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REVIEW

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# Microbial enzymatic production and applications of short-chain fructooligosaccharides and inulooligosaccharides: recent advances and current perspectives

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**Abstract** The industrial production of short-chain fructooligosaccharides (FOS) and inulooligosaccharides is expanding rapidly due to the pharmaceutical importance of these compounds. These compounds, concisely termed prebiotics, have biofunctional properties and hence health benefits if consumed in recommended dosages. Prebiotics can be produced enzymatically from sucrose elongation or via enzymatic hydrolysis of inulin by exoinulinases and endoinulinases acting alone or synergistically. Exoinulinases cleave the non-reducing β-(2, 1) end of inulin-releasing fructose while endoinulinases act on the internal linkages randomly to release inulotrioses (F3), inulotetraoses (F4) and inulopentaoses (F5) as major products. Fructosyltransferases act by cleaving a sucrose molecule and then transferring the liberated fructose molecule to an acceptor molecule such as sucrose or another oligosaccharide to elongate the short-chain fructooligosaccharide. The FOS produced by the action of fructosyltransferases are 1-kestose (GF2), nystose (GF3) and fructofuranosyl nystose (GF4). The production of high yields of oligosaccharides of specific chain length from simple raw materials such as inulin and sucrose is a technical challenge. This paper critically explores recent research trends in the production and application of short-chain oligosaccharides. Inulin and enzyme sources for the production of prebiotics are discussed. The mechanism of FOS chain elongation and also the health benefits associated with prebiotics consumption are discussed in detail.

**Keywords** Fructooligosaccharide · Inulooligosaccharide · Endoinulinase · Exoinulinase · Fructosyltransferase · Degree of polymerization · Inulin

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## Introduction

Fructooligosaccharides (FOS) of the inulin type constitute an important class of carbohydrates that have gained attention in the New Millennium due to their beneficial health effects [36, 48, 58, 88]. Fructooligosaccharides are found naturally in foods such as onion (*Allium cepa* L.), banana (*Musa* sp.), Jerusalem artichoke (*Helianthus tuberosus* L.), chicory (*Cichorium intybus* L.), tomato (*Solanum lycopersicum* L.), cereal plants, and some grasses [49, 52, 63, 90, 99, 124, 130]. It is estimated that more than 36,000 vegetable plants in the *Compositae* and *Asteraceae* families accumulate large amounts of polyfructans as reserve carbohydrates [49, 50, 60, 83, 96, 134]. Generally, these compounds have a number of biofunctionalities which have been documented in several publications though some of the claims have not been thoroughly elucidated and consequently require further research for validation [35, 41, 58, 88, 105, 122].

Fructooligosaccharides have been classified as prebiotics because of their bifidogenic nature and also their health-promoting properties when consumed in sufficient amounts as recommended by health practitioners [48, 73, 79, 89, 117]. There are essentially three main criteria that should be met before a food ingredient can be classified as a prebiotic: (1) it should not be hydrolysed or absorbed in

the upper part of the gastrointestinal tract, (2) it should be a selective substrate for one or a limited number of probiotics, and (3) it should be able to alter the colonic microflora toward a potentially more healthy composition and/or activity [89]. In general terms, probiotics are potentially beneficial bacteria commensal to the colon (for example bifidobacteria and lactobacilli), which are stimulated to multiply by the prebiotics with concomitant suppression of potentially pathogenic bacteria [1, 73, 89, 117]. Recently, a new term, synbiotics, has been introduced, which is the combination of prebiotics and probiotics in food products such as yogurt and other fermented dairy products that have the ability to influence and improve the gastrointestinal health of humans [48, 122]. Health benefits and applications of FOS in human nutrition are well documented and these include the activation of the immune system, resistance to infections, synthesis of B-complex vitamins and enhanced calcium absorption in the gastrointestinal tract [105, 127].

The biofunctional applications of fructooligosaccharides are due to the following four properties that make them important food ingredients [92, 122, 127]. First, FOS have a low sweetness intensity, since they are about one-third as sweet as sucrose and this property is useful in the various foods where the use of sucrose is restricted by its high sweetness [127]. Second, FOS have low calorie levels and they are rarely hydrolysed by digestive enzymes and are not used as an energy source in the body and consequently are safe for consumption by diabetics [92, 122, 127]. Third, FOS are noncariogenic, that is, they are not used by *Streptococcus mutans* to form acids and insoluble β-glucan that are implicated in the formation of dental caries [127]. Fourth, as prebiotics, FOS encourage the growth of the bifidobacteria and discourage the growth of potentially putrefactive microorganisms that have a tendency of causing diarrhoea [6, 127]. The suppression of the growth of harmful bacteria in the gut is possibly due to the formation of lactic, acetic and other short-chain organic acids that may be antagonistic to the potentially pathogenic intestinal competitors [48]. Important physiological roles of FOS in human beings include the reduction of levels of serum cholesterol, phospholipids, and triglycerides [127].

Fructooligosaccharides derived from inulin are referred to as inulooligosaccharides (IOS). Inulin is the storage carbohydrate in the roots and tubers of plants such as Jerusalem artichoke, chicory, and dahlia with exclusive 2 → 1 fructosyl-fructose linkages [55, 81, 130]. In a broader context, fructans are carbohydrate polymers in which fructosyl-fructose linkages constitute the majority of linkages [33, 61]. Fructans include both small oligomers, polymers with more than 10,000 residues and dimeric inulobiose [61]. Currently, the nomenclature of different types of fructans is under intense debate and some fructan names can occasionally be interchanged.

## Classification and chemical structure of fructans

Inulin is a linear compound consisting of β-(2, 1)-linked D-fructofuranose units and one terminal α-(1, 2)-linked D-glucopyranose unit [131]. There are several types of fructans depending on their chemical structure and organisms producing them [19, 132]. Fructans are categorised as follows: inulin, levan, phlein, graminan, kestoses and kesto-*n*-oses. Inulin is a linear plant β-(2, 1)-fructan that is polydisperse with a degree of polymerisation ranging from 2 to 60 fructose units [96, 97, 118]. In most cases, the inulin molecule has a terminal glucose moiety (GFn) but in some instances, it may have fructose molecules only and lacking the terminal glucose moiety (Fm) [44, 73] (Fig. 1).

Levan is primarily found as a microbial exopolysaccharide and is a fructose biopolymer mainly linked by β-(2, 6)-glycosidic bonds, with β-(2, 1)-linked side chains [19, 40, 76, 125]. Levan is also a fructan of higher plants that has mostly the (2, 6) fructosyl-fructose linkage and it consists of high degree of polymerisation (DP), (DP > 100) polymers such as those found in bacterial systems [33, 61]. Phlein are plant-derived compounds which contain mostly the (2, 6) fructosyl-fructose linkage and these polymers are of lower molecular weight (DP < 100) [61]. Grasses possess fructans with a more complicated branched structure containing a preponderance of β-2, 6 linkages of the levan type in addition to the β-2,1 linkages [76, 101]. Graminan has both (2, 1) and (2, 6) linkages, GFn and Fm fructosyl-fructose linkages in significant amounts with branching [61]. Kestoses are trimeric fructans containing glucose

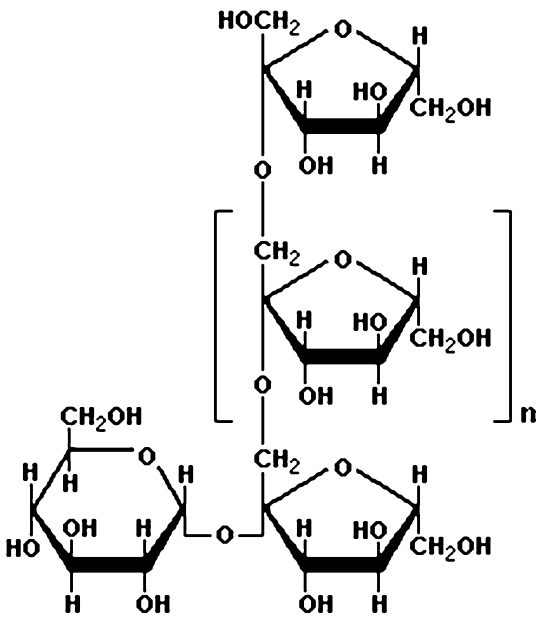


**Fig. 1** Chemical structure of inulin, where $n$ = approx. 20 fructose residues [89]


**Fig. 2** Photographs of flowering chicory plants **a** (*Cichorium intybus* L) and its storage roots (**b**). (http://tcpermaculture.blogspot.com/2012/11/permaculture-plants-chicory.html)

and two fructose units while kesto-*n*-oses are oligomeric fructans of DP $\geq$ 3 that contain a sucrose unit with the DP designated by a Greek root for instance, kestohexaose and kestodecaose [61].

An oligosaccharide is characterised by the degree of polymerisation, type and sequence of its monosaccharide moieties [87]. On average, up to 10 monomeric units are contained in the chain, which can either be linear or branched [87, 89]. The degree of polymerisation is defined as the number of fructose units linked to the terminal glucose moiety. In some plants, for instance *C. intybus*, the degree of polymerisation is determined by the type of cultivar, plant's life cycle and the time of harvest [44, 84, 120]. Early harvesting produces inulin with a higher degree of polymerisation as compared to late harvesting, before the onset of inulinases which can cleave the long molecules into short-chain oligosaccharides [73]. FOS are also defined as lower mass linear molecules that are made up of 2–20 fructose monomers with a terminal glucose moiety that are joined together by glycosidic linkages [89, 118, 123]. Fructooligosaccharides are generally regarded as safe (GRAS) for human consumption [49]. They are not hydrolysed by digestive enzymes in the upper gastrointestinal tract and due to their non-digestibility, they reach the colon intact [89]. Among the non-digestible oligosaccharides are compounds containing fructose, glucose, xylose and galactose [80, 89]. The chain length, presence of the β-link and branching make these compounds resistant to hydrolysis by the digestive enzymes in the upper gastrointestinal tract and consequently are classified as natural fibre [80].

## Sources of inulin and fructooligosaccharides

Fructooligosaccharides are found in trace amounts as natural components in fruits, vegetables and honey [92]. The major sources of inulin are chicory (*C. intybus*) and Jerusalem artichoke (*H. tuberosus*) (Fig. 2). Inulin content on fresh weight basis in these plants ranges from 10 to 20 % [90]. The distribution of inulin from various plant sources is illustrated in Fig. 3. However, a major limitation of chicory as a source of inulin is the presence of fructan 1-exohydrolase (1-FEH, EC 3.2.1.153), an inulinase that degrades fructosyl-fructose linkages at low temperatures, reducing the quality of the inulin at the end of the growth period and during storage [73, 115]. The inulins derived from plant sources are polydisperse and are mixtures of different carbohydrates [65]. The average chain length of inulin varies between plants and in different growth conditions. Due to polydispersity, the molecular weight of inulin varies between ±3,500 and 5,500 [110].

### Other bifidogenic factors

Besides fructooligosaccharides and inulooligosaccharides, there are other types of oligosaccharides which are reported to be bifidogenic [21]. Bifidogenic factors are defined as carbohydrate-bearing materials that survive direct metabolism by the host and are preferentially metabolised by bifidobacteria in the large intestines [73, 121]. There are 12 classes of food-grade oligosaccharides currently in commercial production [21]. Xylooligosaccharides (XOS) are sugar oligomers composed of xylose

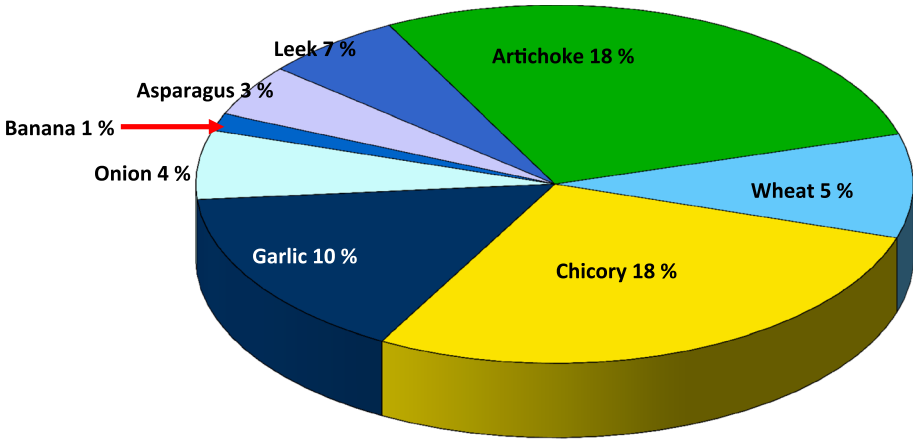


**Fig. 3** A graphical illustration showing the distribution of inulin from various plant sources that are commonly used in human nutrition [89, 118]

monomers with only (1, 4)-β-D-xylopyranosyl linkages that are widely found in bamboo shoots, fruits, vegetables and honey [21, 73]. Xylooligosaccharides are mainly used in pharmaceutical formulations, feed formulations for agricultural purposes and in foods for human consumption [121]. The main advantage of xylooligosaccharides over inulin is that they can resist acid and thermal degradation and consequently have found wide use in low pH fruit juices and carbonated prebiotic drinks [73, 87]. Isomaltooligosaccharides (IOS) are [α-D-Glu (1, 6)] $n$, where $n = 2–7$ and are commercially produced from starch by the action of debranching enzymes such as pullulanase (EC 3.2.1.41) and isoamylase (EC 32.2.1.68) [21, 87]. Intake of isomaltooligosaccharides has been found to improve colonic conditions by reducing the levels of intestinal putrefactive bacteria such as *Clostridia perfringens* and members of the family Enterobacteriaceae [87]. Galactooligosaccharides (GOS) do not occur naturally but are synthesised from lactose using the galactosyltransferase activity of β-galactosidase. Different types of galactooligosaccharides are produced commercially, namely, isogalatobiose, galsucrose and lactosucrose [21, 87]. Galactooligosaccharides are used as prebiotic food components and they are bifidogenic since they enhance the growth of bifidobacteria in the colon [87].

## Industrial and pharmaceutical importance of inulin

The name inulin was used after the compound was first isolated from *Inula helenium* [4]. Many other plants have been shown to make inulin as a storage polysaccharide [4]. Chicory inulin has interesting industrial applications. It is a cheap, renewable and readily available raw material of fructose, which is widely used in the food industry as a sweetener [127]. Inulins are the only class of polysaccharides based on the furanose structure [4]. Inulin is used as a special food for diabetics, as diuretics and is also used in kidney clearance tests because it remains intact in the blood long after injection [4].

**Table 1** The effect of time of harvest on the chain length (average DP and maximal DP) of native chicory inulin [23]

| Date of Harvest | Average DP | $DP_{max}$ |
|---|---|---|
| End of September | 11.7 | 72 |
| Mid November | 9.0 | 60 |
| End of December | 6.0 | 51 |

Inulin solutions have different properties depending on the origin of the inulin [26]. Inulin solubility depends significantly on temperature, degree of polymerisation, distribution of the molecular chains, degree of molecular branching and the processing method [26, 65]. It has been reported that native chicory inulin is soluble to about 60 g/L at 10 °C while at 90 °C it is soluble to about 330 g/L [26]. Native chicory inulin is dispersible in water under normal conditions and has a tendency to clump during hydration due to its hygroscopic character.

## Composition of chicory inulin

Inulin is a polydisperse polymer made of between 2 and 60 fructose units [65]. However, a high sugar content and long-chain inulins are preferred by the sugar industry [23, 120]. In Belgium, it has been observed that inulin has the highest DP early in the harvesting season, from end of September to October as shown in Table 1 [23].

Native inulin is a form of inulin that has been extracted from the plant source with hot water, minimising any hydrolase activity that may lead to its degradation. Commercial inulin preparations obtained from various sources are not native and they should not be considered as products representing the inulin which is typical of the plants from which they were extracted [23]. The composition of

**Table 2** Microbial and plant sources of oligosaccharide producing enzymes [127]

| Plant sources | Microorganism sources |
|---|---|
| *Agave americana* (agave) | *Aureobasidium pullulans* |
| *Agave vera cruze* (agave) | *Aureobasium* sp. |
| *Asparagus officinalis* (asparagus root) | *Arthrobacter* sp. |
| *Allium cepa* (onion bulbs) | *Aspergillus japonicas* |
| *Cichorium intybus* (chicory) | *Aspergillus niger* |
| *Crinum longifolium* (sugar-beet leaves) | *Aspergillus oryzae* |
| *Helianthus tuberosus* (Jerusalem artichoke) | *Aspergillus phoenicis* |
| *Lactuca sativa* L. (lettuce) | *Aspergillus sydowi* |
| *Lycoris radiate* (monocot) | *Claviceps purpurea* |
| *Taraxacum offinale* (dandelion) | *Fusarium oxysporium* |
| | *Penicillium frequentans* |
| | *Penicillium spinulosum* |
| | *Phytophthora parasitica* |
| | *Scopulariopsis* sp. |
| | *Saccharomyces cerevisiae* |

native inulin is GFn molecules, accompanied by a small percentage of Fm molecules [89]. Several methods for inulin extraction from various sources have been documented [63]. The hot water extraction process at 85 °C is one of the most popular methods followed by evaporation and spray drying [108]. Another efficient method for inulin extraction that is widely used at laboratory scale is the precipitation from aqueous solution using ethanol [63]. This method was shown to be uneconomical and not suitable for industrial production of inulin [63]. Lingyun et al. [63] developed an extraction strategy using high-intensity and high-frequency ultrasound waves (sonication). This disrupts plant cell walls thereby facilitating the release of extractable compounds and enhances mass transport of solvent from the continuous phase into plant cells. In addition, the use of pectolytic enzymes allows for the enzymatic disruption of cell walls which leads to release of inulin from plant cells. This enzymatic treatment also reduces the viscosity and clarifies the final inulin solution.

## Enzymes used for oligosaccharide production

A wide range of inulinases and fructosyltransferases are involved in the formation of oligosaccharides and are produced by plant and microbial sources [75, 78, 111], (Table 2). Principal FOS such as 1-kestose, nystose and fructofuranosylnystose are synthesised by a wide range of enzymes. For industrial application, it is technically difficult to source these enzymes from plants. Traditionally, inulinases have been produced by submerged fermentation (SmF) but recently, solid-state fermentation (SSF) is gaining importance though there are only a few reports on the application of this technique [124]. Solid-state fermentation is associated with numerous advantages over SmF such as superior productivity, simplicity, low capital investment, low energy requirement, less water output, and better product recovery [124]. Due to these advantages, SSF is regarded as the most appropriate process [124]. Enzyme properties have been shown to be greatly influenced by the nature of their source.

### Microbial exoinulinases

Inulin hydrolysis is achieved by the use of exoinulinase (2,1-β-D-fructan fructohydrolase, EC 3.2.1.80), which successively cleaves fructose from the non-reducing β-2, 1 end of inulin [3, 53, 75, 82, 131–134]. Microorganisms involved in the production of exoinulinases include *Aspergillus* sp., *Kluyveromyces* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Penicillium* sp., *Chrysosporium* sp., *Bacillus* sp., and others as shown in Table 2. Generally, inulinases sourced from fungal and bacterial sources are more thermotolerant and this property is important for their industrial application, as elevated temperatures prevent microbial contamination of the final product. Inulinases from microbial sources are active under mild conditions and are stable over a broad pH range.

### Microbial endoinulinases

Microbial endoinulinases (2,1-β-D-fructan-fructan hydrolase, EC, 3.2.1.7) hydrolyses the internal linkage of inulin to release intermediates such as inulotriose (F3), inulotetraose (F4) and inulopentaose (F5) [3, 53, 127]. It has been well documented that the two endoinulinases and exoinulinases act either alone or synergistically to produce fructose, but it is not known whether the two enzymes coexist [53, 90, 127].

### Plant fructosyltransferases and mechanism of action

Fructosyltransferases obtained from plants are distinct fructosyltransferases such as sucrose: sucrose 1-fructosyltransferase (1-SST) and fructan: fructan 1-fructosyltransferase (1-FFT). Plants such as *C. intybus* and *H. tuberosus* produce high levels of fructosyltransferases such as 1-SST, (EC. 2.4.1.99) and 1-FFT, (EC. 2.4.1.100). The two enzymes are produced during the fructan accumulation stages and during the cold months. The established method for fructan biosynthesis in Jerusalem artichoke (*H. tuberosus*) tubers was first demonstrated by Edelman and Jefford [29]. They showed that fructan synthesis occurs through the formation of a trisaccharide intermediate by the concerted

action of two distinct fructosyltransferases, 1-SST and 1-FFT [12, 67]. In this model, 1-SST is a key enzyme that initiates fructan biosynthesis from sucrose to form a trisaccharide, 1-kestose, with the concomitant stoichiometric release of glucose and that 1-FFT transfers fructose moieties between fructan molecules to form long chain fructans. The product, 1-kestose, from the initial reaction with 1-SST is reported to be an efficient fructose donor for the formation of long-chain fructans [12, 67, 115]. The mechanism of this reaction is illustrated in reactions 1 and 2.

$$\mathbf{G1},\ \mathbf{2F} + \mathbf{G1}, \mathbf{2F} \xrightarrow{\mathbf{1-SST}} \mathbf{G1}, \mathbf{2F}, \mathbf{2F} + \mathbf{G} \quad (1)$$

$$\mathbf{G1},\ \mathbf{2F}(\mathbf{1},\mathbf{2F})_{\mathbf{m}} + \mathbf{G1}, \mathbf{2F}(\mathbf{1},\mathbf{2F})_{\mathbf{n}} \xrightarrow{\mathbf{1-FFT}} \mathbf{G1},\ \mathbf{2F}(\mathbf{1},\mathbf{2F})_{\mathbf{m-1}} + \mathbf{G1},\ \mathbf{2F}(\mathbf{1},\mathbf{2F})_{\mathbf{n+1}} \quad (2)$$

Where m > 0 and n > 1, G and F represents glucose and fructose molecules, respectively, and 1, 2 represents the β (2, 1) glycosidic bond).

The two enzymes have since been purified and characterised and consequently the model proposed by Adelman and Jefford was validated [12, 57, 67, 112]. An important finding of this method is that the enzymatic de novo synthesis of fructan with a degree of polymerisation >3 from sucrose by a mixture of 1-SST and 1-FFT is impossible, as a result of enzyme inhibition by sucrose [12, 57]. According to Cairns [12], long-chain fructan synthesis in some grasses is not inhibited by sucrose and a high enzyme concentration is a requirement to overcome inhibition by sucrose. Enzymes that are involved in fructan synthesis have been studied [13, 45, 115]. The enzymology of fructan biosynthesis in plants [45, 115] involves the production of 1-kestose (G-F2-1F) and 6-kestose (G-F2-6F), both products of sucrose (G-F) from fructosyltransferase action. The 1-SST catalyses the synthesis of 1-kestose formation and 1-kestose is a substrate for inulin and inulin neoseries synthesis using 1-FFT for chain elongation. Sucrose: sucrose 6-fructosyltransferase (6-SST) catalyses the synthesis of unbranched levan in barley and has the ability to introduce branches into longer chains. Fructan: fructan 6-fructosyltransferase (6-FFT) has chain elongating activity producing branched levan. Fructan: fructan 6-glucose-fructosyltransferase (6-SFT) is involved in the production of fructan neoseries.

Microbial fructosyltransferases and mechanism of action

A microbial fructosyltransferase (Ftase; E.C. 2.4.1.9) catalyses the formation of FOS from sucrose [92]. A wide range of microbial sources for fructosyltransferases (Ftases) have been reported in the literature [58, 88, 92, 133], (Table 2). These enzymes synthesise FOS with different linkages to form several kinds of FOS of varying yields as a direct result of the initial concentration of sucrose in the reaction mixture [15, 92]. Fructosyltransferases obtained from microorganisms are single enzymes with both transferase and hydrolase activities [92, 106].

A mathematical model has been postulated to elucidate the mechanism of FOS synthesis with FTase from a set of disproportionation reactions which is slightly different from the mechanism of plant fructosyltransferases [54]. The mechanism of FOS synthesis by microbial fructosyltransferases has been elucidated and in this reaction 2 mol of sucrose act as fructose donors and acceptors for the formation of 1 mol of glucose and 1 mol of 1-kestose. In turn, the 1-kestose acts as an acceptor for the formation of a tetrasaccharide [54, 127]. The reaction mechanism can be exemplified by reactions 3 and 4. The glucose that is liberated in the reaction mixture acts as an Ftase inhibitor [54]. Consequently, the batch enzymatic synthesis of polysaccharide from sucrose is disadvantageous due to a large loss of enzyme activity by end-product inhibition of the enzyme [46].

$$\mathbf{GF} + \mathbf{GF} \xrightarrow{\mathbf{Ftase}} \mathbf{G} + \mathbf{GF2} \quad (3)$$

$$\mathbf{GF_n} + \mathbf{GF_n} \xrightarrow{\mathbf{Ftase}} \mathbf{GF_{n-1}} + \mathbf{GF_{n+1}} \quad (4)$$

Where n > 1 and G and F represent glucose and fructose molecules, respectively.

A fructosyltransferase from *Aureobasidium pullulans* has been isolated, characterised and optimum reaction conditions for the synthesis of FOS determined [54]. Parameters such as optimal pH, optimal temperature, pH stability and thermal stability and kinetic parameters such as $K_m$ and $V_{max}$ were established and the data generated were used to propose the reaction mechanism [54].

The synthesis of FOS is a kinetically controlled reaction that involves a fructosyl-enzyme intermediate whereby the two nucleophiles; water and sucrose, compete for the fructosyl-enzyme intermediate [36]. When water is the nucleophile, the enzyme acts as a hydrolase to liberate glucose and fructose, and when sucrose is the nucleophile, the enzyme acts as a transfructosidase synthesising high DP FOS [36]. As a result, the first condensation product, 1-kestose, can also be hydrolysed by the enzyme.

## Isolation and purification of inulinases and fructosyltransferases

To produce pure IOS and FOS, it is desirable to purify enzymes used for IOS and FOS to electrophoretic homogeneity so that their properties and mode of action can be studied as well as to find out if the purified enzyme can

enhance the production of FOS or IOS. FTases have been purified from various sources [92]. The purified FTase was found to produce 1-kestose and nystose unlike the crude enzyme which produced GF5 and GF6 oligosaccharides [92].

## Physiological roles of fructans

Fructans are fructose oligomers and polymers synthesised by a wide range of plant and bacterial species, and mainly function as reserve carbohydrates that are hydrolysed by fructan 1-exohydrolase when energy supplies are needed [67, 77, 90, 99–101]. There are other physiological roles that have been postulated by other workers but these have not been fully elucidated [16, 114, 115].

Fructans increase the osmotic pressure of plant cells resulting in osmoprotection which protects the plant against abiotic stress [23, 43, 115]. A wide range of carbohydrates are involved and these consist mainly of hexoses (mostly fructose and glucose), disaccharides (sucrose and trehalose), sugar alcohols (for example inositol and mannitol) and complex carbohydrates (for example raffinose and stachyose) [43]. The presence of fructans allows for frost tolerance and as a result contributes to membrane stabilisation [112, 113, 115]. According to De Leenheer and Hoebregs [23], the average DP is decreased with growth time and results in higher intracellular osmotic pressure, and this leads to cold resistance in the plant. The variation in DP may be induced by external weather conditions [23]. Fructans have been examined for their biological role as an adaptation to low-temperature photosynthesis [113, 123].

## Production of inulooligosaccharides and fructose from inulin

Complex polysaccharides are difficult to synthesise and manipulate. Currently, there is no commercial process that can synthesise these compounds in an automated fashion, which poses a major limitation to detailed study of their biological functions [28, 95]. In addition, there is no method available to biologists for the structural assignment of carbohydrates with DP < 10 as compared to other biological molecules such as nucleic acids which can be synthesised in vitro using the polymerase chain reaction (PCR) [28]. Despite these constraints, however, there are several methods that can be employed for the synthesis of fructose and fructooligosaccharides with DP < 10 from simple inexpensive raw materials. Fructose can be obtained by acid hydrolysis of inulin at elevated temperatures though the product monosaccharide can be easily degraded at low pH and the process can give rise to colouring of the inulin hydrolysate and unwanted by-product formation in the form of inulin anhydrides that lower the yield and require extensive downstream processing [26, 39, 90, 110].

Another major drawback of chemical hydrolysis is that the technique requires refluxing for extended periods which can be expensive and there is need to use expensive acid-resistant equipment [68]. Chemical inulin hydrolysis can be carried out by treatment with organic or mineral acids or by heterogeneous catalysis using solid acidic catalysts such as acid-cation resins, zeolites or oxidised activated carbon [90]. The energy economy and low formation of by-products, mainly those resulting from the cyclisation of glucose and fructose at acid pH and high temperature, suggest the advantages of using enzymes over acid hydrolysis [109].

Enzyme-based processes operate at lower temperatures, produce less toxic and pollutant wastes with fewer emissions and by-products compared to conventional chemical processes [109]. Enzymatic methods for the production of fructooligosaccharides have demonstrated to be the best option for the industrial production of fructose syrups since they are specific and are not associated with the shortcomings of the chemical approach [90]. Fructose can be produced from starch by enzymatic methods involving α-amylase, amyloglucosidase and glucose isomerase (reaction 5) resulting in the production of a mixture consisting of oligosaccharides (8 %), fructose (45 %) and glucose (50 %) [39]. The major drawback of this method is that downstream processing of the final fructose product is costly [26, 39, 97]. The use of inulinases is an alternative enzymatic method which can give rise to 95 % pure fructose and the remainder being a mixture of inulooligosaccharides (IOS) (inulobiose, inulotriose, inulotetraose and inulopentaose) and small amounts of glucose.

Inulin hydrolysis using enzymes is usually carried out at 60 °C and mild pH conditions [128, 129]. The temperature is especially critical because it prevents microbial contamination of the final product, it lowers the viscosity, it improves transfer rates and it allows for the use of higher concentrations of inulin due to increased solubility [8, 39]. Under these conditions it is crucial to employ highly thermostable inulinolytic enzymes for example a thermostable extracellular exoinulinase from *Aspergillus fumigatus* and a heat-stable exoinulinase from *Streptomyces* sp. [97]. For IOS production, the main benefit of using a higher reaction temperature lies in the increase of the substrate concentration that improves the IOS yield. The disadvantages at these elevated temperatures may be the instability of enzymes, substrates or products and the occurrence of side reactions [8].

The production methods for IOS can either be batch or continuous using free or immobilised enzymes. Batch production of IOS has limitations such as requirements for high amounts of enzymes and product purification. Due to

the high cost of enzymes, this method has high production costs [26]. Continuous methods are desirable because the enzymes are amenable to immobilisation, which allows for continual biocatalyst reuse [90]. Continuous mode of operation prevents contamination of the final product [90, 109]. Inulinases or whole microbial cells with inulinase activity can be immobilised on suitable resins such as amberlite, potentially providing effective, cheap and simple procedure required for industrial scale process [90]. The yield of target products such as pentamers and hexamers, however, decreases with progression of hydrolysis. This is because they are intermediates of the reaction and as a result, stricter control of the hydrolysis reaction must be achieved to produce these oligosaccharides more efficiently [59].

## Glucose removal from the reaction mixture

Inhibition of the Ftase by glucose is one important factor limiting maximal FOS yield under batch conditions [46, 92, 100]. This is due to the accumulation of by-products such as glucose in the reaction mixture which may in turn inhibit the fructosyltransferase activity [50, 83, 103]. Techniques for the removal of the reducing sugars involving nanofiltration and microfiltration have been proposed by some workers to eliminate low molecular weight carbohydrates from a mixture of oligosaccharides [26, 41, 42, 93, 102].

The use of immobilised enzymes such as glucose oxidase (GOD) for the bioconversion of D-glucose to D-gluconic acid and hydrogen peroxide has been evaluated with promising results [71, 100, 106]. On the other hand, the use of glucose isomerase for glucose removal from the reaction mixture is reported to be ineffective [106, 127]. *Saccharomyces cerevisiae* and *Zymomonas mobilis* have been used separately for the removal of glucose from the FOS reaction mixture [20, 126]. Glucose and fructose were completely fermented to ethanol and carbon dioxide with minor amount of sorbitol as a fermentation by-product [20, 41, 126]. Degradation of oligosaccharides was not observed due to the selective fermentation characteristics of the yeast towards different sugars [41, 116, 126]. Strains of *S. cerevisiae* and *Z. mobilis* lack carbohydrases that hydrolyse most oligosaccharides [20]. High content FOS (98 %) was obtained by eliminating the released glucose and unreacted sucrose from the reaction mixture [92]. Viscosity and reduced sweetness and hygroscopic properties cause fewer Maillard reactions during heat processing [20]. Furthermore, removal of contaminating simple sugars lowers cariogenicity and caloric value, and allows the oligosaccharides to be included as diabetic foods [20].

## Applications of fructose

High-fructose syrup (HFS) has major applications as a sweetener in food, pharmaceutical industries, and is used as a substitute for sucrose [39, 90, 109]. Functional attributes of fructose include high osmotic pressure, high solubility, source of energy, twice as sweet as sucrose, and prevents crystallisation of sugar in food products. Furthermore, fructose prevents microbial growth, flavour, colour, product stability enhancement and it is organoleptically desirable by human beings [26, 77, 90]. Fructose is an attractive food additive which can replace glucose since it does not involve the metabolic pathway of glucose which requires insulin and hence complications with diabetes [26, 37]. Fructose does not stimulate insulin secretion. Consequently, fructose can be consumed by diabetic patients without compromising their health. Due to the growing need of foods with health benefits, there is a growing need to produce fructose for diabetic people.

The fate of fructose after absorption in the gastrointestinal tract involves its transformation into fructose-1-phosphate and then metabolised into triose phosphate, glyceraldehydes, and dihydroxyacetone [70]. The events that occur after fructose enters the glycolytic pathway are threefold. A negligible portion of the fructose is converted into glucose and this leads to a small but measurable increase in blood glucose level [70]. A large part of the fructose is transformed into lactate and this leads to a threefold increase in the levels of blood lactate [70]. Therefore, fructose bypasses the regulatory processes involved with glucose metabolism in the liver.

## Applications of fructooligosaccharides

Fructooligosaccharides are relatively new functional food constituents that are becoming popular because they have a potential for enhancing the quality of flavour and physicochemical properties of food products [73, 87]. The formation of lactic, acetic, propionic and other short-chain organic acids are thought to be antagonistic to other potentially pathogenic gastrointestinal competitors [48]. As a result, FOS are slowly replacing some harmful sugar products such as sucrose that can cause health problems such as diabetes mellitus. Fructooligosaccharides are produced from natural compounds and this has led to increased interest from consumers across the world [87]. A functional food is defined as any food that has a positive impact on an individual's health, physical performance or state of mind in addition to its nutritional content [64, 92]. Fructooligosaccharides are widely applied in food formulations because of their functional properties. Examples of their

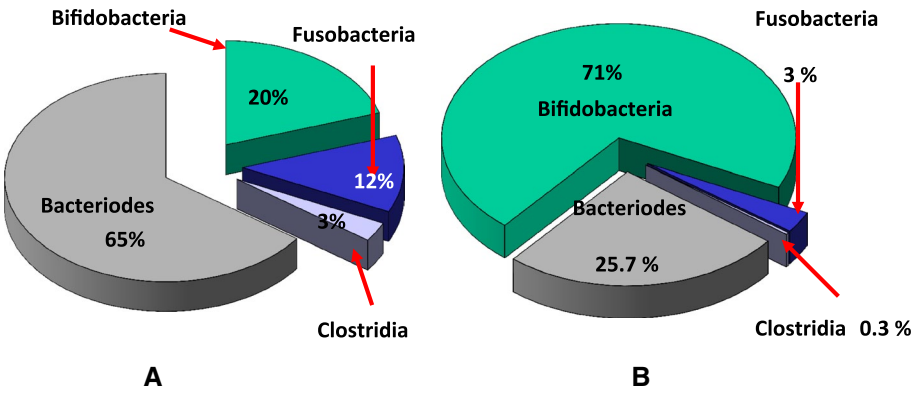


**Fig. 4** Gastrointestinal microbial populations before inulin intake (**a**) and after inulin intake (**b**) [89]

use in food include light jam products, ice cream and confectionery [92].

Application of FOS in food formulations is a new phenomenon. Fructooligosaccharides have different applications depending on the method of production. Fructooligosaccharides synthesised by transfructosylation from sucrose are used as prebiotic ingredients while the longer chain oligosaccharides derived from controlled enzymatic hydrolysis of inulin are used as fat replacers [87]. The functional properties of fructooligosaccharides are greatly dependent upon their molecular weight with pentamers (DP5) and hexamers (DP6) being especially significant because of their effective stimulation of growth of beneficial bacteria in the gastrointestinal tract [59].

## Biofunctional properties of fructooligosaccharides

Investigations have been carried out to confirm that FOS have biofunctional properties [31, 32, 89, 92], (Fig. 4). Published research data indicate that FOS have desirable health effects such as enhancing mineral absorption, defence mechanisms, lipid metabolism, anticancer effects, enhancing gut immune function, control of diabetes among other health claims which still need further research for validation [7, 51, 65, 92].

### Fructooligosaccharides as prebiotics

The bifidogenic activity is reported to be optimum with short-chain FOS in which the fructosyl units ($n = 2$–8) are bound by a β-(2, 1) linkage [5]. The proliferation of bifidobacteria is associated with beneficial effects such as improved digestion and absorption, increased vitamin availability and most importantly prevention of gut colonisation by pathogens and putrefactive bacteria [87]. The prebiotic role of FOS has been promulgated from in vivo and in vitro studies of the metabolism of these compounds by intestinal bacteria [92]. Durieux et al. [27] used two types, Fibruline instant and Fibrulose 97, to demonstrate the prebiotic role of chicory fructooligosaccharides. They found that all the bacterial strains investigated, *Bifidobacterium longum*, *B. infantis* and *B. angulatum* utilised the fructose oligomers in the commercial chicory FOS and this ostensibly proved that FOS can be used as prebiotics [27]. Furthermore, the prebiotic role of FOS was shown by Rycroft and coworkers [91] by carrying out a comparative evaluation of the fermentation properties of prebiotic oligosaccharides by the predominant gut bacterial groups. Researchers [10, 89, 91], found that all prebiotics investigated increased the level of bifidobacteria from 20 to 71 % and clostridia levels dropped from 3 to 0.3 % (Fig. 4a, b). A similar observation was demonstrated in fish [11] and in improving the intestinal health of dogs [85].

### Control of diabetes and other diseases

It has been demonstrated that a daily intake of 20 g of FOS significantly reduced the basal hepatic glucose production in healthy human subjects with no effect on insulin stimulated metabolism [66]. Other compounds such as serum triacylglycerol, total and HDL cholesterol, free fatty acids, apolipoproteins A1 and B were not modified by the consumption of FOS [66]. During the enzymatic synthesis of FOS glucose and sucrose are some of the side products formed and these sugars are implicated in diabetes. Hence, it is imperative to have pure FOS that are free from glucose and sucrose in the mixture to be marketable to diabetic patients [92].

Further, probiotics, prebiotics and synbiotics have been proposed to have a therapeutic role in paediatric surgery, digestive organ surgery, liver disease and systemic inflammatory response syndrome [17, 72, 98]. The most important effect of prebiotics is to increase the body's resistance to invading pathogens, thus preventing episodes of diarrhoea [22, 24]. Lenoir-Wijnkoop et al. [62] demonstrated that the use of infant formula with a specific mixture of prebiotics is an effective strategy in preventing atopic

dermatitis. However, other studies concluded that there is not enough evidence to suggest that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in improved growth or clinical outcomes in term infants, and that there is no data available to establish if synbiotics are superior to probiotics or prebiotics [47, 74, 119]. Hence, more clinical trials are needed to provide a clearer picture of the possible clinical benefits and limitations of current and future prebiotics. This includes proteomic studies of probiotic bacteria that can provide insight on the impact of prebiotic consumption [56].

### Role as anticancer agents

Recent research in experimental animal models has revealed the physiological effects of FOS and inulin in the possible protection against the development of colon cancer and also reduction of chemically induced aberrant crypts [87, 92]. The anticarcinogenic attributes of FOS are reported to be a result of the proliferation of the bifidobacteria [24, 86]. Experiments demonstrated that a 15 % supplementation of basal diets of experimental animals resulted in significant reduction of colon tumours, inhibited the proliferation of transplantable malignant tumours in mice and also lowered the incidence of lung metastases of a malignant tumour implanted intramuscularly in mice [107]. Moreover, Taper and Roberfroid [107] reported that the dietary treatment with FOS or inulin significantly potentiated the effects of sub-therapeutic doses of six different cytotoxic drugs used in cancer treatment in human beings.

### Role as dietary fibre

Dietary fibre is defined as those substances that consist of remnants of edible plant cell polysaccharides that are resistant to hydrolysis by human alimentary enzymes [18]. Fructooligosaccharides are fermented by the bacteria in the large intestines producing short-chain fatty acids mainly acetate, propionate and butyrate which are absorbed efficiently [48, 66]. This has led to FOS being regarded as dietary fibre [18]. By closely monitoring the levels of ingested FOS in individuals with conventional ileostomy, Cherbut [18] found that 90 % of the ingested FOS was recovered at the end of the ileum. In the colon the FOS are completely fermented and as a result of these fermentation properties, FOS affect the intestinal epithelium that may strengthen mucosal protection and reduce the risk of gastrointestinal diseases [18].

### Role in mineral absorption

Research carried out on animal models has shown that non-digestible oligosaccharides (NDO) such as inulin, oligofructose and transgalactooligosaccharide (TOS) stimulate mineral absorption, mainly calcium and magnesium [94]. This role of FOS has been demonstrated by the accumulation of bone mineral content and also the prevention of osteoporosis in ovariectomised rats whereby the addition of 5 % FOS led to a significant increase in bone mineral content [92]. Increase in bone mineralisation is possibly as a result of the enhancement of passive and active mineral transport across the intestinal epithelium, mediated by an increase in metabolites of the intestinal flora and a reduction in pH [94]. In addition, inulin has been shown to increase Fe absorption in rats with iron-deficiency anaemia [34].

### Role in lipid metabolism

Apart from their beneficial effect on the gastrointestinal tract, studies on animal models have shown that inulin and oligofructose modified the hepatic metabolism of lipids [25, 30]. By feeding rats with inulin or oligofructose, it was shown that these carbohydrate rich diets markedly lowered serum triacylglycerols (TAG) and phospholipid concentrations.

### Role in defence mechanisms

Fructooligosaccharides are known to stimulate the growth of beneficial bacteria while suppressing the growth of potentially harmful microorganisms. This is attributed to the low-pH environment as a result of the production of antibiotic like compounds by the beneficial bacteria during the fermentation of FOS [92]. Supplementing the diets of chicken, pigs and rats with oligofructose and other NDO resulted in a reduction of the faecal density of *Salmonella* [92]. Furthermore, a diet consisting of 100 g/Kg of inulin and oligofructose that was fed to mice infected with virulent strains of *Listeria monocytogenes* and *Salmonella typhimurium* was shown to reduce mortality as compared to mice fed with a placebo of cellulose rich diet as a source of fibre [9]. Moreover, the anti-pathogenic effect of prebiotics and probiotics has been illustrated in animals, as well as

**Table 3** Survival requirements of probiotics in the human colon [38]

| Factors influencing the use and viability probiotics |
|---|
| Resistance to the acidic environment of the stomach and the biliary salts |
| High rate of proliferation and affinity for adherence to the intestinal wall |
| Competitive effect for availability of nutrients |
| Production of metabolites deleterious to pathogens (e.g. SCFA) |
| Modulation of metabolic activity (e.g. inactivation of pro-carcinogens) |
| Immuno-modulation (i.e. production of mucins and bacteriocins) |
| Must satisfy GRAS conditions for safety of use |

their potential as alternatives to antibiotic growth-enhancing feed additives [2, 14, 104], and have been found to have a positive effect in the immune system of pre-ruminant calves [69]. However, more studies are essential to understand the mode of action and long-term effects of a diet supplemented with prebiotics. In addition, survival requirements of probiotics in the human colon are presented in Table 3.

## Conclusion

Research in the production and application of FOS and IOS is gaining momentum due to several health benefits and biofunctional properties of these compounds. Prebiotics are produced by crops such as chicory and Jerusalem artichoke. However, FOS can be synthesised in vitro from precursors such as sucrose using fructosyltransferase enzymes. Furthermore, IOS can also be produced from the enzymatic hydrolysis of inulin under controlled conditions. The main drawback of the production process is the low yields of FOS. It is therefore crucial to explore other methods such as molecular methods to improve the efficiency of the enzymes involved in the synthesis of FOS and IOS. More research on the efficacy and mode of action of prebiotics is critical to harness maximum benefits from the preparation and consumption of these oligosaccharides.

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