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Fructosyltransferase Sources, Production, and Applications for Prebiotics Production

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Abstract

Fructooligosaccharides (FOS) are considered prebiotic compounds and are found in different vegetables and fruits but at low concentrations. FOS are produced by enzymatic transformation of sucrose using fructosyltransferase (FTase). Development of new production methods and search for FTase with high activity and stability for FOS production Is an actual research topic. In this article is discussed the most recent advances on FTase and its applications. Different microorganisms have been tested under various fermentation systems in order to identify and characterize new genes codifying for FTase. Some of these genes have been isolated from bacteria, fungi, and plants, with a wide range of percentages of identity but retaining the eight highly conserved motifs of the hydrolase family 32 glycoside. Therefore, this article presents an overview of the most recent advances on FTase and its applications.

Keywords: Enzyme production, Fructooliogosacarides, Fructosyltransferase, 1-kestose, 1-nystose, 1-β-fructofuranosyl nystose

1. Introduction

At the present time, there is a growing consumer demand for healthier and calorie-controlled foods. For this reason, food industry has developed different alternatives for sweeteners, and

among them is the fructooligosaccharides (FOS). Use of these compounds offers multiple health advantages. FOS are fructose oligomers with a terminal glucosyl unit and a general formula of GFn; where typical values of n are 2–4 and are composed of sugars units such as 1-kestose, 1-nystose, and 1- β -fructofuranosyl nystose, which can be found in different fruits and vegetables, but in very low concentrations to exert a beneficial effect on health. In addition, its production is limited by seasonal conditions [1]. FOS cannot be hydrolyzed by the gastrointestinal enzymes and are recognized as prebiotic which selectively stimulate growth and/or activity of bifidobacteria and lactobacilli, microorganisms that promote benefits to human health [2, 3].

FOS can be produced by the action of different types of enzymes with transfructosylation activity (i.e., fructosyltransferase—FTase [EC 2.4.1.9] and/or β-fructofuranosidase—FFases [EC 3.2.1.26]). These enzymes are obtained from different plants and microorganisms [4]. The reaction mechanism of FTase depends on enzyme source [5]. Most of these enzymes have been isolated from different fungal strains such as: *Aureobasidium* spp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. [6]. However, different FTases from bacterial (*Bacillus macerans*, *Lactobacillus reuteri*, and *Zymomonas mobilis*) species have been also reported [7].

2. FTase: an overview

FOS can be synthesized in nature by the catalytic action of enzymes with transfructosylating activity. They are classified as 1^F-FTases (E.C. 2.4.1.9, E.C. 2.4.1. 99, and E.C. 2.4.1.100), or β - FFases (, E.C. 3.2.1.26). FTase catalyzes the transfer of a fructosyl group to a molecule of sucrose or a FOS when a FOS with a chain longer by one fructosyl unit is formed [8]. This enzyme also mediates polymerization reactions, where degree of polymerization (DP) decreases to the maximum by transferring fructosyl units from higher molecular mass fructans [9]. The reaction mechanism of the FTases depends on the enzyme source. In plants and some microorganisms, a series of enzymes act together, whereas a single enzyme works in most of the microorganisms [10].

The FTase that converts sucrose to the shortest β (2–1) linked fructan 1-kestose is called sucrose: sucrose 1-FTase (1-SST) [11]. It is reported that, FTase differs in molecular weight and properties depending on its origin [12]. Properties of FTases can change according to the microorganism and culture medium composition, especially the carbon source, which can play a role as an inductor [1]. FTase can be produced intra- and/or extracellular by different microorganisms, including bacteria and fungi. Despite the large number of microbial FTase producers, only some of them have the potential for industrial application and have been used in several studies about FOS production [1]. The transfructosylating activity is responsible for FOS production from sucrose, although quantitative differences exist because of the microbial strains [13].

FTase has been produced using both solid and liquid fermentation, and FOS obtained by these fermentations have been reported. Factors affecting FOS by fermentation were mentioned, such as temperature, pH, and substrate concentration [14, 15]. FTase has a temperature optimum between 50 and 60°C, and the optimal pH is between 4.5 and 6.5 [1, 8].

2.1. Mechanism of action

The reaction mechanism depends on the enzyme source and purity. The accepted mechanism is a type disproportionate reaction where FTase catalyzes the transfer of a fructosyl moiety of a sucrose or fructooligosacharide donor to another sucrose or FOS acceptor to provide a superior FOS [1]. The reaction mechanism has been expressed as follows:

$$GFn + GF_n GF_{n+1} GF_{n-1} n = 1 - 3$$

where GF is sucrose or FOS n is the number of fructosyl units.

3. Microbial and plant FTases

Several fungal strains, especially those from *Aspergillus* genus, are known to produce extracellular or intracellular FTase. The microbial FTase (Ftase; E.C. 2.4.1.9) catalyses formation of FOS from sucrose; FTases obtained from microorganisms are single enzymes with both transferase and hydrolase activities [12]. Some of the fungi reported as FTase producers are the following: *Aspergillus niger* ATCC 20611 [13], *A. niger* strain AN 166 [16], *Aspergillus foetidus* [17], *Aspergillus oryzae* CFR 202 [15, 18–20], *Aureobasidium pullulans* CFR 77 [18], *A. oryzae* KB [21], and *Aspergillus awamori* GHRTS [22]. Some of these fungal strains have the capacity to produce two types of FTases (Table 1). The enzymatic activity is different because it depends on carbon source and type of microorganism. The FFase with a high transfructosylating activity has been studied extensively in *A. niger* because this fungal specie is used for industrial production of FOS. *A. niger* AS0023 produces two types of FFases, and one of the enzymes has high transfructosylating activity [24].

Source	FTase Activity	Temperature	Reference
Aspergillus niger YZ59	Recombinant Ftase 1020 U/ml	55°C	[23]
Aspergillus awamori GHRTS	6120 U/gds	30°C	[22]
Aspergillus oryzae CFR 202	16.5 U/ml/min	30°C	[19]
Aspergillus niger	107.87 U/mL	30°C	[16]

Table 1. Microbial FTases produced by filamentous fungi, its activity, and fermentation temperature of the maximum enzyme activity reached.

Bacterial strains have been reported to produce different inulinases, but reports on enzymes able to produce FOS are scarce from bacterial strains. Someone bacteria mentioned to produce these enzymes are the following: *B. macerans, L. reuteri,* and *Z. mobilis* [7].

The FTases obtained from plants have other amino acid composition that is different from microbial FTases such as sucrose: sucrose 1-FTase (1-SST) and fructan: fructan 1-FTase (1-FFT).

Plants such as *Cichorium intybus* and *Helianthus tuberosus* produce high levels of FTases such as 1-SST, (EC. 2.4.1.99) [10]. In 1995, Ftase were isolated and purified from barley (*Hordeum vulgare*) [25].

4. Structure of FTase

According to the Protein Data Bank (PDB) of Research Collaborators for Structural Bioinformatics (RCSB) data base, the crystal dimensional structure of FTase from *Aspergillus japonicus* comprises 632 residues that fold into two domains, with a N-terminal five-blade-propeller (residues 21–468), and a C-terminal sandwich domain (480–653), which are linked by a 9-residue short-helix (469–479) [26].

5. FTases properties

5.1. Factors affecting FTase activity

Fructosyltransferase (FTase) participates on FOS/fructan production by catalyzing the transfer of a fructose unit from one sucrose/fructan to another [26]. This enzyme has been included in the glycoside hydrolase family 32 (GH32) and has been isolated from different sources, and the optimal conditions for the enzyme activity have been reported (Table 2). The optimal temperature reported for FTase enzyme activity ranges from 52 to 65°C, while the optimal pH varies widely from 4.5 to 8.0 [27, 28]. There are different reports mentioned about the chemical reagents and amino acids that positively affect FTase activity [29, 30]. On the other hand, there is a controversy in the use of detergents—some authors mention that these compounds enhance FTase activity [29], and in contrast there are others who mention that these compounds negatively affect FTase activity [27].

Source	Temperature	pH	Positive effect	Observation	References
Aspergillus sp.	52 °C	4.5	FeSO4, Fe2+, Fe2+ Ca2+	Intra- and extracellular	[27]
Marine *Aspergillus niger*	65°C	8.0		Intracellular	[28]
Aspergillus aculeatus	60°C	5.0–7.0	Dithiothreitol, 2-mercaptoethanol, sodium dodecylsulphate, Tween 80		[29]
Penicillium purpurogenum	55 °C	5.5	Leucine induced slightly extracellular production	Intra- and extracellular	[30, 31]

Table 2. Optimal conditions for FTase activity from different microbial sources.

5.2. Carbon and nitrogen sources

Different reports mentioned that the preferred carbon source to produce FTase is sucrose. Patil and Butle [31] indicated that *Syncephalastrum recemosum Cohn* preferred sucrose to produce FTase. Similarly, Dhake and Patil [30] employing *Penicillium purpurogenum* found that the best carbon source for FTase production was sucrose. The complete hydrolysis of this carbohydrate was reported by Kumar *et al.* [28] who used a marine *A. niger* strain to degrade sucrose, and the product consisted entirely of D-fructose, although different products from sucrose hydrolysis have been reported depending on the FTase enzyme origin. A recombinant FTase from timothy (*Phleum pratense* L.) and expressed in *Pichia pastoris* produced linear β (2, 6)-linked levans from sucrose [32]. While recombinant FTase from *L. reuteri* for sucrose hydrolysis was used, large amounts of FOS with _(231)-linked fructosyl units, plus a high-molecular-weight fructan polymer (>107) with _-(231) linkages (an inulin) were found [33]. Amount of sucrose in the fermentation reactor may alter the final product. Ghazi *et al.* [29] found that FTase from *A. aculeatus* at elevated sucrose concentrations showed a high transferase/hydrolase ratio. The $k$cat and Km values for transfructosylating and hydrolytic activities vary. Ghazi *et al.* [29] modified sucrose using a microbial FTase and found transfructosylating activity of $1.62 \pm 0.09 \times 104\ s - 1$ for $k$cat and $0.53 \pm 0.05\ M$ for Km, whereas for hydrolytic activity, the $k$cat and Km values were $775 \pm 25\ s - 1$ and $27 \pm 3\ mM$, respectively. On the other hand, the best nitrogen source to produce FTase by *S. recemosum Cohn* is ammonium nitrate [31].

5.3. FTase biochemical properties

Biochemical properties of FTase may change depending on its origin. Ghazi *et al.* [29] reported a dimeric glycoprotein with 20% of carbohydrate content and a molecular mass of around 135 kDa from *A. aculeatus.* In contrast, a FTase from *A. foetidus* was found as extensively glycosylated, with a probable active form of a dimer of identical subunits and an apparent mass of 180 kDa [34]. On the other hand, a FTase which catalyze formation and extension of P-2, 6-linked fructans in barley (*H. vulgare* L.) was mentioned to occur in two closely similar isoforms having both of them two subunits with masses of 49 and 23 kDa [25].

Chuankhayan *et al.* [26] sequenced a recombinant FTase from *Aspergillus japonica.* In this case, they found that this enzyme comprises two domains with an N-terminal catalytic domain containing a five-blade-propeller-fold linked to a C-terminal-sandwich domain. In addition, the same authors reported four substrate-binding subsites (1–3) in the catalytic pocket with shapes and characters distinct from those of clan GH-J enzymes; in this step, they used different FTase mutants. The residue Asp-60 was proposed for nucleophile, Asp-191 for transition-state stabilizer, and Glu-292 for general acid/base catalyst, which govern the binding of the terminal fructose at the 1 subsite and the catalytic reaction. Although to define the 1 subsite for FTase preference of fructosyl and glucosyl, moieties are needed, the residues Ile-143, Arg-190, Glu-292, Glu-318, and His-332 combine the hydrophobic Phe-118 and Tyr-369. On the one hand, to define the _2 subsite for raffinose, Ile-143 and Gln-327 are required, on the other hand, Tyr-404 and Glu-405 are needed to define the 2 and 3 subsites for inulin-type substrates with higher structural flexibilities.

6. FTases gene organization

Genome sequence of different microorganisms and vegetables has allowed identification of some enzymes, development of new products, improvement of strains, and increase of process efficiency. There are some reports of isolation and cloning of the FTase gene. The genes coding for FTase have been isolated from bacteria, fungi, and plants, with a wide range of percentages of identity but retaining the eight highly conserved motifs of hydrolyses family 32 glycoside [35]. Fungal FTase genes have been isolated mainly from *Aspergillus* strains (Table 3), although there are reports about Ftase genes from other fungal genera [37].

Source	Enzyme	Bp	Intron	Size	Molecular weight	Amino acids	References
Aspergillus oryzae N74	FTase	1620	172–224		57 kDa	525	[35]
Aspergillus foetidus	FTase			1.6 kb	59.1 kDa	537	[34]
Aspergillus niger	FTase			1.9 kb	76 kDa		[36]

Table 3. Characteristics of different FTase genes and its enzyme.

The gene that encodes *A. oryzae* N74 FTase accounts for a 525 amino acids protein of 57 kDa, with a signal peptide of 17 amino acids. Alignment of genomic and mRNA sequence from *A. oryzae* N74 strain showed the presence of a 52 bp intron located between 172 and 224 bp [35]. Other authors mentioned that the FTasa was partially purified using a three-step procedure involving anion exchange chromatography, hydrophobic interaction, and ultrafiltration. The *A. sydowi* IAM 2544 FTase gene was expressed in conidia; the gene encodes a protein with a calculated molecular mass of 75 kDa and comprises 682 amino acids [38]. Genes of *Aspergillus* FTases are more homogeneous with a size ranging from 1.6 kb to 2 kb and coding for enzymes about 500–600 amino acids long.

7. Fructooligosaccharides

FOS is a common name for fructose oligomers and corresponds to complex carbohydrates which are nondigestible oligosaccharide food ingredients and are fermentable by the gut microbiota. For this reason, they can be classified as prebiotics, and its commercial production has increased in response to a growing consumer demand for the so-called "health foods" [16, 39]. FOS are mainly composed of 1-kestose (GF2), 1-nystose (GF3), and 1- β-fructofuranosyl nystose (GF4), in which 1–3 fructosyl units (F) are bound at the β (2–1) status of sucrose molecule (GF) (Figure 1) [4].
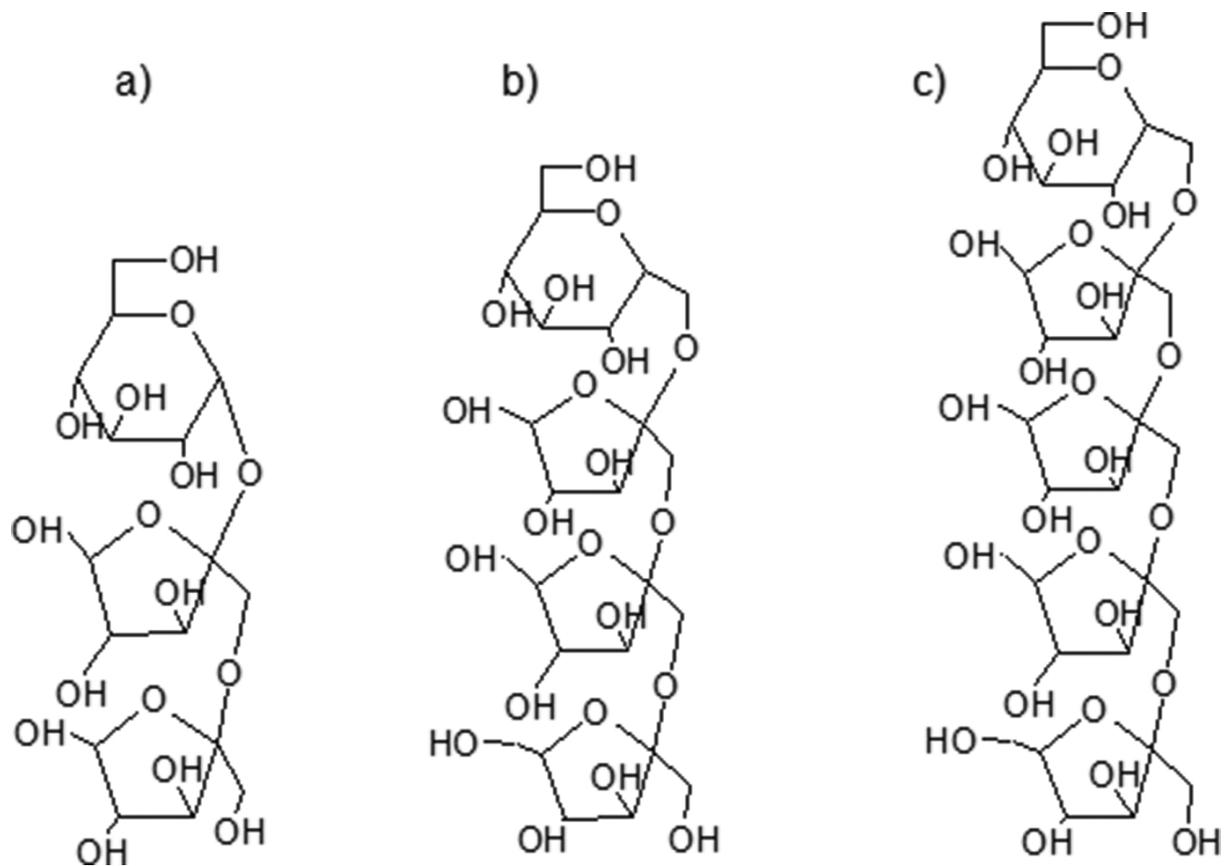
Figure 1. Chemical structures of the most common FOS, where (a) 1-kestose, (b) 1- nystose, and (c) 1-β fructofuranosyl nystose.

FOS can be found in several vegetal sources such as tomato, onion, barley, garlic, Jerusalem artichoke, banana, rye, honey, sugar beet, to name a few; however, FOS concentration in these sources is low, and mass production are limited by seasonal conditions [3, 4]. At the industrial level, FOS are mainly produced from the disaccharide sucrose by action of different microbial enzymes with transfructosylating activity such as FTase (EC 2.4.1.9) and/or β-fructofuranosi-dase (EC 3.2.1.26), [4]. Moreover, FOS compounds have received a generally recognized as safe status (GRAS) from the Food and Drug Administration (FDA) and has been consumed because of the several benefits of FOS to human health such as calorie-free and noncariogenic sweeteners, stimulate bifidobacteria growth, and activation of the immune system; have been claimed to contribute to the prevention of colon cancer and reduce the levels of serum cholesterol, phospholipids, and triglycerides; also promote calcium and magnesium absorption in animals and the human gut [14, 19, 22, 40, 41].

Dominguez *et al*. [7] reported that FOS have low sweetness intensity as they are only about one-third as sweet as sucrose, they supply small quantities of power, about 0–3 kcal/g sugar substitute. Other authors mentioned that FOS are about 0.4–0.6 times sweeter than sucrose and are considered as noncariogenic since no compounds are produced when polyglucanes are passing through the mouth [37]. These last properties are very useful in types of foods in which sucrose use is limited because of its high sweetness [7].

7.1. FOS production

Production of FOS has received particular attention in recent years, so there is necessity for the development of new enzymatic systems [42]. FOS represent one of the major classes of bifidogenic oligosaccharides in terms of production volume. Kestose and nystose are the main prebiotic compounds, which can be principally produced by hydrolysis of inulin or by transfructosylation of sucrose [24]. The enzymes that are potentially useful for high production of FOS were reported about three decades ago. Hidaka *et al.* [13] reported an *A. niger* enzyme with transfructosylating activity which reached a maximum FOS conversion of 55–60% (w/w) based on total sugars, and this enzyme was successively used for industrial production of FOS. Almost at the same time, McCleary *et al.* [43] investigated the other *A. niger* enzyme with transfructosylating activity, which could compete with other enzymes for industrial production of FOS because of its considerably high activity. Chien *et al.* [39] mentioned a FOS production process using *A. japonicus* enzymes, while Antosova *et al.* [44] reported a FOS production process using enzymes from *A. pullulans* CCY 27-1-1194.

8. Use of FTAse for FOS production

FOS have demonstrated important properties for improving human health, thus they have attracted an increased interest mainly as ingredients for food applications. They contribute to 10% of the natural sweeteners, and their demand has risen rapidly (about 15% per year) in the last 15 years [45]. Consequently, establishing sustainable and economically viable industrial process for the production of FOS with high yields and productivities is strongly desirable [46]. These can be manufactured by three methods: (1) extraction from inulin-rich plant materials, (2) by enzymatic synthesis from sucrose, and (3) by enzymatic degradation of inulin [45, 47]. Most of the FOS marketed as food ingredients/nutritional supplements are synthesized either from sucrose by the action of FTases [48, 49] or by enzymatic degradation of inulin [50, 51]. In this section, we will discuss the production of FOS through FTase.

Commercial production of FOS was first developed using enzymatic fructosyl transfer on sucrose by Hidaka *et al.* [52], and since then, β-fructofuranosidase has been isolated especially from fungi: *A. niger* [13, 53], *A. japonicus* [54, 55], *A. oryzae* [21, 56], *A. pullulans* [57, 58], and *Fusarium oxysporum* [59].

Nowadays, to reduce cost, enzyme immobilization techniques have been applied. Fungal β-fructofuranosidase has been covalently immobilized onto inorganic supports such as porous glass or porous silica [54, 60]. *Aspergillus* FTase was immobilized in methacrylamide-based polymeric beads and various linocellulosic materials to produce FOS from sucrose [55]. Ganaie *et al.* [49] evaluated immobilization of *Aspergillus flavus* FTase with sodium alginate and chitosan forming gel bead for continuous production of FOS, showing that recycling efficiency of alginate beads was more successful as compared to chitosan beads. In addition, formation of FOS by FTase entrapped alginate beads was higher than by chitosan beads in 36 h of enzyme-substrate reaction according to HPLC analysis (66.75 and 42.79%, respectively).

However, it has been observed that enzymes immobilized on a porous support decrease apparently its enzymatic activity because of diffusion resistance. Instead, the use of magnetic nanoparticles is proposed, which offers (a) a higher specific surface area that permits binding of a larger amount of enzyme, (b) relatively low mass transfer resistance and (c) selective separation from a reaction mixture by application of a magnetic field [61, 62]. Chen *et al.* [63] evaluated that β-fructofuranosidase from *A. japonicus* was immobilized in Fe_3O_4-CS nanoparticles, retaining up to 88% of its activity. The recovery of enzyme activity was inversely proportional to enzyme concentration. The main oligosaccharide products were 1-kestose and nystose. After 10 days, it was observed that the consumption of sucrose appear to have stopped because of the accumulation of glucose, which inhibited transfructosylating reactions [64]. The immobilized enzyme can easily be recovered by applying a magnetic field and reused it for FOS production.

Another alternative for FOS production is the use of solid-state fermentation (SSF). Most investigations on FOS production are based on submerged fermentation systems, but SSF is attractive because of low capital cost and low demand of water, generating less wastewater as a consequence. Besides, higher productivities and yields could be obtained at industrial scale [14]. Mussatto *et al.* [46] evaluated the economic and environmental impact of three different fermentation processes for FOS production: (1) submerged fermentation of sucrose solution by *A. japonicus* using free cells (FCF) or (2) using immobilized cells in corn cobs (ICF), and (3) SSF using coffee silver-skin as support material and nutrient source (SSF). In this study, an annual productivity goal of 200 t was established. Based on parameters such as productivity, product concentrations, yields, and thermo-physical data, SSF was the most attractive process with higher annual productivity of FOS (232.6 t) and purity (98.6%) against 148.9 t and 96.6% for FCF and 158.3 t and 98.4% for ICF. The SSF also produced greater amounts of the shorter chain FOS (GF2 and GF3), which have more prebiotic activity and stronger sweetness [65]. Although, the three processes are economically feasible, SSF has the highest potential to be implemented on an industrial scale not only because of productivity and purity but also because of the lowest payback time, wastewater generation, carbon footprint, and highest annual profit.

9. Properties of FOS

General structure of FOS can be depicted as GFn, where "*n*" is the number of fructosyl units (F) that are bound by β (2→1) position of sucrose with the last one attached to a terminal glucose (G) [51]. FOS produced from sucrose have lower DP (DP◎4) than those from inulin (DP > 9) [47]. This is relevant because DP plays an important role in the gut fermentation of FOS. The short-chain FOSs are fermented by the bacteria present in the proximal colon, while the long-chain FOS are fermented in distal colon [66]. Structurally, FOSs produced from sucrose are kestose (GF2), nystose (GF3), and 1-β-fructofuranosyl nystose (GF4).

FOSs contain several qualities that make its usage possible as an alternative sweetener in the food market. They are water soluble and one-third as sweet as sucrose [67]. However, their

viscosity and thermal stability is higher than sucrose. They are stable in a pH range from 4.0 to 7.0 and can be refrigerated for a period of one year. Their high moisture-retaining capacity provides prevention of excessive drying besides controlling microbial contamination owing their low water interacting activity [68].

They can be considered as noncariogenic sugar substitutes in confectionary, gums, and drinks since they cannot serve as a substrate of *Streptococcus mutans*, *Lactobacillus acidophilus*, and other bacteria to form insoluble β-glucans implicated in plaque formation, which causes dental cavities [69]. As FOSs possess β-configuration in anomeric carbon, C_2 in the fructose monomers, they are nondigestible by human digestive enzymes which are mostly specific for α-glycosidic bonds and therefore, are not utilized as energy source in the body [70]. Consequently, they are safe for consumption by diabetics [65, 71, 72].

10. FOS as prebiotic

The most widely used definition for prebiotic is "nondigestible food ingredient that beneficially affects host's health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" [73]. In 2004, the definition was updated, and it was defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" [74]. According to this, prebiotics are a major part of the functional foods and among them, the FOS are in focus due to their functional properties and economical potential [65, 75].

A prebiotic must fulfill three criteria: (1) no hydrolysis or absorption in the upper part of the digestive system, (2) selective substrate for one or more desired bacteria species in the colon and stimulation of that species regarding growth and activation and (3) able to positively influence the numeric proportion of different bacteria species in the colon [76]. FOS selectively stimulates the growth of *Bifidobacteria* and *Lactobacillus* sp. in the colon, and these bacteria show commensalism association in the host body. These colon-specific anaerobic bacterial groups degrade FOS producing short-chain fatty acids (SCFA) such as acetate (C2), propionate (C3), and butyrate (C4), decreasing colon pH and subsequently enhancing the absorption of mineral ions (Ca^{2+} and Mg^{2+}) and nutrients in the host body [77, 78].

Many Bifidobacteria and *Lactobacillus* sp. are resistant to acidic pH, but it is harmful to those antagonist bacteria in colon like *Clostridium* sp. [79]. Furthermore, compared with other anaerobes in the gastrointestinal tract, lactobacilli and bifidobacteria have enzymes with lower activities, such as β-glucosidase, β-glucuronidase, urease, azoreductase, and nitrate reductase, which are involved in the formation of mutagens and carcinogens [80].

Antiinflammatory and antitumorigenic roles of SCFA have been reported [81, 82]. As a result of the prebiotic function, a decrease in inflammatory markers such as phagocytosis and interleukin (IL)-6 production by increasing CD^{3+}, CD^{4+}, and CD^{8+} populations has been observed [83]. In case of antitumorigenic roles, especially in the context of colon cancer, the

action mechanism is yet unclear [80]. However, it is known that butyrate has an important role on DNA methylation thus modifying gene expression so it may directly enhance cell proliferation of normal cells, but suppress cell proliferation of transformed cells. Furthermore, in the presence of butyrate, apoptosis may be enhanced in transformed cells but inhibited in normal cells [84]. Thus, the regular intake of FOS as a part of diet could help to improve health and over all well-being by providing resistance against the intestinal/extra intestinal pathogens, enhancing the growth of the colon microbiota which have metabolic activities and biochemical processes with a tremendous influence in human host [85].

11. Applications of FOS

FOS are components of functional food that are becoming popular in the society because they have a potential for enhancing flavor quality and physicochemical properties of food products, besides FOS offer various benefits for human health and are also of industrial interest [34]. FOS are used in different food applications and other areas because of its positive impact on human health, physical performance, or state of mind [12], and the most relevant uses in food formulations are the following: beverages (fruit drinks, coffee, cocoa, tea, soda, health drinks, and alcoholic beverages), dairy products (fermented milk, instant powders, powdered milk, and ice cream), also in light jam products and confectionary [86].

12. Functional foods

The growing interest of consumers in the relationship between nutrition and health has increased demand among the population for food products that improve or benefit their health beyond basic nutrition [87, 88]. Because of this demand, both the academic community and the food industry have focused on developing products that meet these characteristics, which are now called functional foods.

The term "functional foods" was first coined in Japan after a group of scientists and nutritionists conducted numerous studies and defined them as "Food for specified health uses" (FoSHU) [89]. Though there is a great number of definitions, Doyon and Labrecque [90] identified four key concepts after reviewing more than 20 definitions: (a) health benefits, (b) the nature of the food, (c) level of function, and (d) consumption pattern. The definition of functional food has evolved, and the latest is that proposed by the European Commission for concerted action Functional Food Science in Europe (FUFOSE) that mentions that "a food can be regarded as 'functional' if it is satisfactorily demonstrated to beneficially effect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain as food and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern" [91, 92].

The challenges for the food industry are great so that the biological value of the functional ingredient is not disturbed and sensory characteristics of the food are acceptable [93]. According to various investigations, the success and acceptance of these foods is influenced by factors such as clarity and understanding of the information about nutrition and health benefits that are provided to consumers, especially in elderly consumers [87].

Experts recognize that there are scientific evidence on the effectiveness of various functional foods, which can be useful to balance a poor diet or assist in avoiding health problems in some cases [94]. In general, the consumers' attitude towards functional foods is positive and have great potential in the food industry.

13. Applications of FOS in food formulations

FOS are ingredients that have been applied in a variety of food matrices, their prebiotic potential has been proven, and its technological properties allow easy incorporation into foods, mainly those that are probiotics [95]. The FOS have comparable glucose syrups and sugar properties and proved approximately a 30–50% sweetness compared with sugar table. Therefore, their application has a dual benefit: (1) as a substitute for sugar and (2) for their prebiotic properties [96].

Akalin and Erisir [97] evaluated the effect of supplementation of oligofructose or inulin in symbiotic ice cream, in the rheological properties and probiotics survival. They found that the survival rate of the probiotics during storage at 30, 40, and 90 days was better with oligofructose. The FOS were evaluated in cookies and Quicks bread and found that consumers had preference equal to or greater for products supplemented with FOS [96, 98]. Although the FOS are easy to incorporate into foods such as yogurt, processing conditions such as acidity and temperature should be considered since they have reported low prebiotic activities under acidic conditions and high thermal processing times [95].

New applications in different food matrices are being evaluated. Valencia *et al*. [99] supplemented a creamy milk chocolate dessert with FOS and probiotics. They found positive results in the consumer acceptability test and in the survival probiotics. Moreover, in a cooked ham, FOS as substitutes of dextrose were added; the appearance of the ham did not change and the addition of FOS in ham transformed it into a healthier product [100].

There is an innovative trend in the FOS application in different types of food and, undoubtedly, to maximize the benefits that can confer the FOS, factors as type of food matrix, processing conditions, and added amount should be considered.

14. Future trends

Because of the importance of this enzyme in the modern industry, it is important to relate a set of FTases from different organisms to allow the identification of features that could be used for the identification and classification of new FTases, and also it is necessary to improve the

conditions and costs of FTases production process. Further studies of gene sequencing will allow distinguishing among the set of FTase and β FFase enzymes.

15. Conclusions

The studies on production and application of FOS are of high interest for food industry because of several health benefits and biofunctional properties that these compounds provide. FOS can be synthesizing from precursors such as sucrose using FTase enzymes. These enzymes can be obtained from different microorganisms (bacteria and fungi) and plants. The main disadvantage of this production is the low yields of enzymatic activity and FOS. Thus, search for new microbial sources of FTase enzymes is a very important research topic as well as studies about the evolution of FTase genes from different sources, and relate their function with the nucleotide sequence using functional genomics studies.

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