofuranosidase from *Aspergillus niger* IMI303386. *Process Biochemistry, 40*, 2461–2466.

Nilsson, A. C., Ostman, E. M., Holst, J. J., & Bjorck, I. M. E. (2008). Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. *The Journal of Nutrition, 138*, 732–739.

Nishizawa, K., Nakajima, M., & Nabetani, H. (2001). Kinetic study on transfructosylation by β-fructofuranosidase from *Aspergillus niger* ATCC 20611 and availability of a membrane reactor for fructooligosaccharide production. *Food Science and Technology Research, 7*, 39–44.

Nobre, C., Teixeira, J.A., & Rodrigues, L.R. (2013). New trends and technological challenges in the industrial production and purification of fructo-oligosaccharides. *Critical Reviews in Food Science and Nutrition*. doi:10.1080/10408398.2012.697082.

Pandey, A. (2003). Solid-state fermentation. *Biochemical Engineering Journal, 13*, 81–84.

Park, J.-P., Oh, T.-K., & Yun, J.-W. (2001). Purification and characterization of a novel transfructosylating enzyme from *Bacillus macerans* EG-6. *Process Biochemistry, 37*, 471–476.

Pierre, F., Perrin, P., Champ, M., Bornet, F., Meflah, K., & Menanteau, J. (1997). Short-chain fructo-oligosaccharides reduce the occurrence of colon tumors and develop gut-associated lymphoid tissue in Min mice. *Cancer Research, 57*, 225–228.

Piñeiro, M., Asp, N. G., Reid, G., Macfarlane, S., Morelli, L., Brunser, O., et al. (2008). FAO Technical meeting on prebiotics. *Journal of Clinical Gastroenterology, 42*(Suppl 3), S156–S159.

Playne, M. J., & Crittenden, R. G. (2004). Prebiotics from lactose, sucrose, starch, and plant polysaccharides. In J.-R. Neeser & J. B. German (Eds.), *Bioprocesses and biotechnology for functional foods and nutraceuticals* (pp. 99–135). New York: Marcel Dekker.

Prata, M. B., Mussatto, S. I., Rodrigues, L. R., & Teixeira, J. A. (2010). Fructooligosaccharide production by *Penicillium expansum*. *Biotechnology Letters, 32*, 837–840.

Qiang, X., YongLie, C., & QianBing, W. (2009). Health benefit application of functional oligosaccharides. *Carbohydrate Polymers, 77*, 435–441.

Raschka, L., & Daniel, H. (2005). Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. *Bone, 37*, 728–735.

Risso, F. V. A., Mazutti, M. A., Treichel, H., Costa, F., Maugeri, F., & Rodrigues, M. I. (2012). Comparison between systems for synthesis of fructooligosaccharides from sucrose using free inulinase from *Kluyveromyces marxianus* NRRL Y-7571. *Food and Bioprocess Technology, 5*, 331–337.

Roberfroid, M. (1993). Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition, 33*, 103–148.

Roberfroid, M. B. (2000a). Defining functional foods. In G. Gibson & C. Williams (Eds.), *Functional foods: trends and prospects* (pp. 9–25). Cambridge: Woodhead Publishing.

Roberfroid, M. B. (2000b). Prebiotics and probiotics: are they functional foods? *The American Journal of Clinical Nutrition, 71*(Suppl), 1682S–1687S.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., et al. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition, 104*(Suppl), S1–S63.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT—Food Science and Technology, 50*, 1–16.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2004a). Production of fructo-oligosaccharides by fructosyl transferase from *Aspergillus oryzae* CFR 202 and *Aureobasidium pullulans* CFR 77. *Process Biochemistry, 39*, 753–758.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2004b). Production of fructosyl transferase by *Aspergillus oryzae* CFR 202 in solid-state fermentation using agricultural by-products. *Applied Microbiology and Biotechnology, 65*, 530–537.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2005a). Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of *Aspergillus oryzae* CFR 202. *Process Biochemistry, 40*, 1085–1088.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2005b). Maximization of fructooligosaccharide production by two stage continuous process and its scale up. *Journal of Food Engineering, 68*, 57–64.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2005c). Recent trends in the microbial production, analysis and application of fructooligosaccharides. *Trends in Food Science & Technology, 16*, 442–457.

Sanz, M. L., Polemis, N., Morales, V., Corzo, N., Drakoularakou, A., Gibson, G. R., et al. (2005). In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *Journal of Agricultural and Food Chemistry, 53*, 2914–2921.

Scheppach, W., & Weiler, F. (2004). The butyrate story: old wine in new bottles? *Current Opinion in Clinical Nutrition Metabolic Care, 7*, 563–567.

Schley, P. D., & Field, J. C. (2002). The immune-enhancing effects of dietary fibres and prebiotics. *British Journal of Nutrition, 87*, 221–230.

Sheu, D. C., Lio, P. J., Chen, S. T., Lin, C. T., & Duan, K. J. (2001). Production of fructooligosaccharides in high yield using a mixed enzyme system of β-fructofuranosidase and glucose oxidase. *Biotechnology Letters, 23*, 1499–1503.

Sheu, D.-C., Duan, K.-J., Cheng, C.-Y., Bi, J.-L., & Chen, J.-Y. (2002). Continuous production of high-content fructooligosaccharides by a complex cell system. *Biotechnology Progress, 18*, 1282–1286.

Shimizu, M., & Hachimura, S. (2011). Gut as a target for functional food. *Trends in Food Science & Technology, 22*, 646–650.

Shin, H. T., Baig, S. Y., Lee, S. W., Suh, D. S., Kwon, S. T., Lim, Y. B., & Lee, J. H. (2004a). Production of fructo-oligosaccharides from molasses by *Aureobasidium pullulans* cells. *Bioresource Technology, 93*, 59–62.

Shin, H. T., Park, K. M., Kang, K. H., Oh, D. J., Lee, S. W., Baig, S. Y., et al. (2004b). Novel method for cell immobilization and its application for production of oligosaccharides from sucrose. *Letters in Applied Microbiology, 38*, 176–179.

Shiomi, N. (1982). Purification and characterisation of 1F-fructosyltransferase from the roots of asparagus (*Asparagus officinalis* L.). *Carbohydrate Research, 99*, 157–169.

Simmering, R., & Blaut, M. (2001). Pro- and prebiotics—the tasty guardian angels? *Applied Microbiology and Biotechnology, 55*, 19–28.

Singh, R. S., & Singh, R. P. (2010). Production of fructooligosaccharides from inulin by endoinulinases and their prebiotic potential. *Food Technology and Biotechnology, 48*, 435–450.

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—a review. *Appetite, 51*, 456–467.

Straathof, A. J. J., Kieboom, A. P. G., & Bekkum, H. (1986). Invertase-catalysed fructosyl transfer in concentrated solutions of sucrose. *Carbohydrate Research, 146*, 154–159.

Szajewska, H. (2010). Probiotics and prebiotics in preterm infants: where are we? Where are we going? *Early Human Development, 86*, S81–S86.

Tokunaga, T., Oku, T., & Hosoya, N. (1986). Influence of chronic intake of new sweetener fructooligosaccharide (Neosugar) on growth and gastrointestinal function of the rat. *Journal of Nutritional Science and Vitaminology (Tokyo), 32*, 111–121.

van Hijum, S. A. F. T., van Geel-Schutten, G. H., Rahaoui, H., van der Maarel, M. J. E. C., & Dijkhuizen, L. (2002). Characterization of a novel fructosyltransferase from *Lactobacillus reuteri* that synthesizes high-molecular-weight inulin and inulin oligosaccharides. *Applied and Environmental Microbiology, 68*, 4390–4398.

Vandáková, M., Platková, Z., Antošová, M., Báleš, V., & Polakovič, M. (2004). Optimization of cultivation conditions for production of fructosyltransferase by *Aureobasidium pullulans*. *Chemical Papers, 58*, 15–22.

Voragen, A. G. J. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science & Technology, 9*, 328–335.

Wallis, G. L. F., Hemming, F. W., & Peberdy, J. F. (1997). Secretion of two β-fructofuranosidases by *Aspergillus niger* growing in sucrose. *Archives of Biochemistry and Biophysics, 345*, 214–222.

Wang, L.-M., & Zhou, H.-M. (2006). Isolation and identification of a novel *A. japonicus* JN19 producing β-fructofuranosidase and characterization of the enzyme. *Journal of Food Biochemistry, 30*, 641–658.

Yamamoto, Y., Takahashi, Y., Kawano, M., Iizuka, M., Matsumoto, T., Saeki, S., et al. (1999). In vitro digestibility and fermentability of levan and its hypocholesterolemic effects in rats. *The Journal of Nutritional Biochemistry, 10*, 13–18.

Yoon, S. H., Mukerjea, R., & Robyt, J. F. (2003). Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation. *Carbohydrate Research, 338*, 1127–1132.

Yoshikawa, J., Amachi, S., Shinoyama, H., & Fujii, T. (2006). Multiple β-fructofuranosidases by *Aureobasidium pullulans* DSM2404 and their roles in fructooligosaccharide production. *FEMS Microbiology Letters, 265*, 159–163.

Yoshikawa, J., Amachi, S., Shinoyama, H., & Fujii, T. (2007). Purification and some properties of β-fructofuranosidase I formed by *Aureobasidium pullulans* DSM 2404. *Journal of Bioscience and Bioengineering, 103*, 491–493.

Yoshikawa, J., Amachi, S., Shinoyama, H., & Fujii, T. (2008). Production of fructooligosaccharides by crude enzyme preparations of β-fructofuranosidase from *Aureobasidium pullulans*. *Biotechnology Letters, 30*, 535–539.

Yu Wang, M. A., Tao Zeng, M. D., Shu-e Wang, M. A., Wei Wang, M. A., Qian Wang, M. A., & Hong-Xia Yu, M. A. (2010). Fructooligosaccharides enhance the mineral absorption and counteract the adverse effects of phytic acid in mice. *Nutrition, 26*, 305–311.

Yun, J. W. (1996). Fructooligosaccharides—occurrence, preparation, and application. *Enzyme and Microbial Technology, 19*, 107–117.

Yun, J. W., & Song, S. K. (1993). The production of high-content fructooligosaccharides from sucrose by the mixed-enzyme system of fructosyltransferase and glucose oxidase. *Biotechnology Letters, 15*, 573–576.

Yun, J. W., Kim, D. H., & Song, S. K. (1997). Enhanced production of fructosyltransferase and glucosyltransferase by substrate-feeding cultures of *Aureobasidium pullulans*. *Journal of Fermentation and Bioengineering, 84*, 261–263.

Ziemer, C. J., & Gibson, G. R. (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal, 8*, 473–479.

Zuccaro, A., Götze, S., Kneip, S., Dersch, P., & Seibel, J. (2008). Tailor-made fructooligosaccharides by a combination of substrate and genetic engineering. *ChemBioChem, 9*, 143–149.