SHUBHANGI S. KHANVILKAR, SHALINI S. ARYA*
*Corresponding author
Institute of Chemical Technology, Food Engineering and Technology Department
NM Parikh Marg, Matunga, Mumbai, 400019, India


Shalini S. Arya

Fructooligosaccharides: applications and health benefits
A review

KEYWORDS: Non- digestible, enzymes, functional ingredient, product formulation, industrial production, health benefits.

Abstract Fructooligosaccharides (FOSs) are non-digestible carbohydrates with functional and physiological attributes like low sweetness, non-carcinogenicity, low caloric value, prebiotic, hypolipidemic and hypocholestrolemic properties. Although fructooligosaccharides are present in trace amounts in natural foods like onions, asparagus, wheat, banana, tomato and honey, commercial production is accomplished, using microbial transferase such as fructosyl transferase or β-fructofuranosidase. The industrial production of fructooligosaccharides (FOS) is expanding rapidly due to the food and pharmaceutical importance of these compounds. Due to its health benefits and functional properties, FOS will continue to exist in market as functional food ingredient for Product formulations.

ABBREVATIONS

FOS- Fructooligosaccharides, DP- Degree of polymerization, DPav- Average Degree of polymerization, SCFOS- Short Chain Fructooligosaccharides FTase- Fructosyl Transferase, FFase- β-fructofuranosidase, SmF- Submerged Fermentation, SSF- Solid State Fermentation, SCFA- Short Chain fatty Acids

INTRODUCTION

Fructooligosaccharides (FOS) are composed of a mixture of Kestose (GF2), Nystose (GF3) and Fructosyl nystose (GF4), obtained from sucrose by enzymatic addition of fructosyl moieties, and having an average degree of polymerization (DPav) of 3.6, and are named as short-chain fructooligosaccharides. They are different from natural fructans having degree of polymerization (DP) (only 10 % of native chicory Inulins have a DP between 2 and 5) (1), and from oligofructoses formed by inulin hydrolysis (DP from 2 to 7, DPav 4) by the systematic presence of a glucose moiety (2). FOS is found in trace amounts as natural components in fruits, vegetables, and honey. FOS is present in more than 36,000 plant sources as reserve carbohydrates (3). It is present in various plant sources such as asparagus, jerusalem artichoke, chicory, sugar garlic, onion, wheat, honey, banana, barley, tomato, and rye (3). Its concentration ranges from 0.15% to 0.75% in natural sources. The highest concentration is present in jerusalem artichoke (up to 20%) and chicory (between 5 and 10%). These fructose oligomers have beneficial functional properties, low caloric value, non-cariogencity and can be used in diabetic formulation. In addition, it exhibits many health benefits such as decrease levels of phospholipids, cholesterol and triglycerides in blood, helps in gut absorption of minerals such as calcium and magnesium, and stimulate the growth of bifidobacteria in the human colon (4-5-6). Therefore, utilization of fructooligosaccharides in food and pharmaceutical formulations is of great interest. Thus, developing economically feasible process of production of fructooligosaccharides to get high content FOS has become major research area (Table 1).

Source	% FOS
Barley	0.15
Tomato	0.15
Onion	0.23
Banana	0.30
Brown Sugar	0.30
Rye	0.50
Garlic	0.60
Honey	0.75

Table 1. Concentration of FOS in Natural Foods (5).

CHEMISTRY

FOS can be obtained in two different ways, which result in slightly different end products. First method involves transfructosylation of sucrose by fructosyl transferase to

produce Short Chain fatty Acids (SCFOS), while the second method involves enzymatic hydrolysis of the polysaccharide inulin (inulin oligofructose) (Figure 1).
In the first method, The FOS formed in this process are the fructose oligomers that consist of a chain of two to four fructosyl moieties linked by a β (2→1) glycosidic bond and a single glucose unit at the non-reducing end, linked by an α(1→2) glycosidic bond (7). These are named l-Kestose (Glu-Fru,), l- nystose (Glu-Fru,) and l^F-fructofuranosyl nystose (Glu-Fru,). These types of FOS are considered as SCFOS.
The transfructosylation of sucrose takes place via the cleavage of the β-(2→1) glycosidic bond and the transfer of the fructosyl moiety onto any acceptor other than water, such as sucrose or a FOS. This synthesis is a complex process in which several reactions occur simultaneously, both in parallel and in series, because SCFOS are also potential substrates of fructosyltransferases. Glucose formed as by-products in the reaction acts as inhibitor for the further production. Therefore, by-products and un-reacted sucrose formed during reaction is removed using chromatographic procedures to get high purity product. These SCFOSs are quickly fermented in the colon to give prebiotic benefits (8).
In the second method, FOS production takes place by the controlled enzymatic hydrolysis of the polysaccharide inulin (inulin oligofructose). The FOS mixture obtained by this process is different from the mixture produced by the transfructosylation process. The oligosaccharide mixture obtained is longer than that produced by transfructosylation of sucrose. Additionally, not all of the β(1→2)-linked fructosyl chains end with a terminal glucose in the oligofructoses prepared by inulin hydrolysis. The inulin extracted from chicory roots contains some fructooligosaccharides in addition to polysaccharides. These longer-chain oligosaccharide products have applications as fat replacers (8).

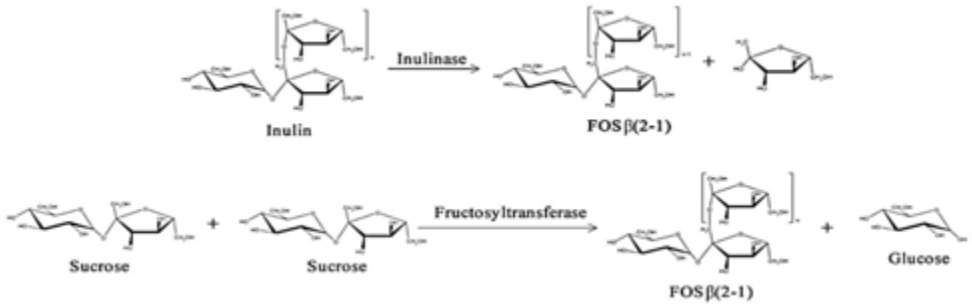

Figure 1. Chemical synthesis of FOS (9).

Enzyme sources of fos synthesis
The enzyme source of FOS synthesis can be classified into two; one is plants such as asparagus, jerusalem artichoke sugar beet, onion, etc.(4) and other consists of bacterial and fungal origins such as *Aspergillus sp., Aureobasidium sp., Artrobacter sp., Fusarium sp*. etc. (4). The production yield of FOS using plant enzymes is low and dependent on seasonal conditions; therefore, industrial production depends chiefly on fungal enzymes from either *Aureobasidium sp*. or *A. niger*. Fructosyl Transferase (FTase) or β-fructofuranosidase (FFase) is responsible for the microbial production of FOS. FTase produces FOS (GFn) from sucrose (GF) in a disproportionate mode, thereby forming 1-kestose (GF2) initially, then1-nystose (GF3), followed by 1-fructofuranosyl nystose (GF4). Microbial FTases are derived from bacterial and fungal sources.

PRODUCTION OF FOS

Fermentative methods of microbial production of fructooligosaccharides
There are two methods of FTase production by fermentation- Submerged Fermentation (SmF) and Solid State Fermentation (SSF). Production of enzymes by SSF has several advantages over SmF with respect to simplicity in operation, high productivity during fermentation, less contamination and concentrated product formation. SSF requires less space capital and operating costs, simpler equipment and easier downstream processing compared to SmF. In addition, it utilizes agro-industrial residues as substrates, which are converted in to bulk chemicals and fine products of high commercial value. (10)

Continuous production of fos
A continuous process using an immobilized enzyme or an immobilized microorganism is preferred due to high production rate compared to batch process. The immobilization of biocatalyst is more advantageous due to easy separation of enzyme and product, the enhancement of volumetric productivity of the reactor. Immobilization of FTases by chitosan and alginate for efficient production of FOS was studied by Ganaie et al (2014). In this study the authors had carried out enzyme immobilization with sodium alginate and chitosan to form gel beads. The alginate beads were more stable by maintaining its spherical conformation in presence of 0.3% (w/v) sodium alginate and 0.1% (w/v) of $CaCl_2$ solution to obtain a product with highest transfructosylating activity. Further, the study described the effect of sodium alginate & $CaCl_2$ concentration on entrapment of FTase for transfructosylating activity and effect of temperature and pH on free and immobilized FTase. The yield obtained was as high as 67.75% (w/w) FTase entrapped alginate beads and 42.79% (w/w) by chitosan beads in 36 h of enzyme substrate reaction (11).
Production of FOS using β-fructofuranosidase immobilized onto chitosan-coated magnetic nanoparticles was reported by Chen et al., 2013(12). The study described immobilization of β-Fructofuranosidase (EC 3.2.1.26) enzyme onto chitosan-coated magnetic nano-particles due to its various advantages such as a higher specific surface area which permit binding of a larger amount of enzymes, mass transfer resistance is relatively low, and the immobilized enzyme can be selectively separated from a reaction mixture by application of a magnetic field (13). The immobilized enzyme retained 55% of its initial activity

after 10 batches of FOS production and it achieved 59.5% yield on a dry weight basis from 50% (w/v) sucrose.
FOS and β–fructofuranosidase production by *Aspergillus japonicas* immobilized on lignocellulosic materials studied by Mussatto et al, 2009(14). Authors have reported immobilization of FFase on various lignocellulosic materials including brewer's spent grain, wheat straw, corn cobs, coffee husks, cork oak and loofa sponge. It was found that corn cobs was the best support material gave highest results of immobilization with highest productivity and yield than those obtained by free cell system
Authors have reported Immobilization conditions of Pectinex Ultra SP-L to produce FOS (15). Pectinex Ultra SP-L is a commercial enzyme preparation containing FTase activity and ability to produce SCFOS. It was immobilized onto anionic ion exchange resin. In the experiments, the optimal immobilization conditions were determined. Moreover, an integrated reactor system was constructed for simultaneous FOS production synthesis and glucose elimination to enhance the product yield. The system achieved ~74% Product yield.

PROPERTIES OF FRUCTOOLIGOSACCHARIDES

FOS delivers nutritional advantages as well as technological properties which makes it important food ingredient.
Sweetness: FOS exhibits sweetness about one third of sucrose, deliver small amount of energy, estimated to be 1.5kcal/g (16). It can be used in various kinds of foods instead of sucrose to avoid excessive sweetness.
Non – cariogenicity: FOS is not utilized by oral microflora unlike starch and simple sugars to form acids, which serve as a matrix for plaque formation and ultimately results in dental caries (17). Hence, FOS is presently utilized as non – cariogenic sugar substitutes in chewing gums, confectionery, yoghurts and drinks,
Solubility: It is water soluble which makes it useful in variety of food applications for enhancing nutritional value of product (10). In particular functional beverages, dessert and sweets, bakery products and dairy products
Viscosity of FOS solution: The viscosity FOS solution is higher than that of sucrose at same concentration. The higher molecular weight of oligosaccharides provides increased viscosity compared with mono and disaccharides, results in improved body and mouthfeel (4).
Thermal/ freezing stability: It is highly stable than sucrose and control the amount of browning due to maillard reactions in heat - processed foods. It can be used to alter the freezing temperature of frozen foods. It is highly stable at refrigerated temperatures over one year. (4)
pH range: It is highly stable in the normal pH range for food (4.0 – 7.0) (4).
Water activity: FOS- is highly hygroscopic. It provides high moisture retaining capacity, preventing excessive drying, and a low water activity, which is convenient in controlling microbial contamination (18).
Bulking agent: Bulking agents can be any reduced-calorie or non-calorie carbohydrate. This can be achieved by replacing the usual sweetening carbohydrate with an intense sweetener such as aspartame, phenylalanine or sucralose, thereby masking the aftertaste produced by some of these intense sweeteners (18) plus a bulking agent

APPLICATIONS OF FOS IN FOOD FORMULATIONS

FOS provides improved organoleptic, nutritional, functional properties which make it important functional food ingredient.
Fructooligosaccharides synthesized 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. An example of the use of FOS in food formulations includes jam products, Ice cream, confectionery applications.(4)
Padma Ishwarya et al., (19) studied the effect of addition of FOS in syrup and powder form in biscuits to provide nutritional and technological benefits. FOS in powder form had better stability and comparatively less adverse effects on the biscuit properties
Renuka et al., (20) studied the effect of fortification of FOS in selected fruit juice beverages. The physicochemical and sensory properties of fruit juices were studied during 6 months storage at ambient and refrigerated conditions. The FOS retained in 6 month storage at refrigerated temperature was significant than the ambient temperature. FOS fortified fruit beverages was acceptable up to 4 and 6 months of storage at ambient or refrigerated storage.

HEALTH BENEFITS

Low calorific value

The presence of β-glycosidic linkage makes FOS nondigestible by human digestive enzymes hence it is not utilized in the body as an energy source. The caloric contribution of FOS is due to colonic fermentation producing short-chain fatty acids (SCFA). It has energy contribution to food of about 1.5 kcal/ g.
The addition FOS leads to caloric reduction which can be achieved by replacing the usual sweetening carbohydrate with an intense sweetener and FOS. Therefore, it can be utilized in low calorie diet ,sweets, diabetic food. In sweet foods, it can be used along with intense artificial sweeteners to mask its bitter after taste (18).

Dietary fiber effect

FOS produces a soluble fiber-like effect and fits well within the concept of dietary fibre (21). It shows various physiological effects being soluble-fermentable fiber which includes resistance to hydrolysis by digestive enzymes, fermentation by the intestinal micro flora, reduced gastrointestinal transit time, increased fecal weight, lowered fecal pH, predictable reduction in caloric value, reduced plasma cholesterol and delay or decrease in glucose absorption. In addition, it may strengthen mucosal protection and reduce the risk of gastrointestinal diseases.

Prebiotic effect

FOS is not hydrolyzed by the human digestive enzymes; it undergoes fermentation in the colon. It encourages the growth of favorable bacteria in the colon; this in turn discourages the growth of potentially putrefactive microorganisms in the colon resulting in a healthy gut environment.

In human clinical study on pre biotic effect of FOS, the effect of ingestion of low dose of FOS (5 g/day) was investigated (22). There was a selective growth of certain groups of bacteria such as bifidobacteria was obsereved, thus confirming the prebiotic properties of oligofructose.

Effect on mineral absorption

Colonic fermentation of FOS leads to decrease in pH in the colon and this facilitates the absorption of mineral ions from the intestine, mainly calcium and magnesium (5).

Human Clinical Studies to assess the effect of mineral absorption

Abrams et al (23) studied effect of consumption of combination of prebiotic short- and long-chain inulin-type fructans on mineral absorption in young adolescents. In this study Design, 8 g/d of a mixed short and long degree of polymerization inulin-type fructan product (fructan group) or maltodextrin placebo (control group) randomly assigned to pubertal adolescents. Results were observed after 8 week and 1 year of supplementation, group of people consuming fructan had increased whole body mineral content and whole body mineral density than control group. It was concluded that there is increase in calcium absorption and enhances bone mineralization during pubertal growth with daily consumption of a combination of prebiotic short and long-chain inulin-type fructans but effect can be altered due to genetic modifiers of calcium absorption.
It was assumed that the effect may be due to enhancement of passive and active mineral transport across the intestinal epithelium, facilitated by an increase in certain metabolites of the intestinal flora and a reduction in pH (24).

Role of fos in defense functions

FOS is known to prevent growth of pathogenic microorganisms by encouraging the growth of beneficial bacteria. This effect is attributed to the low pH environment created during fermentation of FOS in the colon and due to the secretion of antibiotic like substances by the beneficial bacteria.

In vivo Study

Buddington, Kelly-Quagliana, Buddington, & Kimura et al. (25) studied the effect of FOS diet on colon health of mice infected with virulent strains of *Listeria monocytogenes* and *Salmonella typhimurium*. It was observed from the study that supplementing the diet of mice with inulin and oligofructose reduced the densities of *Candida* in the small intestine of mice, 7 days after infection. Mice infected systemically with virulent strains of *Listeria monocytogenes* and *Salmonella typhimurium* after being fed a diet with inulin and oligfructose (at 100 g/kg) had lower mortality due to enhanced T-lymphocyte functions than mice fed a diet with cellulose as the source of fiber. It also lowered the incidence of the infection of *Listeria* and *Salmonella* and growth of tumors after exposure to carcinogens and transplanted tumor cells.

Lipid metabolism

FOS lowers the level of serum lipids, serum cholesterol, triglyceride in blood; thus reducing the risk of obesity and diabetes (26).There are various mechanisms have been proposed to explain the effect of lipid metabolism.The first involves modulating glucose or insulin concentrations in blood. FOS decreases the glucose peaks in blood that occur after eating and, as a result, there is a significant decline in the production of glucose and insulin induced lipidic enzymes (27).The second mechanism includes the production of SCFAs in the colon. Propionate inhibits the cholesterogenesis and lipogenesis pathways, while acetate encourages the same. Therefore, the pattern of FOS fermentation and, specially, the quotient between acetate and propionate that reaches the liver may considered to be potential factor for lipid reduction (27).The third proposed mechanism involves reduction of serum cholesterol due to the precipitation and excretion of bile acids in the intestine, which suggests that the liver uses cholesterol to synthesize bile acids. On the other hand, changes in the concentration of serum cholesterol have been correlated to changes in intestinal microflora. Few strains of *Lactobacillus acidophilus* assimilate the cholesterol present in the medium, while others inhibit cholesterol absorption through the intestinal wall (28).There has been a correlation between the level of lipids in serum and the incidence of cardiovascular diseases, which makes FOS a possible tool for their prevention (27).

Anti-diabetes effect

Alles et al., (29) studied Consumption of FOS on serum lipids, serum acetate, blood glucose in 20 patients with type 2 diabetes. In a single-blind, crossover, randomized study design, patients consumed either glucose as a placebo (4 g/d) or FOS (15 g/d) for 20 d each. It was concluded that 20 d of dietary supplementation with FOS had no major effect on serum lipids, or serum acetate, blood glucose in patients with type 2 diabetes.

Anticancerous effect

Studies with inulin and FOS have shown reduction of chemically induced aberrant crypts and prevention of colon cancer. According to Pool-Zobel, van Loo, Rowland, and Roberfroid, (30) in rats, a prebiotic effect resulting in the proliferation of bifidobacteria (with the major metabolites lactate or acetate) as well as of other bacteria could be responsible for the observed anticancer effects.

CONCLUSION

FOS has emerged as functional food ingredient in market due to growing demand for healthy food. Research work has been done on production of FOS using different microbial FTase for production yield. Development of continuous methods for FOS production using immobilization has led to increased industrial production. Nutritional and Functional properties has resulted in incorporation in variety of products such as Bakery products, Beverages, Confectionery, Fruit based and dairy products. The health benefits associated with the consumption of these FOS has led to its increased popularity as food ingredients and they are also being promoted as functional food ingredient for product formulations.

REFERENCES

1. Roberfroid MB, 2007. Inulin-type fructans: Functional food ingredients. Journal of Nutrition, 137, 2493s-2502s
2. Fructooligosaccharides (FOS) from sucrose related health claims, European Food Safety Authority (EFSA), Journal 2011
3. Carpita, N.C., Kanabus, J., &Housley, T.L. (1989).Linkage structure of fructans and fructan oligomers from Triticum aestivum and Festuca arundinace a leaves. Journal of Plant Physiology,134,162–168
4. Yun, J. W. (1996). Fructooligosaccharides—occurrence, preparation, and application. Enzyme and Microbial Technology, 19, 107 –117.
5. Sangeetha, P. T., Ramesh, M.N., & Prapulla, S.G.(2005).Recent trends in the microbial production, analysis and application of fructooligo-saccharides. Trends in Food Science & Technology, 16, 442 –457.
6. Mussatto, S. I., Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. Carbohydrate Polymers, 68, 587–597
7. L'Homme,C., Puigserver, A.,& Biagini, A.(2003). Effect of food-processing on the degradation of fructooligosaccharides in fruit. Food Chemistry, 82, 533–537
8. Prapulla SG, Subhaprada v, Karanth NG (2000) Microbial production of oligosaccharides: a review. Adv Appl Microbial 47:243–299
9. http://www.mdpi.com/2218-273X/3/4/812/htm accessed on October 2014
10. Sangeetha P.T. (2003). Microbial Production Of Fructooligosaccharides, PhD Thesis submitted to University of Mysore, 23-37
11. Ganaie, M. A., Rawat, H. K., Wani,O. A., Gupta,U. S., Kango,N. (2014). Immobilization of fructosyltransferase by chitosan and alginate for efficient production of Fructooligosaccharides, Process Biochemistry ,49, 840–844
12. Chen,S.C., Sheu,D.C., Duan, K. J., (2014). Production of fructooligosaccharides using b-fructofuranosidase immobilized onto chitosan-coated magnetic nanoparticles. Journal of the Taiwan Institute of Chemical Engineers 45 1105–1110
13. Halling PJ, Dunnill P. Magnetic supports for immobilized enzymes and bioaf-finity adsorbents. Enzyme Microbio Technology 1980; 2:2–10.(24)
14. Mussatto, S., Aguilar,C. N., Rodrigues,L. R., Teixeiraa, J. A.(2009). Fructooligosaccharides and β-fructofuranosidase production by Aspergillus japonicas immobilized on lignocellulosic materials. Journal of Molecular Catalysis B: Enzymatic 59, 76–81
15. Csanadi, Z., Sisak, C., (2008) Production of short chain fructooligosaccharides. Hungarian Journal of Industrial Chemistry, 36(1-2), 23-26
16. Roberfroid MB, 2007. Inulin-type fructans: Functional food ingredients. Journal of Nutrition, 137, 2493s-2502s
17. Oku T, 1994. Special physiological functions of newly developed mono and oligosaccharides. In: Functional foods – designer foods, pharma foods, nutraceuticals Goldberg I Eds., Chapman & Hall, New York, pp. 202-217
18. Crittenden RG and Playne MJ, 1996. Production, properties and applications of food – grade oligosaccharides. Trends in Food Science and Technology, 7, 353-360
19. Padma Ishwarya, S., Prabhasankar, P., (2013) Fructooligosaccharide–Retention during baking and its influence on biscuit quality, Food Bioscience , 4 , 68–80
20. Renuka B et al. 2009. Fructooligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics .LWT-Food Science and Technology. 42(5): p. 1033-1031.
21. Cherbut C, (2002). Inulin and oligofructose in the dietary fibre concept. British Journal of Nutrition, 87, S159-S162
22. Rao, V. A. (2001). The prebiotic properties of FOS at low intake levels. Nutrition Research, 21, 843-848
23. Abrams SA, Griffin IJ, Hawthorne KM, et al. A 50. combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr 2005;82:471-476.
24. Ahrens, S.K.E., & Schrezenmeir ,J. (2002).Inulin, oligofructose and mineral metabolism-experimental data and mechanism. British Journal of Nutrition,87,S179–S186
25. Buddington, R.K., Kelly-Quagliana, K., Buddington, K. K.,& Kimura,Y.(2002). Non-digestible oligosaccharides and defense functions: lessons learned from animal models. British Journal of Nutrition,87,S231–S239
26. Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: a review. Carbohydrate Polymers, 68, 587–597.
27. Sabater-Molina, M., Larqué, E., Torrella, F., Zamora, S. (2009). Dietary fructooligosaccharides and potential benefits on health. J Physiol Biochem, 65 (3), 315-328
28. Delzenne N.M. (2003) Oligosaccharides: state of the art. Br. J. Nutr.,62, 177–182.
29. Alles M.S., de Roos M., Bakx J.C., van den Lisdonk E., Zock P.L., Hautvast J.G. (1999) Consumption of fructooligosaccharides does not favorably affect blood glucose and serum lipid concentrations in patients with type 2 diabetes. Am. J. Clin. Nutr., 69, 64–69.
30. Pool-Zobel,B.,vanLoo,J.,Rowland,I.,& Roberfroid, M.B.(2002). Experimental evidences on the potential of prebiotic fructans to reduce the risk of colon cancer. British Journal of Nutrition,87, S273–S281.