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Vandana Bali [a], Parmjit S. Panesar [a], Manab B. Bera [a] & Reeba Panesar [a]
[a] Biotechnology Research Laboratory, Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, 148106, Punjab, India
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Fructo-oligosaccharides: Production, purification and potential applications

Vandana Bali, Parmjit S. Panesar*, Manab B. Bera and Reeba Panesar

Biotechnology Research Laboratory,

Department of Food Engineering and Technology,

Sant Longowal Institute of Engineering and Technology,

Longowal - 148106, Punjab, India

***Corresponding author**

E. mail: pspanesarrr@yahoo.com

Phone: +91-1672-253252; Fax: +91-1672-280057

ABSTRACT

The nutritional and therapeutic benefits of prebiotics have attracted the keen interest of consumers and food processing industry for their use as food ingredients. Fructo-oligosaccharides (FOS), new alternative sweeteners, constitute 1-kestose, nystose, and 1-beta-fructofuranosyl nystose produced from sucrose by the action of fructosyltransferase from plants, bacteria, yeast and fungi. FOS has low caloric values, non-cariogenic properties, and help gut absorption of ions, decrease levels of lipids and cholesterol and bifidus-stimulating functionality. The purified linear fructose oligomers are added to various food products like cookies, yoghurt, infant milk products, desserts and beverages due to their potential health benefits. This review is focused on the various aspects of biotechnological production, purification and potential applications of fructo-oligosaccharides.

KEYWORDS

Fructo-oligosaccharides, prebiotics, fructosyltransferase, production, applications

1.0 INTRODUCTION

There is increasing interest of people in the health improvement and good diets, thus, nutraceuticals or functional foods have attained a special attention in the development of new food products during the last few decades (Bitzios *et al.,* 2011). Many neutraceutical products like fructo-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, lactulose etc. having prebiotic properties have been developed and are available commercially. Among the prebiotics, fructo-oligosaccharides (FOS) are of major focus due to their properties and great economic potential for sugar industry (Godshall, 2007). Being 0.4 to 0.6 times sweeter then sucrose, these are being used in pharmaceutical industry as artificial sweeteners (Biedrzycka and Bielecka, 2004). They are basically oligosaccharides of fructose possessing a single glucose moiety or bound to sucrose. These sweeteners are non-cariogenic, reduce cholesterol, phospholipids and triglyceride levels in blood (Tokunaga *et al.,* 1986), help gut absorption of calcium and magnesium, and have low caloric value. These act as prebiotics by hydrolyzing the gastro-intestinal enzymes and promoting the growth of probiotic bacteria i.e. Bifidobacteria in the colon (Moore *et al.,* 2003) and inhibit the growth of harmful microorganisms in the gut of the human preventing colon cancer and are useful as diabetic products (Gibson and Roberfroid, 1995; Sanchez *et al.,* 2010). These escape digestion in the upper part of intestine and are fermented to products such as lactate, short chain fatty acids like butyrate, propionate and acetate and gas with calorific value approximately 2 Kcal/g in the colon (Bornet *et al.,* 2002). FOS is composed mainly of 1-kestose (GF2), nystose (GF3) and fructofuranosyl nystose (GF4) (Figure 1). They occur naturally in some vegetables like onion, wheat, rye, shallots, tomatoes, bananas, garlic, Jerusalem artichokes (Wang *et al.,* 1999) or can be produced from sucrose or inulin by

microbial enzymes (bacterial and fungal sources) i.e. β-D-fructofuranosidase or fructosyltransferase. Recently there is increase in the use of fructo-oligosaccharides in the food industry which has resulted in the exploration of "new" microorganisms and enzymes for the production of fructo-oligosaccharides.

2.0 ENZYMES INVOLVED IN THE PRODUCTION OF FRUCTO-OLIGOSACCHARIDES

The FOS-producing enzymes which carry out the transfructosylation reaction (Figure 2) are classified into two categories (Chien *et al.,* 2001)

i) β-D-fructofuranosidase (FFase, EC 3.2.1.26) possessing both transfructosylating and hydrolytic activity. It releases glucose molecule from sucrose by cleaving the β-1, 2 linkage, thus, transferring the fructosyl group to sucrose and fructo-oligosaccharides. Composition of FOS, thus produced, is 1-kestose (GF2), nystose (GF3), and 1-β-fructofuranosyl nystose (GF4) having fructosyl units (F) linked at β(2→1) position of sucrose molecule (GF) (Hidaka *et al.*, 1988; Kaplan and Hutkins, 2000; Sangeetha *et al.*, 2005b; Yun, 1996).

ii) Fructosyltransferase (FTase, EC 2.4.1.9) possessing high transfructosylating activity producing FOS (2-4). FTase catalyse the transfer of fructosyl moiety from one sucrose molecule to other, thus, yielding higher FOS units (Maiorano *et al.,* 2009). 91 sequences of FTases belonging to glycoside hydrolase 32 (GH32) and 68 (GH68) families grouped in 7 clades (5 for plants, 1 for fungi and 1 for bacteria) have been reported from various plant, fungal and bacterial sources (Almeciga-Díaz *et al.,* 2011). Two types of β-fructofuranosidases F1 and F2 are produced by *Aspergillus oryzae*; F1 through transfructosylation hydrolysed sucrose (>2%) to

produce 1-kestose, nystose, and fructosyl nystose while F2 produced glucose and fructose at concentration <2% sucrose (Kurakake *et al.,* 2010).

3.0 PRODUCTION OF FRUCTO-OLIGOSACCHARIDES

Industrial production of FOS involves the action of enzymes with transfructosylating activity isolated from microbial sources like fungi such as *Aspergillus japonicus, A. niger, A. sydowi, A. foetidus, A. oryzae, A. pullulans, Penicillium citrinum, P. frequentans,* and *Fusarium oxysporum, Aureobasidium* sp.; bacteria like *Arthrobacter* sp., *Zymomonas mobilis, Lactobacillus reuteri* and *Bacillus macerans*; and yeasts like *Kluyveromyces* and *Candida* (Hernalsteens and Maugeri, 2008; Maugeri and Hernalsteens, 2007; Sangeetha *et al.,* 2005b; Sguarezi *et al.,* 2009; Yun, 1996). Commercially, food grade FOS is produced from sucrose or inulin using intracellular enzymes. Application of enzymes from plant sources is less due to seasonal variations in conditions therefore microbial enzyme is preferred for the production of FOS. These can be produced by the application of enzymes by different methods like by using whole cells producing enzymes, isolated enzymes or by using immobilization technology (either whole cell or enzyme immobilization).

3.1 Whole cell synthesis

Microbial β-fructofuranosidases with transfructosylating activity can catalyze the transfructosylation of substrates like sucrose or inulin and synthesize fructo-oligosaccharides (Table 1). Prebiotic trisaccharide, neokestose, was produced from sucrose by *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) (Kritzinger *et al.,* 2003). There was increase in the production of neoketose with cells harvested at exponential phase whereas reduction in the rate and level of production was observed on recycling the cells. A mixture of functional prebiotics FOS and

sweetening power has been produced by controlled acid thermal hydrolysis of fructans from agave (*Agave tequilana Weber* var. *azul*) (Avila-Fernandez *et al.,* 2011). Molasses has been used as sucrose equivalent by *Aureobasidium pullulans* cells for the production of FOS resulting in 166 g/L yield from 360 g/L molasses sugar (Shin *et al.,* 2004). It has been reported that oligomers can be produced from other polysaccharides like FOS can be obtained from hydrolysis of levan which is a polysaccharide produced from *Zymomonas mobilis* (de Paula *et al.,* 2008). *A. pullulans*, being a major source of FTase, produces both intracellular and extracellular ezymes. Ultrasonication has been used to release intracellular FTase from *A. pullulans* CFR 77 for the subsequent production of FOS (Lateef *et al.,* 2007). Substrate feeding of *A. pullulans* can lead to the enhanced production (25-47%) of fructosyltransferase and also increase in the final concentration and specific activity of the enzyme (Yun *et al.,* 1997). Inulinase has been produced from *Kluyveromyces marxianus* var. *Bulgaricus* using Yacon (*Polymnia sanchifolia*) as medium (Cazetta, 2005).

Extracellular β-fructofuranosidase with broad substrate specificity from yeast *Schwanniomyces occidentalis* yield prebiotic 6-kestose accounting 16.4% (w/w) FOS of total carbohydrate from sucrose, 1-ketose, nystose and raffinose (Alvaro-Benito *et al.,* 2007). Biotransformation has also been carried out from sucrose by *Aspergillus oryzae* KB producing F1 (producing 1-kestose, nystose, and fructosyl nystose) and F2 (producing glucose and fructose) β-fructofuranosidase (Kurakake *et al.,* 2008). Production of 1-ketose has also been reported from fungal strain *Scopulariopsis brevicaulis* with theoretical yield of 85% whereas crystallization and recrystallization yield of 71.0% and 78.0% with 99.9% purity (Takeda *et al.,* 1994).

Prebiotic 6-kestose has been synthesized from β-fructofuranosidase producing *Schwanniomyces occidentalis* with kinetic maximum of 101 g/L corresponding to 16.4% (w/w) of the total carbohydrates in the reaction mixture (Alvaro-Benito *et al.,* 2007). High-content fructo-oligosaccharides (80%) was produced from *Aspergillus japonicus* CCRC 93007 or *Aureobasidium pullulans* ATCC 9348 mycelia (with β-fructofuranosidase activity) and *Gluconobacter oxydans* ATCC 23771 (with glucose dehydrogenase activity) in a 7 day continuous bioreactor system equipped with microfiltration module yielding more than 160 g/L/h FOS (Sheu *et al.,* 2002). FOS production by *Penicillium expansum* in shake flasks yielded 0.58 g FOS/g sucrose (3.25 g FOS/L/h) (Prata *et al.,* 2010). Comparing batch and two step batch culture of *Aureobacterium* sp. ATCC 20524, the latter produced higher fructosyltransferase (80.5% higher) and FOS (Salinas and Perotti, 2009).

3.2 Enzymatic synthesis

Different enzymes like FFase and FTase have been employed for the synthesis of FOS (Hang and Woodams, 1996). FOS enzymes are basically isolated from microbial sources, although, they also occur in some plants (Yun, 1996). The purification of the enzyme is carried out by different methods applied solely or in combination with each other (Table 2). The cell separation from the fermentation media is generally done by centrifugation followed by recovery of the enzyme by different precipitation methods (ethanol precipitation, ammonium sulphate precipitation *etc.*). Further purification is achieved by using different column chromatography techniques.

There has been production of novel FOS's by enzymatic methods (Grizard and Barthomeuf, 1998) and the attempts for the optimization of enzymatic production have been carried out for

the maximum production of FOS (Table 3). Industrial enzymes used for the synthesis of FOS have been isolated from fungi *Aureobasidium pullulans* (Hayashi *et al.,* 1990; Yun 1996) and *Aspergillus niger* (Hidaka *et al.,* 1988). Also, β-fructofuranosidases are produced by *Aspergillus oryzae* (Kurakake *et al.,* 1996; Sangeetha *et al.,* 2004 a,b) and *Aspergillus japonicus* (Chien *et al.,* 2001). The production of FOS was achieved by FTase isolated from *Aspergillus oryzae* CFR 202 under submerged fermentation conditions with the activity of 15 ± 2 U/ml/min up to six recycles (with 53% FOS yield) using 60% sucrose as substrate at 55 °C at pH 5.15, thus, leading to development of advantageous and economical process (Sangeetha *et al.,* 2004a). Synthesis of novel fructo-oligosaccharides by substrate and enzyme engineering and random mutagenesis has also been reported (Beine *et al.,* 2008).

Prebiotic, low calorie, non carcinogenic FOS enriched with leached out bioactive components of fruits and vegetables has been produced by fructosyl transferase enzyme from the value addition of spent osmotic sugar solution (SOS) recovered from the osmotic dehydration of carrot cubes (Aachary and Prapulla, 2009). Attempts have also been made to produce fructans (1-kestose, 6-kestose, neokestose and nystose, as well as other non identified fructo-oligosaccharides) from ethanol producing bacteria *Zymomonas mobilis* from sucrose syrup (Bekers *et al.,* 2002). Yeast strains of *Candida* sp. (LEB-I3), *Rhodotorula* sp. (LEB-U5.), *Cryptococcus* sp. (LEB-V2) and *Rhodotorula* sp. LEB-V10 isolated from fruits and flowers (from Brazilian tropical forests) produced approximately 100 g/L of FOS from 500 g/L sucrose solution (Maugeri and Hernalsteens, 2007). Mixed enzyme system of β-fructofuranosidase and glucose oxidase has been used in stirred tank reactor for the batch production of high content FOS (upto 98%) from sucrose and glucose as substrate (Yun *et al.,* 1994).

Studies have also been carried out to investigate the role of enzyme's active site for catalytic action, scope and limitation of substrate in the synthesis of sucrose analogues by the action of *Bacillus subtilis* fructosyltransferase (Seibel *et al.,* 2006). Central composite experimental design (CCD) and response surface methodology (RSM) were employed to study the effect of sucrose and yeast extract on β-fructofuranosidase production isolated from *Aspergillus japonicas* TIT-90076 at pH 5.0 and 55°C using sucrose, yeast extract, $MgSO_4.7H_2O$ and K_2HPO_4 as enzyme production media (Chen and Liu, 1996).

3.3 Immobilized Biocatalysts Based Synthesis

Many bacterial and fungal strains or their enzymes bearing transfructosylation activity, isolated from different sources, have been immobilized on different materials for the maximum production of FOS under optimum conditions. *Aspergillus japonicas* isolated from soil has been immobilized on calcium alginate beads for FOS production and used for 23 cycles without the loss in activity (Cruz *et al.,* 1998). The production obtained from 5.75% (cellular weight/ volume) mycelia (122·4 Ut/g) and 65% sucrose solution (w:v) under optimum conditions in the final product were 61·28% of total FOS containing GF2 (30·56%), GF3 (26·45%), GF4 (4·27%), sucrose (9·6%) and glucose (29·10%) whereas fructosyl-transferring enzyme from *Aureobasidium* sp. ATCC 20524 has been immobilized on a *shirasu* porous glass for the production of 1-kestose from sucrose (Hayashi *et al.,* 1991). Two step production process has also been reported for the maximum yield of FOS from sucrose in an internal-loop airlift bioreactor using calcium alginate-immobilized mycelia of *A. japonicas* (1%) and *Aspergillus niger* (7%) (Lin and Lee, 2008). Initially 55% (w/w) FOS was produced from total sugars by *A. japonicas* followed by removal of generated glucose by *A. niger* resulting in the production of

90% (w/w) FOS fraction. Calcium alginate gel was used to immobilize fructofuranosidase producing cells of *Aspergillus niger* St-0018 and *A. foetidus* St-0194 for the production of FOS under periodic and continuous conditions (Markosian *et al.*, 2007).

Different lignocellulosic materials including brewer's spent grain, wheat straw, corn cobs, coffee husks, cork oak, and loofa sponge were used as support material for FOS and FFase production from *A. japonicus* ATCC 20236 (Mussatto *et al.*, 2009a). Corn cobs, coffe silverskin and cork oak (agro-industrial residues) were used in solid-state fermentation of *A. japonicus* as support and nutrient source for the production of FOS out of which coffee silverskin yielded FOS 127.7 g/L and 71.3 U/ml β-fructofuranosidase activity (Mussatto and Teixeira, 2010). *In situ* immobilization by absorption of *Aspergillus japonicus* ATCC 20236 was done on different synthetic materials (polyurethane foam, stainless steel sponge, vegetal fiber, pumice stones, zeolites, and foam glass) for the production of FOS from sucrose out of which vegetable fibre proved to be the potential and economical support matrix for the production of FOS at industrial scale (Mussatto *et al.*, 2009b). Using sucrose as substrate, corn corbs proved to be the best immobilizing matrix, as compare to free cell system, with 1.49 g/g carrier microorganism immobilization, 6.61 g/L/h FOS productivity and yield (0.66 g/g based on total substrate; 0.73 g/g based on consumed substrate).

Transfructosylating activity of pectinases (Pectinex Ultra SP-L, from *Aspergillus aculeatus*, and Rapidase TF, from *Aspergillus niger*) has also been studied for the production of 387 g/L (61.5% w/w) FOS by covalently immobilized polymethacrylate-based polymer from total carbohydrate in the reaction mixture (Sepabeads® EC) (Ghazi *et al.*, 2005). Also, covalent immobilization of Pectinex Ultra SP-L on Eupergit C resulted in 96% relative activity without any decrease in

activity upto 20 batch reactions (Tanriseven and Aslan, 2005). It produced approximately 57% FOS of the total sugars from 60% (w/v) sucrose solution with 96% efficiency as compared to free enzyme system.

Aspergillus niger ATCC 20611 and *Aspergillus japonicus* TIT-KJ1 isolated β-fructofuranosidases, immobilized covalently on methacrylamide-based polymeric beads, produced approximately 60% FOS of the total sugars in the reaction mixtures from 50% (w/w) sucrose solution (Chiang *et al.,* 1997). It was also reported that with the increase in the initial sucrose concentration there was increase in the total FOS fraction. The production of FOS from β-D-fructofuranosidase producing *A. japonicus* has been carried out by immobilizing on gluten (Chien *et al.,* 2001). The maximum reaction velocity was achieved with 20% (w/w) of cell content producing 61% FOS of the w/w of total sugars in the reaction mixture. With the increase in the residence time and decrease in flow rate, there was increase in the FOS mass fraction. Immobilization of exo-fructosyltransferase produced from *B. subtilis* NCIMB 11871 has been done on Eupergit® C 250 L and Trisopor®-Amino at 50 °C for the synthesis of sucrose analogue galactosyl-fructoside (Baciu *et al.*, 2005).

Packed bed plant scale reactor have been studied for the continuous production of FOS from calcium alginate immobilized *Aureobasidium pullulans* giving productivity of 180 g FOS/L/h for more than 100 days (Jung *et al.,* 2011). The same system was run for immobilizing thermostable β-Fructofuranosidase from *Aspergillus japonicus* for 35 days with only 17% loss in the activity during the period (Cheng *et al.,* 1996). 50 day continuous production of neo-FOS yielding 49 g/L was carried out using whole cell immobilization of *Penicillium citrinum* KCCM 11663 in packed bed reactor (Park *et al.,* 2005). Co-immobilization of whole cells (*Penicillium citrinum*) together

with neo-fructosyltransferase increased the production of neo-FOS (Lim *et al.,* 2007). Various studies of immobilization of fructosyltranferase from *Rhodotorula* sp. by adsorption on niobium ore was reported (Aguiar-Oliveira and Maugeri, 2010, 2011; Alvarado-Huallanco and Filho, 2010).

4.0 FACTORS EFFECTING FRUCTO-OLIGOSACCHARIDE PRODUCTION

The optimum conditions and the effect of process parameters like substrate concentration, temperature and pH on FOS production differs from one microorganism to other microorganism. With the increase in substrate concentration above the optimum level, there is decrease in FOS production rate. Kinetic analysis of competitive product inhibition predicted that sucrose reaction rate was inhibited at concentration greater than 20% (w/v) (Duan *et al.,* 1994). A rapid conversion of sucrose to glucose and fructose was observed at low sucrose concentration (10 g/dm^3) and very low concentrations of FOS were obtained whereas there was reduction in the concentrations of hydrolysed product at sucrose concentrations higher than 216 g/dm^3 (Fernandez *et al.,* 2004). In a two stage continuous production process of FOS from FTase producing *Aspergillus oryzae* CFR 202, maximum yield was obtained using response surface methodology based on shell design grown initially on media containing sucrose followed by the production of FOS by enzyme using sucrose as substrate (Sangeetha *et al.,* 2005a). Cell concentration negatively affects the maximum neo-FOS yield by *Xanthophyllomyce dendrorhous* (Ning *et al.,* 2010).

Media optimization for increasing the β-fructofuranosidase production from *Aspergillus japonicus* TIT-90076 has been done resulting in 180% increased production (Chen, 1998). FOS has also been produced from plantation white sugar (PWS) - table sugar with ICUMSA 200-300

by using *Aspergillus* FTase (Toharisman *et al.,* 2009). Attempts have been made to produce β-fructofuranosidase from *Aspergillus japonicus* from industrial waste like soyabean residue for the production of FOS (Hayashi *et al.,* 1992). Sugarcane molasses (3.5-17.5% w/v total sugar) and yeast powder (1.5-5% w/v) were used for FTase and FOS production from *Aspergillus japonicus*-FCL 119T and *Aspergillus niger* ATCC 20611 (Dorta *et al.,* 2006).

Mostly the FOS production is optimum at pH 5.5 and temperature 60 °C but variations are observed with enzymes isolated from different sources. The FOS yield was stable from 4-8 with temperature between 50–65 °C (Madlova *et al.,* 2000). A sharp decrease in the enzyme activity is also observed above the optimum temperature (Cazetta *et al.,* 2005). Incubation time also effects the FOS production. Due to self hydrolase activity of transfructosylating enzyme, prolonged incubation results in the conversion of major product GF_4 into GF_2 (Nemukula *et al.,* 2009). FOS proportion of 600-620 g/kg in the reaction mixture was observed at pH 5.5 and temperature 40°C where as the optimum transfructosylating activity was observed at pH 5.5-6.0 at °60 C and that for hydrolytic activity at pH 4.0 at 55 °C (Fernandez *et al.,* 2004).

Immobilization of whole cells or enzymes, increase the FOS production rate but it has been reported that higher productivity was achieved in some cases with free cells as compared to immobilized ones (Ning *et al.,* 2010). Decrease in the enzyme activity has also been reported by the repeated cycles in the immobilized batch fermentation systems (Mussatto *et al.,* 2009b).

5.0 PURIFICATION OF FRUCTO-OLIGOSACCHARIDES

Purification and estimation of FOS is achieved by different quantitative methods. Chromatographic separation has been performed by using mobile and stationary chromatographic system for the separation of FOS. Glass-packed precoated silica gel with

sodium acetate was used for optimal separation of FOS in dietetic products (Reiffova and Nemcova, 2006). Onion (*Allium cepa* L.) soluble non-structural carbohydrates i.e. sugars and FOS (degrees of polymerisation (DP) in the range of 3–19) have been separated using liquid chromatography (LC) with acetonitrile (ACN) as a mobile phase (Vagen and Slimestad, 2008). Alternative economical methods have been developed to quantify sugars without the use of ACN. Using Ethanol as mobile phase, separated and comparable concentrations of all FOS are eluted with that of ACN followed by LC based isocratic water mobile phase separation of sugars (Downes and Terry, 2010). As compared to ethanol, methanol based extraction proved to be more efficient, eluting both sugars and FOS at higher concentrations. FOS have been separated from mixture of sugars with activated charcoal fixed bed column with separation coefficient of 3.99 ± 0.07 between fructo-oligosaccharides and glucose (ES(fructoolig/gluc)) and 2.89 ± 0.36 between fructose and fructo-oligosaccharides (ES(fructoolig/fruct)) with 80% degree of purification and 97.8% recovery of FOS by using 2^2 central composite design (Kuhn and Filho, 2010).

The application of high-performance anion-exchange chromatography along with pulsed amperometric detection (HPAEC-PAD) and capillary zone electrophoresis (CZE) has been reported for the detection of fermentation properties of FOS in pure *Bifidobacterium* cultures (Corradini *et al.*, 2004). HPAEC-PAD method has also been reported to selectively determine short chain FOS in prebiotic foods such as fermented milk (Borromei *et al.*, 2009). Similarly, fractionation/gradient elution of oligosaccharides can also be achieved by hydrophilic interaction chromatography followed by detection through pulsed amperometric detection (Feste and Khan, 1993). Single-column chromatographic separation of FOS has been reported using Amberlite™

CR1320Ca, a cation-exchange resin, with 86% yield (Vankova and Polakovic, 2010). Improved separation of mono- and disaccharide mixtures can be achieved by using Zeolite fixed bed columns. Ba^{2+}-exchange Y zeolites packed in fixed bed columns were used to identify and quantify GF2 (kestose), GF3 (nystose) and GF4 (frutofuranosyl nystose) in an ion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Kuhn and Filho, 2010).

FOS has also been isolated from different plant parts. FOS has been isolated from roots and leaves of *Stevia rebaudiana* by hot aqueous extraction following ethanol precipitation (de Oliveira *et al.,* 2011). The separation and quantification of high molecular weight plant extract fructans, ranging from degree of polymerization (DP) 3 to 49, has been achieved by reverse-phase liquid chromatography coupled with negative electrospray ionization mass spectrometry (Harrison *et al.*, 2009). Both high and low molecular weight fructans has been isolated and their structure has been elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy from garlic (*Allium sativum*) for detecting their immuno-modulatory properties (Chandrashekar *et al.,* 2011). 80% yield with 90% purity of enzymatically transformed FOS from sucrose of sugarcane molasses has been achieved by using two stage nanofiltration process (Kuhn *et al.,* 2010). Different techniques like NMR, MALDI-MS, MALDI-TOF, GC-MS and ESI-MS are used for structure prediction of purified FOS (Arrizon *et al.,* 2010; Wang *et al.,* 1999).

Purity of FOS can be increased by a two step strategy. FOS was synthesised using extracellular β-fructofuranosidase produced by *Aspergillus japonicus* (yields higher FOS content) followed by cultivation of *Pichia pastoris* (exhaust all monosugars), thus, increasing FOS purity (Yang *et al.,* 2008). Microarrays have also been used for the identification of prebiotic FOS metabolism in microorganisms (Saulnier *et al.,* 2007).

6.0 APPLICATIONS

Fructo-oligosaccharides from sucrose have a number of desirable characteristics such as low calories, no cariogenicity, safety for diabetics, and bifidus-stimulating functionality (Yun, 1998).

6.1. Dietary modulation of the human colonic microbiota

The chicory fructo-oligosaccharides, a natural food ingredient in most European countries, is one of non-digestible oligosaccharides and obtained by enzymatic synthesis using sucrose. In healthy humans, these oligosaccharides are only slightly digested in the small intestine and fermented in the colon, resulting in reduced energy production (Molis *et al.,* 1996). These stimulates the growth of Bifidobacteria (Fahey, 2010; Losada and Olleros, 2002) thereby modifying the composition of the colonic microbiota and also led to a significant decrease in the number of potentially pathogenic bacteria thus making them good candidate as functional foods. Besides bifidogenic effect, the chicory fructo-oligosaccharides have additional nutritional properties as dietary fibres and effect digestive physiological parameters like colonic pH and stool bulking. In experimental model organisms, scientists reported the improvement of the bioavailability of essential minerals and reduce serum triglyceridemia by lowering hepatic lipogenesis. Such effects demonstrate interactions between the chicory fructo-oligosaccharides and key functions in the body but their significance for humans is to be proved (Roberfroid, 1997). Moreover, these prebiotics modulate lipid metabolism, mostly by fermentation products. These properties prove FOS as health-enhancing functional food ingredient (Gibson and Roberfroid, 1995). Though FOS has usually been regarded as a selective substrate for bifidobacteria, lactobacilli are uses

these oligosaccharides can out-compete bifidobacteria in continuous culture at pH 5.2-5.4 when FOS is the primary carbon and energy source (Sghir *et al.*, 1998). FOS produces a mild prebiotic effect without any gastrointestinal discomfort in pediatric patients with cancer (Zheng *et al.*, 2006). Shadid *et al.*, (2007) studied the effect of galacto-oligosaccharide and long-chain fructo-oligosaccharide supplementation during pregnancy on maternal and neonatal microbiota. Bifidobacteria in the maternal gut were significantly higher in the supplemented group than control however in neonates, bifidobacteria and lactobacilli percentages, diversity and similarity indexes, and foetal immune parameters did not differ significantly thereby indicating that maternal gut microbiota is not transferred to neonates. The increased maternal bifidobacteria did not affect foetal immunity as measured by a comprehensive examination of cord blood immunity. Effect of short-chain FOS supplementation in equine intestinal microflora and its effectiveness on microbial disturbances related to sudden diet changes i.e. an abrupt incorporation of barley in the diet was carried out (Respondek *et al.*, 2008). The addition of barley to the control diet increased the concentration in total anaerobes, lactobacilli, streptococci, and lactate-utilizing bacteria. The scFOS supplementation reduced the barley intake-related changes and increased the colonic D-lactate concentration. 4 g FOS per day promotes the proliferation of bifidobacteria in the intestine (Buddington *et al.*, 1996; Howard *et al.*, 1995; Oyarzabal and Conner, 1996). As compared to supplementation of acidophilus, or LAC + FOS, addition of FOS alone may improve gut health by decreasing certain faecal protein catabolites (Swanson *et al.*, 2002).

6.2 Immuno-modulatory effect

The proper functioning of immune system is dependent on sufficient supply of nutrient. Fermentation products of FOS may modulate the gut associated lymphoid tissue (GALT) as well as the systemic immune system (Seifert and Watzl, 2007). Clinical trials indicated decreased appearance of clinical inflammation, reduced level in cytokines interleukuin (IL)-1α and necrosis factor α in ulcerative colitis (Furrie *et al.,* 2005) by supplementation with *Bacillus longum*, inulin and FOS. Similarly in Crohn's disease patients, intake of inulin and FOS led to increased IL10 positive mucosal dendtritic (Lindsay *et al.,* 2006). The effect of FOS in healthy and endotoxemic animals was investigated (Manhart *et al.,* 2003). The FOS-enriched diet increased the total cell yield in healthy and endotoxemic mice. Similarly, B lymphocytes were increased in both groups. In contrast, T lymphocytes were unaltered in healthy mice but increased in LPS-challenged mice after FOS enrichment. As compared to control animals, the endotoxemic mice showed increase of CD^{4+} cells as compared to CD^{8+} cells, thereby increasing the CD4:CD8 ratio. Besides the large intestine, FOS administration also affects the inductive part of the mucosal immune system in the small intestine. A number of studies reported the immuno-modulation effect of FOS (Bunout *et al.,* 2004; Delgado *et al.,* 2011; Guigoz *et al.,* 2002; Hosono *et al.,* 2003; Morrison *et al.,* 2006; Nakamura *et al.,* 2004; Osman *et al.,* 2006; Roller *et al.,* 2004; Swanson *et al.,* 2002).

6.3 Fortification of selected fruit juice beverages

Fortification of various fruit juice especially pineapple, mango and orange juice with fructo-oligosaccharides has also been carried out (Renuka *et al.,* 2009). Experimental results suggested that sucrose which is used as fruit juice sweetener can be partially substituted with FOS without significantly affecting the overall quality. Changes in various physicochemical and sensory parameters during storage of fortified juices, at room temperature and under refrigerated

conditions, were evaluated. The pH, total soluble solids, titratable acidity, and colour did not change significantly during storage. The sensory properties indicated the acceptance of fortified juices.

6.4 Antioxidant properties

The effect of heating at high temperature and proteolysis on antioxidant properties of Maillard reaction mixtures of soy protein isolates (SPI) and FOS with suitable controls was studied (Mesa *et al.*, 2008). Glycation and cross-linking of protein was estimated. *In-vitro* gastro-intestinal conditions generated by hydrolysis were determined by LDL oxidation and oxygen radical absorbance capacity assays. Studies indicated that neo-antioxidants formed by thermal degradation of FOS (caramelization) prevented the LDL oxidation and scavenged peroxyl-alkyl radicals. Peptides derived from soy protein scavenged peroxyl radicals and did not protect LDL against copper oxidation.

6.5 Potential sweetener for diabetics

Diabetes mellitus is one of the major disorders associated with changing life style and involves mal functioning of endocrine system. In severe cases, it can lead to cardiovascular diseases, renal failure, diabetic foot, blindness and even premature death. Hydrolysis of sucrose by action of FTase from *Aspergillus oryzae* results in mixture containing glucose (28–30%), sucrose (18–20%) and FOS (50–54%). Identification of oligomers present in the mixture of FOS using NMR spectroscopy and LC–MS (Mabel *et al.*, 2008) indicated the presence of mono to pentasaccharides with none of toxic microbial metabolites, thereby, increasing the possibility of its application as a food ingredient.

Results of streptozotocin-induced diabetic rats fed on FOS (5 and 10%) indicated its use as an alternative non-nutrient sweetener without any deleterious effects. Despite the high free-sugar content associated with it, FOS did not increase the hyperglycemia and glucosuria in diabetic animals, even at 10% levels. The decrease in loss of blood protein is also observed in diabetic rats (Mabel *et al.,* 2008).

6.6 Supplementation of oral electrolyte solutions for treatment of diarrhoea

Secretory diarrhoea disturbs the normal densities and relative species abundance of the microbiota in the intestine. Diarrhoea caused significant declines in total bacterial counts in mid small intestine, cecum and distal colon. Oral electrolyte solutions (OES) replenish salts and water lost during the disease. Oli *et al.,* (1998) evaluated the efficiency of an OES with and without FOS for treatment of pigs with acute diarrhoea induced by cholera toxin. Both treatment with OES and FOS accelerates the recovery of total bacterial counts; pigs treated with OES showed increase in densities of *Enterobacteriaceae* while FOS promoted lactobacilli growth. Studies on the prebiotic effect of FOS intake on weight gain and acute diarrhoea in 150 children of urban slum in Bangladesh concluded that daily intake of FOS significantly reduced the duration of diarrhoea days and the duration per episode (Nakamura *et al.,* 2006).

6.7 Absorption of Fe^{2+}, Mg^{2+}, Ca^{2+} and other ions

In intestine, fermentation of FOS decreases the pH and help in the absorption of various minerals including Mg^{2+}, Fe^{2+} and Ca^{2+}. In gastrectomized experimental animals, the intake of FOS in diet led to an increased iron absorption thereby helping in recovery from anaemia (Ohta *et al.,* 1998). Prevention of osteopenia was also obtained in totally gastrectomized rats. Major portion (99%) of the body's calcium is stored in bone and its salts are responsible for the hardness of bones

(Heaney, 1996) along with Mg^{2+} (Rude, 1996). The positive effects of FOS on intestinal calcium and magnesium absorption have been well documented (Morohashi *et al.,* 1998, Ohta *et al.,* 1998, Takahara *et al.,* 2000). Rats fed with Mg-deficient diet exhibited auricular and facial peripheral hyperemia and haemorrhage (Ohta *et al.,* 1995). Increased concentrations of Ca^{2+} or P in the experimental diets significantly decreased the apparent absorption of Mg^{2+}. FOS increased the Mg^{2+} absorption in rats fed a low-Mg^{2+}, high-Ca^{2+}, and high-P diet. Moreover, FOS reduced inflammation in Mg^{2+}-deficient rats, such as peripheral hyperemia and haemorrhage. FOS-feeding improved recovery from anaemia and increased the absorption of Ca^{2+} and other metal in Fe^{2+}-deficient anaemic rats (Gudieal-Urabano and Goni, 2002; Ohta *et al.*, 1994 a,b). Increased true calcium absorption and balance produced by FOS feeding might improve bone calcification (Morohashi *et al.,* 1998). FOS also effects the expression of genes transient receptor potential vanilloid type 6 (TRPV6), calbindin-D9k, and plasma membrane calcium-ATPase 1b (PMCA1b) involved with calcium absorption in rat colorectal mucosa cells (Fukushima *et al.,* 2009) suggesting that FOS increase calcium absorption by increasing mRNA expression of TRPV6. Addition of FOS at 5% along 1% calcium prevented the bone loss in rats while 10% FOS with 0.5% calcium increases the bone mineralization (Scholz-Ahrens and Schrezenmeir, 2002).

6.8 Reduction of Lipids

Dietary fibers may modulate insulin resistance and glucose homeostasis. However, their efficacy is dependent on their origin, physical properties, and fermentability. Studies in dogs indicated progressive appearance of insulin resistance with fattening and the rate of glucose infusion during euglycemic clamp was lower at the end of the fattening period (Respondek *et al.*, 2008). In obese dogs, FOS increased the rate of glucose infusion compared with control. FOS increased

uncoupling protein 2 and also carnitine palmitoyl transferase 1 adipose mRNA levels rises during the postprandial period. Further adding 1% scFOS to the diet of obese dogs decreases insulin resistance and help to modulate the transcription of genes involved in fatty acid or glucose metabolism. Further, fermentation of FOS increases the SCFA (propionic acid) in the intestine which in turn reduces the levels of TAG and cholesterol associated with LDL and VLDL, thereby, indicating the application of FOS against hypercholesterolemia (Mabel *et al.,* 2008).

6.9 Enhancing *Salmonella* Vaccine Efficacy

The effect of FOS: inulin mix on murine response to *Salmonella* vaccine and effect on protection against *Salmonella* infection was studied (Benyacoub *et al.,* 2008). Balb/c mice were fed 5% FOS: inulin mix diet with suitable control before oral immunization with a suboptimal dose of live attenuated *Salmonella typhimurium* vaccine. Infection of mice with LD100 of virulent *S. typhimurium* after sufficient time interval showed increased specific blood *Salmonella* immunoglobulin G and faecal immunoglobulin A in mice fed the diet containing fructo-oligosaccharide and inulin as compared with control mice. Peritoneal macrophage phagocytic activity also significantly increased in FOS: inulin-fed mice at 1 week post-immunization as compared with control mice. However, the improved response to *Salmonella* vaccine was concomitant with an increase in the survival rate of FOS: inulin-fed mice upon challenge with virulent *Salmonella,* thereby, suggesting that a diet supplemented with FOS: inulin mix stimulates mucosal immunity and seems to improve efficacy of an oral vaccine.

6.10. Antibiotic Therapy

Treatment of various aliments with antibiotics especially penicillin, cephalosporin, and clindamycin are found to be associated with acute diarrhoea due to loss of normal protective intestinal microflora (Fekety and Shah, 1993; Mcfarland and Bemasconi, 1993). In a double blind randomized controlled trials, patients taking fructo-oligosaccharides were less likely to develop diarrhoea during antibiotic treatment (Madeo *et al.,* 1999). While determining the efficacy of FOS-*Lactobacillus sporogenes* preparation in the prevention of diarrhoea due to antibiotics treatment in children, La Rosa *et al.,* (2003) found 71% diarrhoea prevention in under treatment children as compared to 38% in control. Many of commercial products also contain FOS which enhance and promote the therapeutic benefits of the probiotic organisms.

6.11 Cancer Treatment

FOS may help protect against cancers especially colorectal cancer (Pierre *et al.,* 1997). Protective action of FOS in the colon might be through activity of butyrate - a short chain fatty acid produced by FOS in the colon. Butyrate also play role in prevention of tumor growth, cell differentiation and up-regulate apoptosis. Feeding FOS reduced the development of intestine tumor, while development of lymphoid nodules in the gut-associated lymphoid tissue (GALT) was increased in a study carried out in human intestinal cancer model *Min* mice (having mutation in the *Apc* gene) as compared to immuno-compromised animals (Pierre *et al.,* 1997, 1999). Similarly, the decrease in AOM-induced colon cancer in F344 rats by oligofructose diet has also been observed (Femia *et al.,* 2002). Immuno-modulation in Peyer's patches reported to be responsible for anti cancer effect of FOS (Roller *et al.,* 2004).

6.12 Miscellaneous applications

Due to non caloric nature supplementation of diet with FOS help in decreasing fat mass and help in controlling the caloric intake in overweight and obese adults (Parnell and Reimer, 2009). Children with atopic dermatitis (AD) a chronic itching skin disease, have a high risk of developing asthma. Effect of early intervention of *Bifidobacterium breve* M-16V and Immunofortis® (a galacto/fructooligosaccharide mixture) in 90 infants with atopic dermatitis has also been reported (van der Aa *et al.*, 2011). 70.7% of patients showed protection against the asthma-like symptoms like frequent wheezing' and 'wheezing and/or noisy breathing apart from colds'. An elevation of one or more of the cholesterol, cholesterol esters, phospholipids, or triglycerides led to hyperlipidemia. Development of coronary heart diseases is associated with elevated level of total and low-density lipoprotein cholesterol (LDL) and reduced high-density lipoprotein cholesterol (HDL). Three groups of people with hyperlipidemia were fed with soy food, soy food with FOS and low fat dairy diet plus FOS respectively. Combination of soy and prebiotics lowered LDL cholesterol levels more than either soy or prebiotics alone, thereby, indicating the beneficial effects of oligosaccharides (Wong *et al.*, 2010). There are high incidences of impaired oral function, bowel function and chronic in elder people. FOS has been commonly used to modulate bowel function. FOS in healthy young adults significantly improves defecation frequency and a feeling of incomplete defecation (Gibson *et al.*, 1995; Liu *et al.*, 1994). Fermentation of fructo-oligosaccharides by bifidobacteria exerts excellent eliminating effects on free radicals (Wang *et al.*, 2008). FOS supplementation significantly increased dry faecal mass in group of constipated elderly men followed by partly decreased at the end of the post-FOS period (Yen *et al.*, 2011). It has been suggested that continuous supplementation is

essential to maintain significant improvement of bowel function in older persons with long-term constipation. *In vitro* fermentation of FOS by lactic acid bacteria increases the radical scavenging ability and decreases lipid peroxide, thereby, indicating the antioxidative effects of FOS in humans. Studies have reported the antioxidative effect of FOS supplementation is closely associated with its bifidogenic effect. The decrease in total serum cholesterol level in normolipidemic rats by dietary supplementation of inulin while decreased hepatic cholesterol level (Fiordaliso *et al.,* 1995) and atherosclerotic lesion area in aortic sinus in apolipoprotein E–deficient mice was observed (Rault-Nania *et al.,* 2006). Hypocholesterol effects of FOS might be due to inhibition of hepatic cholesterogenesis by propionate produced during fermentation of FOS (Demigne *et al.,* 1995) mechanism. *In vitro* study showed that lactic acid bacteria assimilate cholesterol from the culture medium (Pereira and Gibson, 2002) leading the theory that FOS might increase faecal cholesterol excretion by stimulating bifidobacteria growth, which in turn decreases the blood cholesterol level.

FOS-induced increase in faecal bifidobacteria excretion and decrease in plasma cholesterol level, indicating that bifidobacteria partly mediate the hypocholesterolemic effect of FOS in elderly subjects (Yen *et al.,* 2011).

7.0 COMMERCIAL PRODUCTION

Fructo-oligosaccharides are reported to be present in many natural foods; however they occur in low concentration. Due to wide applications and to produce it at larger scale, a number of companies are producing them by application of enzymes. Commercial use of FOS started in 1980s due to consumer demand for healthy and calorie-reduced foods. There is tremendous growth of fructo-oligosaccharide market and according to Global Industry Analysts Inc. (GIA),

the U.S. market for prebiotics of which fructo-oligosaccharides is part, will reach $225.1 million by 2015, while European sales are expected to hit $1.17 billion (Roberts, 2011). Production of Beneshine™ (P-type Powder and liquid scFOS (> 95% purity) from sucrose by applications fructosyltransferase (FTS for possible application in can be taken as the specific food for diabetic patients, overweight individuals, and weight-losing persons) is being carried out by Shenzhen Victory Biology Engineering Co., Ltd., China (http://www.vitafos.com/company.asp). Commercial FOS syrups exhibit sweetness levels between 30- and 50-percent of sugar. FortiFeed® P-95 (http://www.fortifeed.com/PDF/ Fortifeed.pdf), a powdered soluble prebiotic fiber, produced from cane sugar using non-GMO raw materials and a natural fermentation method, contains minimum of 95% short-chain FOS on a dry basis (Corn Products International, Inc.). Highly bifidogenic, fructo-oligosaccharides Actilight® selectively nourishes friendly gut bacteria (http://www.actilight.com). Allergy Research Group LLC developed and producing FOS as a dietary Eliminex®, a FOS derived from chicory root consists of a special kind of pure soluble fibre and carry moisture through the digestive system.

8.0 CONCLUSIONS

Fructo-oligosaccharide are non-cariogenic, low caloric, fibrous, and strong bifidus-factors, which have scientifically proven health benefits including role in absorption of calcium and minerals, replacement of sugar and fat in food products, reduction in cholesterol, controlling various diseases like cancer, prevention of necrotizing enterocolitis (have shown promising results in animal models only) etc. Even change in the gut microbiota of infants and alteration in the large bowel function by application of FOS has been observed, but large clinical trials are needed to prove their potential. Novel microorganisms producing potential transfructosylating enzymes are

needed to be explored for their application in FOS production, the scale up studies should be made for their industrial applications.

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TABLE 1: Micro-organisms employed for Fructo-oligosaccharides production

Source of enzyme	Mode of production	Reaction conditions	Max FOS	Reference
Scopulariopsis brevicaulis N-01	Fermentation	40°C ; pH 6–9	NA	Hatakeyama *et al.*, 1996
Aspergillus oryzae CFR 202	Submerged fermentation	60% sucrose; 55 °C ; pH 5.15	53%	Sangeetha *et al.*, 2004a
Aspergillus oryzae MTCC 5154	Submerged fermentation	60 °Bx sucrose ; 55 °C	50.63 to 57.86 %	Aachary and Prapulla, 2009
A. japonicus NTU-1249	Submerged fermentation	50% (w/v) sucrose ; 50 ^{o}C ; pH 5.0	60%	Su *et al.*, 1991
Pectinex® Ultra SP-L) from *A. aculeatus*	NA	300 mM Sucrose; 60 °C; pH 5.6	88%	Nemukula *et al.*, 2009
Microbacterium laevaniformans ATCC 15953	Submerged fermentation	5% sucrose; 30 ^{o}C; pH 6.5	Variable	Park *et al.*, 2003
Gluconacetobacter diazotrophicus (recombinant)	Submerged fermentation	250 mM sucrose; 60°C; pH 5.0	100 g l^{-1}	Trujillo *et al.*, 2001
Penicillium frequentans (*P. glabrum*)	NA	15% (w/v) sucrose	NA	Usami *et al.*, 1991
Aspergillus sp. N74	Agitated airlift reactor	70% (w/v); 60 °C; pH 5.5	69-70%	Sanchez *et al.*, 2008
Rhodotorula	Submerged fermentation	70% (w/v) sucrose; 50 °C ; pH 4.5	NA	Alvarado-Huallanco and Filho, 2011
Aureobasidium pullulans KCCM12017	Submerged Production	Sucrose; 55^{o}C; pH5.5	GF_2 :120g $L^{-1}h^{-1}$; GF_3 :86g $L^{-1}h^{-1}$; GF_4 :9.6g $L^{-1}h^{-1}$	Shin *et al.*, 2004
Aureobasidium pullulans KFCC 10524	Fed batch Fermentation	Sucrose; 55^{o}C; pH5.5	NA	Yun *et al.*, 1997

Aspergillus sp 27H		615 g L^{-1} sucrose ;40^{o}C; pH5.5	376 g dm(-3)	Fernandez *et al.*, 2004

TABLE 2: Microbial sources, purification and properties of FOS producing enzymes

Enzyme	Micro-organism	Purification	Properties	Reference
β-Fructofuranosidase I (FFase I)	*Aureobasidium pullulans* DSM 2404	Centrifugation	Not affected by various metal ions; shows high transfructosylating activity	Yoshikawa *et al.*, 2007
Transfructosylating enzyme	*Bacillus macerans* EG-6	Ammonium Sulphate Precipitation (20–60%), CM-Sepharose CL 6B and fast protein liquid chromatographies on Resource Q, Phenyl-Superose HR 5/5 and Mono S	Stable at pH 5.0-7.0, optimum pH 5.0 and temp 50 °C	Park *et al.*, 2001
β-fructofuranosidase (EC 3.2.1.26)	*Aspergillus niger* IMI303386	$(NH_4)_2$ SO_4 precipitation, DEAE Sepharose fast flow ion-exchange chromatography and Ultrogel AcA44 gel filtration	Stable at pH 4.0-8.0, optimum pH 5.5 and temp 50 °C	Nguyen *et al.*, 2005
β-fructofuranosidase	*Bifidobacterium longum* KN29.1	NA	Stable at pH 5.7-9.1 temperature up to 45 °C	Jedrzejczak-Krzepkowska *et al.*, 2011
Fructosyltransferase	*Aspergillus aculeatus*	Pectinex Ultra SP-L	Stable at pH 5.0-7.0 and temp 60 °C	Ghazi *et al.*, 2007
Sucrose 1F-β- -fructosyltransferase (EC 2.4.1.99)	*Allium cepa* L.	Ammonium sulphate and followed by chromatography on DEAE-cellulose, CM-cellulose, octyl-Sepharose and Sephadex G-200	Optimum pH of 5.4 stable at 20–37° for 10 min; inhibited by Hg^{2+}, Ag^{+}, Mn^{2+} and p-chloromercuribenzoate	Shiomi *et al.*, 1985

Fructosyltransferase (EC.2.4.1.9)	*Aspergillus niger* AS0023	Successive chromatographies on DEAE-sephadex A-25, sepharose 6B, sephacryl S-200, and concanavalin A–Sepharose 4B columns	Optimum pH 5.8 and temp. 50°C; inactivated by 1 mM Hg^{2+} and Ag^{2+}	L'Hocine *et al.*, 2000
Fructosyltransferase	*Aureobasidium pullulans*	Anion-exchanger Sepabeads FP-DA	Both hydrolytic and transferase activity activity coefficient decrease with decreasing sucrose concentration	Antosova *et al.*, 2008
β-Fructofuranosidase	*Scopulariopsis brevicaulis* N-01	Column chromatographies on Butyl Toyopearl 650M, DEAE-Sephadex A-50, Toyopearl HW-55F, and Hydroxyapatite	Optimal activity at 40°C and pH 6–9	Hatakeyama *et al.*, 1996
^{6}G-fructofuranosidase	*Xanthophyllomyces dendrorhous* 269	DEAE-52 cellulose chromatography	Optimum activity at pH 6.4 and 45 °C; stable at pH 4–7 and at 45 °C	Chen *et al.*, 2011
Fructosyltransferase	*Aspergillus oryzae*	High performance liquid chromatography (HPLC)	Optimal activity at 50 °C, pH 6.2	Han *et al.*, 2011
Transferase	*Aspergillus aculea*	Dialysis, concentration with polyethyleneglycol (30%) an DEAE-Sephacel Chromatography	Optimal activity at 60 °C, pH 6.0	Nemukula *et al.*, 2009
Fructosyltransferase	*Rhodotorula*	Alcohol precipitation, Q-Sepharose ion-exchange chromatography and ultrafiltration	Optimal activity at pH 4.5 and 50 °C	Alvarado-Huallanco and Filho, 2011
β-fructofuranosidase	*Aspergillus japonicus* CCRC 38011	Acetone precipitation followed by centrifugation	Optimal activity at pH5.0 and 65-70 ^{o}C	Su and Sheu, 1993
Fructosyltransferase and	*Rhodotorula* sp. LEB-V10	Cell separation by centrifugation, recovering by ethanol precipitation	Optimal activity at pH 4.0 and 72-75°C (FFase); pH 4.5 and	Hernalsteens and Maugeri, 2008

fructofuranosidase		and purification by anion exchange chromatography	65-70°C (FTase)	
β-Fructofuranosidase	*Bifidobacterium infantis*	Ammonium sulphate fractionation, and DEAE-cellulose, butyl-Toyopearl and Sephacryl S-300 column Chromatographies	Optimal activity at pH 6.0-6.2, 40°C	Imamura *et al.*, 1994
β-fructofuranosidase	*Aspergillus oryzae* ATCC 76080	Ultrafiltration, DEAE-Sepharose CL-6B ion-exchange chromatography, preparative isoelectric focusing electrophoresis and Sephacryl S-200 gel filtration	Optimal activity at pH 5-6, 50°C	Chang *et al.*, 1994

(*NA: Not Available)

TABLE 3: Various enzymes employed in synthesis of fructo-oligosaccharides

Enzyme	Reaction Condtions	FOS yield	Reference
Fructosyltransferas	55% sucrose; pH 5.5; Temp. 55°C; 220 rpm.	46% (Extracellular Enzy 54% (intracellular Enzyr	Ganaie *et al*., 2011
Fructosyltransferas	60% sucrose; pH 5.15; Temp. 55°C	53%	Sangeetha *et al*., 2005c
Fructosyltransferas	770 g sucrose/litre; Temp. 55°C; shaking condition	54.9%	Yun *et al*., 1997
Fructosyltransferas (Rohapect CM)	2.103 M sucrose; pH 5.5; Temp.50°C; 150 rpm.	68.2%	Vegaa and Zúniga-Hansena, 2(
Fructosyltransferas	750.73 g/litre coconut sug (71% sucrose); pH 5.5; 54.34°C	32.52%	Noormazlinah, 2010
β-fructofuranosidas	615 g dm^{-3} sucrose; pH 5.5, Temp.40 °C	376 g /dm^3	Fernández *et al*., 2004
β-fructofuranosidas	600 g/litre; pH 5.6; Temp. 50°C; 650 rpm	101g/L (16.4% w/w)	Alvaro-Benito *et al*., 2007
β-fructofuranosidas	515 g/ litre sucrose; pH 5. Temp.40 °C; 150 rpm	87.9 g/L (75%)	Gutierrez-Alonso *et al*., 2009
β-fructofuranosidas	300 g/ litre sucrose; pH 5. Temp.30°C	134.60 g/L (54%)	Mussatto *et al*., 2009
Fructosyltransferas	50% (w/v) sucrose; pH 4.! Temp.50°C	220 g/L (90%)	Hernalsteens and Maugeri, 2010

FIGURE LEGENDS:

Figure 1: Chemical structures of fructo-oligosaccharides

Figure 2: The proposed transfructosylation reaction mechanism

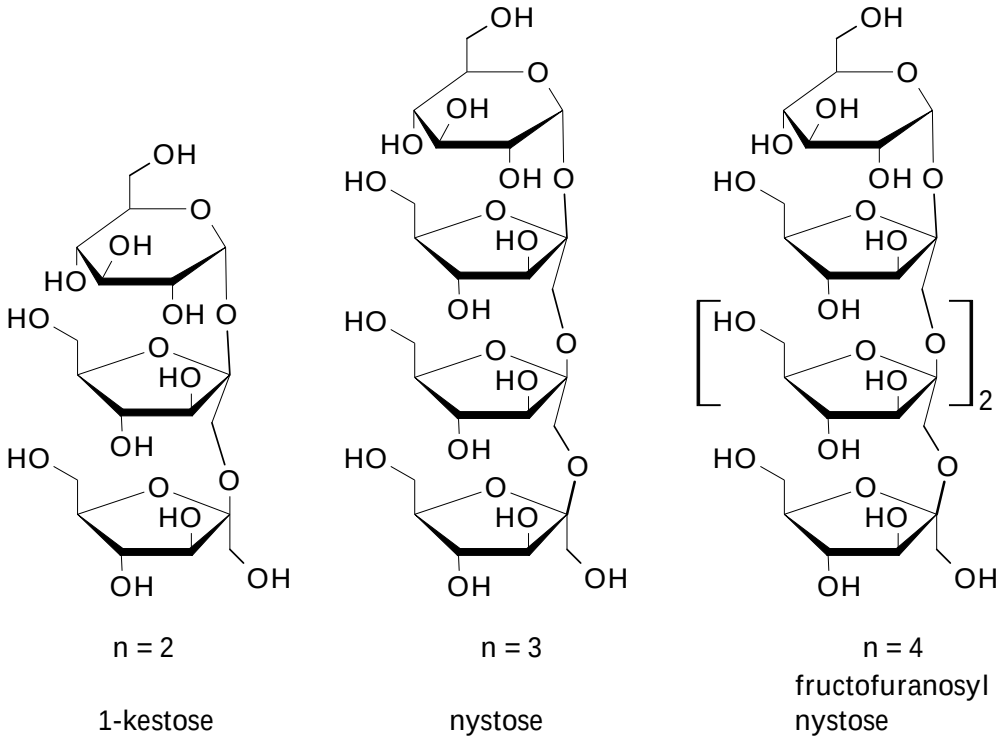

Figure 1: Chemical structures of the fructo-oligosaccharides (FOS)

(Source: Ohta *et al.*, 1998)

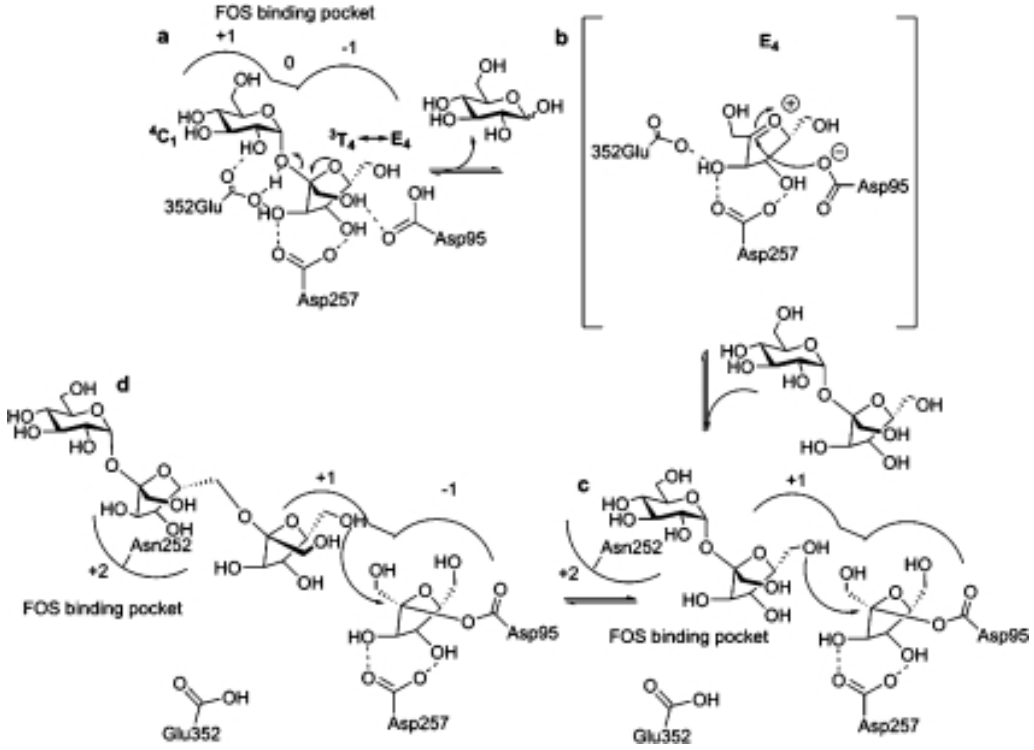

Figure 2: The proposed transfructosylation reaction mechanism

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