Journal of
Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2021-111

Research Article

Open Access

Technological Aspects of Fructo-Oligosaccharides (FOS), Production Processes, Physiological Properties, Applications and Health Benefits

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Received: February 15, 2021
Accepted: May 01, 2021
Published: May 04, 2021

Citations: Ibrahim O. 2021. Technological Aspects of Fructo-Oligosaccharides (FOS), Production Processes, Physiological Properties, Applications and Health Benefits. *J Food Chem Nanotechnol* 7(2): 41-46.

Published by United Scientific Group

Abstract

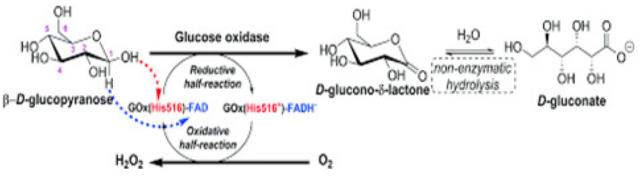
Fructo-oligosaccharides (FOS) has two other names oligofructose and oligofructan are naturally presents in fruits and vegetables but are mainly produced enzymatically from sucrose or inulin as enzymes substrates. Its chemical structures are short chain of fructose polymer with terminal glucose unit comparing to inulin that has similar chemical structure but at higher degree of fructose polymerization (DP). Inulin is present at high level in Chicory roots and Jerusalem artichoke. The enzyme fructosyl-transferase or the enzyme ß-fructo-furanosidase are used for the production of FOS from sucrose as substrate, while the enzyme endo-inulinase is used for the production of FOS from inulin as a substrate. Fructo-oligosaccharides (FOS) are used in food applications, dietary supplements and nutrition bars as low-calorie sweetener for diabetes in replacement to sucrose, also used in pharmaceutical drugs and cosmetics products formulation. FOS for health benefits is used in dietary fiber and as prebiotics for constipation and to enhance the growth of beneficial bacteria in the colon respectively. Other health benefits for FOS are increase mineral absorption in digesting system, and decrease, serum cholesterol/triglycerides. FOS are General Recognized as Safe (GRAS) in United States, and also approved for use by other international regulatory agencies in food products, infant formulas, pharmaceutical and in other applications.

Keywords

Oligofructose, Oligo-fructan, Functional oligosaccharides, Chicory roots, Jerusalem artichoke, sucrose, Fructosyl-transferase, β-fructo-furanosidase, Inulin, Endo-inulinase, Exo-inulinase, Prebiotics

Introduction

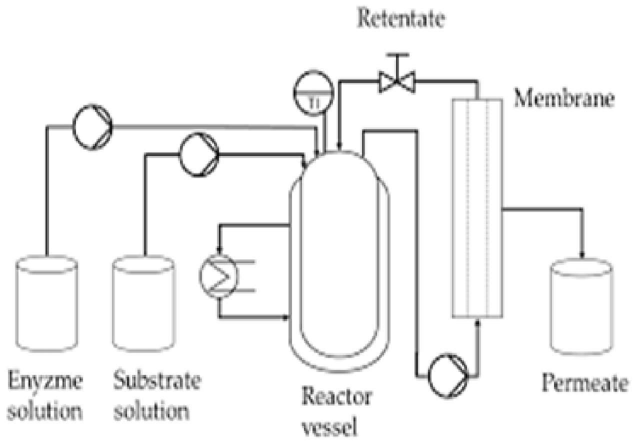
Chemical structures of Fructo-oligosaccharides (FOS) are [G-(F) n-F], composed of fructose units (F), united together with β (2→1) glycosidic bonds, and one terminal glucose unit (G), joint to a fructose unit (F) with α (1→2) glycosidic bond. Fructo-oligosaccharides (FOS) are manufactured enzymatically [1] from sucrose or inulin as substrates. Fructosyl-transferase and ß-fructo-furanosidase enzymes are derived from molds or bacteria such as, *Aspergillus niger* and *Lactobacillus bulgaricus*, respectively and utilize sucrose as a substrate for FOS production on large scale. Enzymatic reaction for these two enzymes on sucrose produced fructo-oligosaccharides (FOS), and free glucose (G) units. Free glucose units in this enzymatic reaction are enzymes inhibitor causing poor bioconversion efficiency of sucrose and low yield of FOS. To improve the enzymes efficiency and FOS yield, the released free glucose in the enzymatic reaction is removed by incorporating the enzyme glucose oxidase (Figure 1) in the enzymatic process or



Glucose oxidase enzyme

Figure 1: Oxidize β-D-glucose first into D-glucono-1,5-lactone, then hydrolyzes it to gluconic acid. Glucose oxidase requires a cofactor, flavin adenine dinucleotide (FAD). FAD is a common enzyme cofactor for biological oxidation-reduction [24].

by using cross flow ultrafiltration membrane in the continuous enzymatic process to remove the free glucose from the reaction while retaining the enzyme (fructosyl-transferase, or ß-fructofuranosidase) in the reaction (Figure 2).



Continuous FOS production process

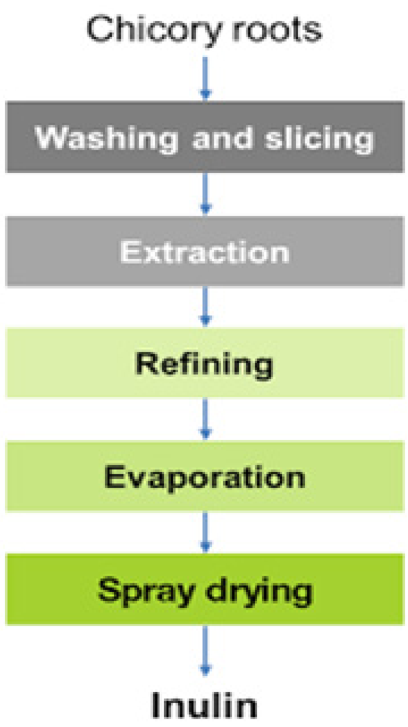
Figure 2: The process scheme is: enzyme solution tank (buffer solution), substrate solution tank (sucrose or inulin), the reactor vessel (immobilized enzyme), the membrane module (to separate end product from the substrate, and glucose) and the permeate tank (FOS product) [25].

Fructo-oligosaccharides (FOS), also produced enzymatically at high yield from the polysaccharide inulin as a substrate using the microbial enzyme endo-inulinase derived from molds such as *Aspergillus niger*. The enzyme endo-inulinase randomly hydrolyze long chain of fructose polymer in inulin chemical structure into short chains of FOS [2]. Inulin as a substrate for the enzyme endo-inulinase is produced at high yield from higher plants such as chicory roots or Jerusalem artichoke by extraction method in hot water. The row extracted inulin is refined from impurities such as sugars and starches using ion exchange technology. The refined inulin is concentrated by evaporation methods and dried using spray dryer to produce dried inulin in a powder form that is acceptable for food applications use and as a substrate for FOS production (Figure 3).

Enzyme's sources and mechanism of actions

Fructosyl-transferase (EC 2.4.1.9)

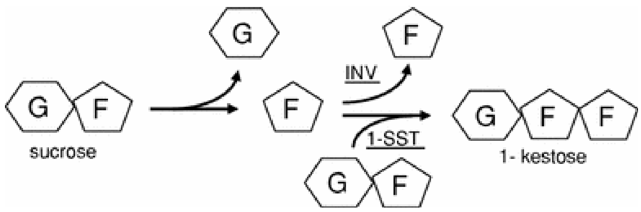
Fructosyl-transferase enzyme occurs in higher plants such as asparagus, chicory roots, onion, and Jerusalem artichoke.



Inulin production

Figure 3: Production process of inulin from Chicory roots. [Novasep.com-services and technologies for life science and chemical industries].

This enzyme is also produced by microbial fermentation as intracellular or extracellular enzyme from bacteria, molds and yeasts such as, *Bacillus macerans*, *Aspergillus niger* and *Saccharomyces cerevisiae* respectively. This fructosyl-transferase enzyme has hydrolytic activity, catalyzes the transfer of fructosyl group (fructose) from the disaccharide sucrose as a donor to a second disaccharide sucrose or to a short chain of FOS as an acceptor (Figure 4). Released fructose from the disaccharide sucrose as a substrate in this enzymatic activity plays a dual role as a donor and as an acceptor. The mechanism of this enzyme is reverse hydrolysis in equilibrium process in which the reaction equilibrium is shifted from the hydrolysis of sucrose towards the synthesis of fructo-oligosaccharides (FOS) [3].

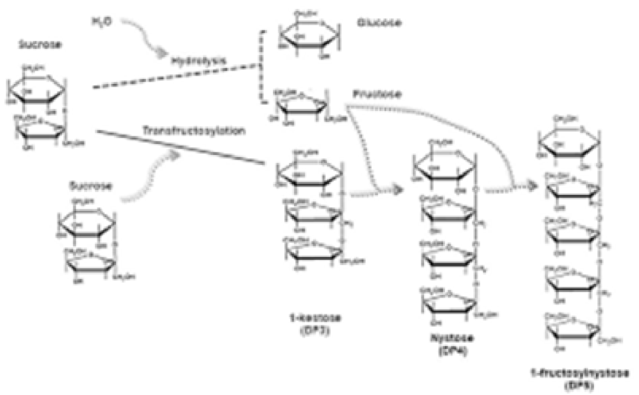


Fructosyl transferase

Figure 4: Sucrose is hydrolyzed into glucose and fructose. Glucose is released and fructose is bound to the enzyme fructosyl-transferase, fructose bound to the enzyme is released and became a donor to another sucrose molecule to form 1-kestose a shorter chain of FOS (as shown in the scheme) continue enzymatic reaction produce longer chains of FOS molecules [26].
1-SST = β-fructotransferase from plant

ß-fructo-furanosidase (EC 3.2.1.26)

ß-fructo-furanosidase enzyme also occurs in higher plants such as asparagus, chicory roots, onion, and Jerusalem artichoke and is produced from bacteria, molds and yeasts such as *Bacillus cereus*, *Aspergillus niger* and *Saccharomyces cerevisiae* This enzyme is mainly invertase enzyme, hydrolyze sucrose into glucose and fructose, also, in the same time exhibit a significant transfructosylating activity depending on the enzyme source and appropriate reaction conditions. The mechanism for the production of FOS by this enzyme is reverse hydrolysis and transfructosylation. (Figure 5). This invertase enzyme is a reverse hydrolysis enzyme with an equilibrium reaction in which the equilibrium is shifted from the hydrolysis mechanism towards the synthesis of high yield of FOS at optimum conditions of sucrose concentration as a substrate and at lower water activity [4].



β-fructo-furanosidase

Figure 5: This enzyme is invertase enzyme, hydrolyze sucrose into glucose and fructose, also, in the same time exhibit a significant transfructosylating reactions to a second sucrose as an acceptor [27].

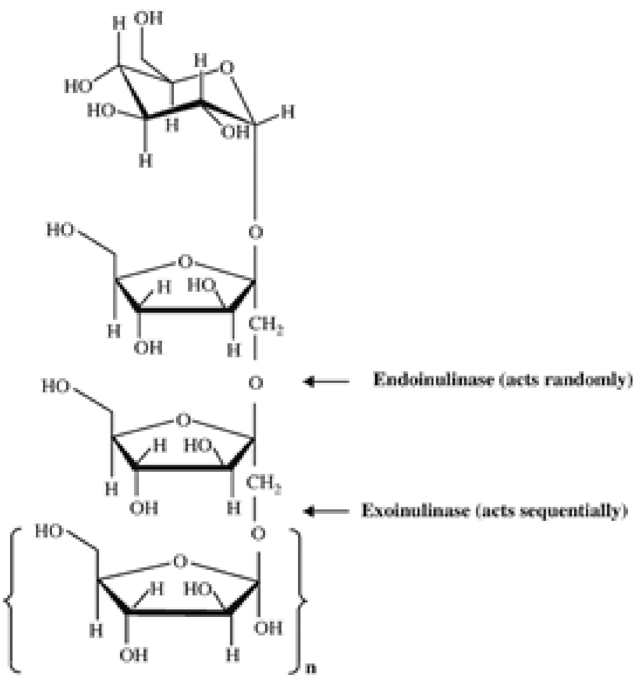
Endo-Inulinase (EC3.2.1.7)

Endo-inulinase enzyme is β-fructan-fructanohydrolase occurs in bacteria, molds and yeasts such as *Bacillus sp*., *Aspergillus sp*, and yeasts such as *Kluveromcen sp*. respectively. This enzyme hydrolyzes β (2→l) D-fructose glycosidic bonds in polysaccharide inulin into a short chain of FOS. Enzymes inulinases are classified into exo-inulinase and endo-inulinase (Figure 6). Exo-inulinases (EC 3.2.1.7) produce fructose from inulin as the main end product by releasing terminal fructose unit from inulin and is not the enzyme used for the production FOS. Endo-inulinase (EC3.2.1.7) is the enzyme used for FOS production from inulin. This endo-inulinase enzyme hydrolyze the 2,1-β-D-glycosidic bonds in inulin chemical structure for the production of FOS as end products [5].

Enzymatic processes for the production of fructo-oligosaccharides (FOS)

Disaccharide sucrose as a substrate

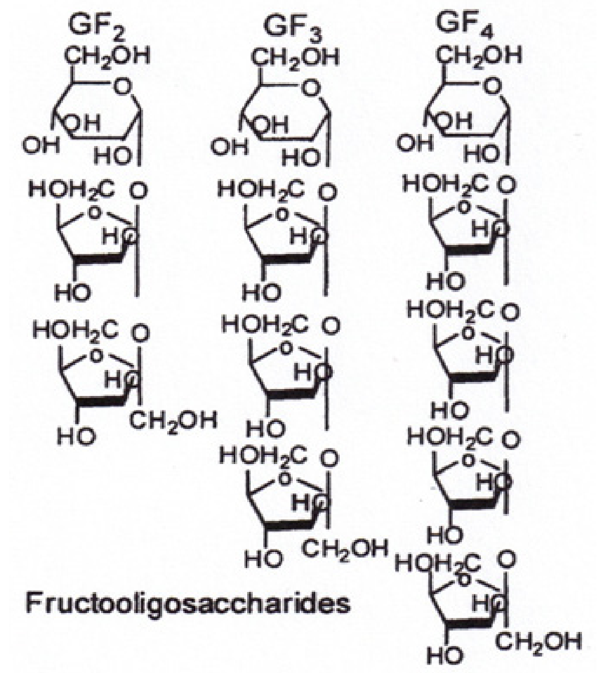
Enzymatic process from sucrose as a substrate using the enzyme fructosyl-transferase or ß-fructo-furanosidase require optimum conditions for higher enzymatic efficiency and higher FOS yield. These conditions are; sucrose concentration,



Enzymatic hydrolysis of Inulin

Figure 6: There are two classes of inulinase enzymes. Exo-inulinase removes the terminal fructose units from inulin to produce fructose as end product and endo-inulinase hydrolyze the internal linkages of inulin to produce fructo-oligosaccharides as end product [28].

high enzymatic activity, optimum enzyme property (pH and temperature), optimum enzymatic reaction time, and controlling the enzymatic process by analytical testing. In general, the optimum pH for enzymatic activity and stability is in the range of 5.5 - 6.5, and optimum temperature is in the rage of 55- 80 ˚C depends on the enzyme properties and the source. Under these optimum condition over 90% yield can be produced from sucrose as a substrate. Sugar concentration and reaction time in the enzymatic reaction is varied based on the enzyme activity used in the process. This enzymatic process for FOS production is more efficient in enzymes immobilization technology in a continuous process [6]. End products from this enzymatic reaction are fructo-oligosaccharides (FOS) and free glucose (G). Free glucose is a by-product in the reaction and enzyme inhibitor causing low yield of FOS. To improve the enzymatic reaction and yield, released free glucose must be removed from the enzymatic reaction by adding in the process, the enzyme glucose oxidase to convert free glucose into gluconic acid, or by utilizing the cross-flow ultrafiltration membrane technology in the process to remove glucose from the enzymatic reaction, while retaining the immobilized enzyme to continue reacting with the substrate for FOS production. This continuous enzymatic process is a cost saving for enzyme use and most efficient process for FOS production. End products from this enzymatic process using sucrose as a substrate are 1-kestose (GF2), nystose (GF3), and 1-β-fructofuranosyl nystose (GF4). Chemical structure of these three FOS is a polymer of 3 to10 fructose units joint together with α (1→2) glycosidic bond and the terminal fructose is linked to a glucose unit by β-(1→2) glycosidic bonds (Figure 7).



FOS chemicals structure from sucrose

Figure 7: FOS is a mixture of 1-Kestose (GF2), Nestose (GF3) and Fructo-furanosyl nystose (GF4) of which fructose units (F) are linked together at β (2→1) glycosidic bonds and a glucose terminal unit (G) linked to terminal fructose unit at α (1→2) glycosidic bond [16].

Inulin as a substrate

Enzymatic process using inulin as a substrate is a second approach for the production of FOS [2]. This process is based on controlling the hydrolysis of inulin using the enzyme endo-nuclease in batch or in immobilizes process [7]. The enzyme endo- inulinase randomly cleaves inulin β (2→l) glycosidic bonds yielding a mixture of FOS consist of 2 to 9 fructose units joint together with α (1→2) glycosidic bonds, and the terminal fructose is linked to a glucose unit by β-(1→2) glycosidic bond. Optimum condition for this enzymatic process depends on microbial source and the enzyme properties. In general inulin concentration as a substrate is varied based on the enzyme activity, temperature is in the range of 35-55 ˚C and the PH is in the range of 6.0 - 7.0. Under these optimal hydrolysis conditions, the expected FOS yield is in the range from 60 to 86% from inulin as a substrate. These optimum conditions are important factors for the enzyme efficiency and higher yield of FOS as the end product. It is important to highlight that FOS produced from inulin as a substrate has different degree of fructose polymerization (DP) than FOS produced from sucrose as a substrate by one of the two enzymes fructosyl-transferase or ß-fructo-furanosidase [8]. Also, it is important to highlight that FOS produced from inulin is marketed under different trade name than FOS produced from sucrose.

Fructo-oligosaccharides (FOS) properties, applications, and regulations

FOS Properties in general are water soluble and have a sweetness in the range of 0.3 to 0.6 time comparing to sucrose. This sweetness range depends on the ratio of the three chemical structures of FOS, which are 1-kestose, nystose, and 1-β-fructo-furanosyl nystose. These three FOS chemical structures have higher molecular weight than sucrose causing higher viscosity at the same concentration as sucrose. This higher viscosity of FOS must be taken in consideration in foods formulation as a replacement to sucrose. FOS are thermostable than sucrose, this thermostability of FOS is important in food processing at higher temperature. The PH of FOS are in the range of 4.0 to 7.0. This range of PH is acceptable for foods applications and for other products formulations. FOS are recognized as a low calories' sweetener, with calories in the range of 1.5 to 2.0 kcal/gram which is about half the caloric values of sucrose [9]. This low-calorie value of FOS is an advantage for the production of low calories food products.

These above properties, makes FOS qualified for multiple applications in foods, beverages, pharmaceutical drugs, and in cosmetics products. In the case foods, FOS are used in low calories foods as a substitute for sucrose in diet beverages, and other low calories food products for weight loss, and as a sweetener in food products for diabetes. Other health benefits for FOS applications are in dietary supplements as a source of fiber for constipation and as prebiotics in probiotics microbial products to enhance the growth of beneficial bacteria such as *bifidobacterial* and l*actobacillus species* in the colon. In addition, FOS are being demonstrated to improve mineral absorption, and decrease cholesterol / triglycerides in blood serum [10].

Fructo-oligosaccharides (FOS) are accepted in United Stated by Food and Drug Administration (FDA) as General Recognized as Safe (GRAS) [11] and accepted by other international regulatory agencies for the use in food products and pharmaceutical drugs formulations. In addition, FOS are accepted the European Union for use in infant formulas in combination with galacto-oligosaccharide [12].

The accepted daily intake of FOS is not more than 20 grams/day for adults and not more than 4.2 grams/day for children. Exceeding these accepted limits for FOS intake could have side effects such as, intestinal gas and bloating, stomach cramps and diarrhea [13]. In addition, excess FOS intake could have negative effects on patients suffering from irritable bowel syndrome (IBS), patients suffering from small intestinal bacteria overgrowth syndrome (SIBO), and might lead into chronic health conditions or other serious symptoms [14].

The use of FOS in infant food formulations has been extensively reviewed in the year 2013, by Food Standards of Australia, and New Zealand and concluded that no adverse effects on healthy infants fed FOS up to 3.0 grams per liter for periods ranging from one week to three months. Currently acceptable daily intake of FOS for infants less than one year old is 4.2 grams per day [15].

Discussion

Fructo-oligosaccharides (FOS) are one of functional oligosaccharides, that includes galacto-oligosaccharides, Xylo-oligosaccharires, soy-oligosaccharides and others.

These functional oligosaccharides are intermediate chemicals in molecular weight between monosaccharides, and polysaccharides [16] and are present naturally in small quantities in fruits and vegetables and in other higher plants.

FOS present at higher concentration in chicory root, and Jerusalem artichoke and at lower concentrations in some grains such as barley and wheat. Unique properties of FOS such as low calories, low sweetness and health benefits elevated its market demand in the year 1980s for commercial use in healthy foods, dietary supplements, infant formulas, and over the counter's pharmaceutical products [17]. Due to continue increasing demands of FOS and other functional oligosaccharides, they are produced commercially by enzymatic processes at higher yield and lower costs instead of extraction methods from higher plant that are costly and lower yield [18].

Commercially FOS is produced enzymatically from two different carbohydrates as a substrate. These two natural carbohydrates are the disaccharide sucrose, and the polysaccharide inulin. Sucrose is the substrate for tow enzymes fructosyl-transferase and ß-fructo-furanosidase. These two enzymes are the major enzymatic process for FOS production. The second substrate is inulin for the enzyme endo-inulinase. End products from sucrose as a substrate are a mixture of FOS constitute mainly of 1-kestose (GF2), nystose (GF3), and 1-β-fructofuranosyl nystose (GF4). These FOS end products from sucrose contains 2 o 4 degree of fructose polymerization (DP). While the end products from inulin are a mixture of FOS constitute of 2 to 9 degree of fructose polymerization (DP).

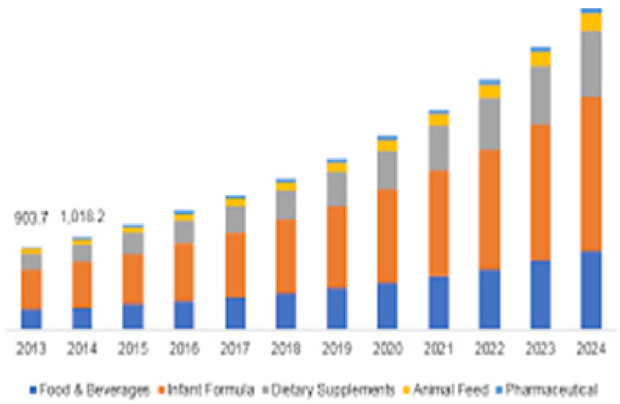
These two classes of FOS from these two substrates (sucrose and inulin) are non-digestible and prebiotics in nature that pass intact (undigested) from small intestine to large intestine, where it promotes the growth of healthy beneficial bacteria of *Bifidobacterium* and *lactobacillus species* in the colon. Growth of these beneficial bacteria in colon inhibits harmful bacteria growth via competitive exclusion [19]. Intermediate metabolites such as butyric from fermented FOS by gut microbiota in large intestine enhance the health of gastrointestinal tract and preventing from colorectal cancer as it has been claimed [20]. In addition, to colon cancer prevention [21], other health benefits from FOS consumption as dietary supplement and healthy foods includes but not limited to increase the digestion of lactose metabolism which benefits lactose intolerance patients , increase calcium and other minerals absorption which benefits elderly people and pregnant women, decrease serum lipids, decrease blood cholesterol, improve HDI/LDL ratio [10] which are heart attack prevention, improve blood pressure, enhance immune response, trigger anti-inflammatory response, decrease fecal PH and eliminating the effect of toxic, or carcinogenic chemicals in digestion system**.**

Because of the chemical structure of FOS is with different glycosidic bonds configuration than other carbohydrates such as starch it resist hydrolysis by the saliva enzyme and by intestinal digestive enzymes. In other words, FOS have a lower caloric value and does not cause the increase in a person's blood sugar as in the case other natural sweeteners such as sucrose, glucose, and fructose. This FOS properties, is an ideal for diabetes and for people looking for an alternative sweetener. This low caloric value with low sweetness properties makes its useful in low calories food formulations, dietary supplements, infants' formulas, and in combination with other high-intensity artificial sweeteners that have after taste such as the artificial sweetener Stevia to mask it's after taste and to improves the sweetness taste. In addition, FOS bulking property make its useful for constipation, diarrhea symptoms, and stimulates healthy bowel movements [24]. Humectant property of FOS is due to high moisture retaining capacity without increasing foods water activity. This humectant property has multiple applications for FOS in foods, pharmaceuticals, and cosmetics formulations [22].

This safety status recognition for FOS in United States, European union and Asia is due to its naturally present at low concentrations in our daily diets, and because commercially manufactured enzymatically from a natural carbohydrate (sucrose and inulin) as enzymes substrates, by using natural microbial enzymes that are also granted safety (GRAS) status for FOS manufacturing.

FOS was emerged as new product in the 1980s, and since then its demand continue rising (Figure 8) with global market reached $ 1.2 billion in the year 2015, with annual growth rate at 10.4%. It is expected that FOS market will reach $ 3.9 billion by the year 2027 [23].

Major key players in global fructo-oligosaccharide (FOS) manufacturing and marketing includes; Cargill Incorporation and Ingredion Incorporated both located in United States, Beneo-Orafti SA and Cosucra-Groupe Warcoing SA both located in Belgium and Beghin Meiji located in Japan.



2013- 2024 FOS market size ($ Million) by applications

Figure 8: Shows the largest market size for FOS is in infant formula, the second is in food and beverages, the third is in dietary supplements, the fourth is in animal feeds and the smallest market size is in pharmaceutical [29].

Conclusion

FOS are manufactured enzymatically from sucrose or inulin as enzymes substrates. These two substrates are available from natural sources and are in our daily diets. Unique properties of FOS and health benefits, increase its market demands with wide applications in diet foods and beverages for diabetes and obesity. Plus, in other healthy applications includes dietary supplements, probiotics, infant formulas, pharmaceutical, cosmetics, animal feeds and agriculture chemicals.

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