Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2018-060

Review Article

Open Access

Functional Oligosaccharides: Chemicals Structure, Manufacturing, Health Benefits, Applications and Regulations

Osama O. Ibrahim*

Consultant Biotechnology/ Food Safety, Bio Innovation, USA

***Correspondence to:**
Osama O. Ibrahim, PhD
Consultant Biotechnology/Food Safety
Bio Innovation, USA
Tel: 847-682-0655
E-mail: bioinnovation04@yahoo.com

Received: July 30, 2018
Accepted: October 04, 2018
Published: October 06, 2018

Citation: Ibrahim OO. 2018. Functional Oligosaccharides: Chemicals Structure, Manufacturing, Health Benefits, Applications and Regulations. *J Food Chem Nanotechnol* 4(4): 65-76.

Published by United Scientific Group

Abstract

Functional oligosaccharides are carbohydrates that have two to ten monosaccharides units linked together with glycosidic bonds. The two linked monosaccharides (disaccharides) of maltose, sucrose, and lactose are digestible oligosaccharides by human gut enzymes. These, digestible disaccharides are sugars and are not classified as functional oligosaccharides.

Functional oligosaccharides are non-digestible by human gut enzymes and providing health benefits as fibers and prebiotics [1]. The common known functional oligosaccharides are fructo-oligosaccharides, galacto-oligosaccharides, lacto-sucrose, malto-oligosaccharides, isomalto-oligosaccharides, trehalose, cyclodextrins, xylo-oligosaccharides, and soy-oligosaccharides.

Functional oligosaccharides have mildly sweet taste and other characteristics such as, mouth feeling. This mouth feeling characteristic interest food industry to incorporate these functional oligosaccharides in foods as a partial substitute for fat and sugars, and to improve food texture. With the exception, of malto-oligosaccharides and trehalose, functional oligosaccharides are non-digestible in small intestine digestive enzymes and reached large intestine (colon) where it acts as a growth factor (prebiotics) to enhance the growth of beneficial bacteria (probiotics) and inhibit pathogenic bacteria in the colon via competitive exclusion. These benefits to colon and for other health benefits, plus unique characteristics have increased the global market of functional oligosaccharides applications in foods, pharmaceuticals, and in other industrial sectors.

Due to, the increase demand of functional oligosaccharides for their health benefits and characteristics, functional oligosaccharides are currently produced enzymatically at higher yield, and lower cost from different natural sources of carbohydrates as a replacement of costly plants extraction methods.

Two of these enzymatically produced functional oligosaccharides are cyclodextrins and trehalose. In addition, to their highlighted health benefits, both have an important pharmaceutical application in drugs delivery systems, in the case of cyclodextrins, and as cryoprotectants of biological materials, viable cells, and foods, in the case of trehalose.

Keywords

Oligosaccharides, Polysaccharides, Monosaccharides, Fructo-oligosaccharides, Galacto-oligosaccharides, Lacto-sucrose, Malto-oligosaccharides, Trehalose, Isomalto-oligosaccharides, Cyclodextrins, Xylo-oligosaccharides, Soy-oligosaccharides, Probiotics, Prebiotics, Drugs delivery systems, Cryoprotectant

Introduction

Functional oligosaccharides are short chain of carbohydrate polymers that not exceeds ten monosaccharides. They are important groups of polymeric carbohydrates that are found in all living organisms in the form of lipopolysaccharides through the reaction of O-glycosidic or N-glycosidic bonds with lipids, or in the form of glycoprotein through the reaction on N-glycosidic bonds with the amino acid asparagine in proteins structure to function like cell to cell recognitions, interactions or adhesions [2].

In higher plants, functional oligosaccharides are one of fibers chemical structure in plant tissues, and naturally present in some plants tissue with large amounts such as, in Jerusalem artichokes from where commercial inulin is extracted [3]. Functional oligosaccharides are also, found in small amounts in onions, garlic, legumes, wheat, asparagus, and other plants as food source. All functional oligosaccharides except malto-oligosaccharides and trehalose are indigestible with prebiotics property due to, the lack of enzymes in digestive system that break down these functional oligosaccharides. Undigested functional oligosaccharides reached the large intestine (colon) where beneficial bacteria in the colon are capable to hydrolyse, and ferment these functional oligosaccharides providing energy and absorbable nutrients such as, short chain fatty acids (SCFAs) that may have beneficial effect on large intestine limning in colon cancer prevention [4]. This, property of functional oligosaccharides in the colon gave them the name prebiotics. Prebiotics definition are non-digestible nutrients that selectively promote the growth of beneficial normal intestinal microorganisms (microbiota) that may have beneficial health effects [5].

Functional oligosaccharides have mildly sweet taste with mouthfeel and texture characteristic [6] that interest food industries in utilizing these oligosaccharides to improve food texture, and as, partial replacement for fats and sugars [7] in healthy foods. Other common properties for functional oligosaccharides are zero or low calorie, low sweetener intensity that are about one third of sucrose sweetness, high soluble in water than sucrose, heat stable, hydrolyse only at high acid environments, and non-carcogenic.

Common health benefits of functional oligosaccharides are prebiotics that enhance beneficial bacteria such as *Bifidobacterium* species and *Lactobacillus* species in the colon, increase the digestion of lactose metabolism, enhance minerals absorption, improve the good ratio of HDL/LDL, decrease serum lipids, decrease blood cholesterol, improve blood pressure, decrease fecal PH, remove toxics from the body, and have lower glycemic index.

Functional oligosaccharides have been used extensively in foods, and as dietary supplements for health benefits to regulate and control blood glucose for diabetes, reduce lipid level for patients with hyperlipidemia symptoms, control body weight, and for healthy colon. In addition, functional oligosaccharides have applications in pharmaceuticals, cosmetics, agrichemical formulations, and in encapsulation technology [8].

Due to, the great interest of these types of oligosaccharides for health benefits and other applications, enzymatic process has been developed to produce functional oligosaccharides at lower cost with high yields and purity in replacements to natural extraction methods of these oligosaccharides from plants tissue that are costly due to, lower yields.

Functional oligosaccharides are classified into four groups:

1. Sucrose-related oligosaccharides.
2. Lactose-related oligosaccharides.
3. Starch-related oligosaccharides.
4. Others-oligosaccharides.

Sucrose Related Oligosaccharides

Fructo- oligosaccharides

Fructo-oligosaccharides (FOS) also, known by the name oligofructose or oligo fructan are naturally presents in higher plants such as, fruits and vegetables [9]. They are short chain of fructose polymer that are different from inulin in the degree of fructose polymerization (DP). Inulin has higher degree of fructose polymerization (DP), and is present in higher plants such as, in Jerusalem artichoke.

Fructo-oligosaccharides are manufactured enzymatically [10] using the enzyme fructosyl-transferase (EC 2.4.1.9), or the enzyme ß-fructo-furanosidase (EC 3.2.1.26). Both enzymes are derived from mold or bacteria such as, *Aspergillus niger* or *Lactobacillus bulgaricus*, respectively. These two enzymes utilize sucrose as a substrate for the production of Fructo-oligosaccharides chemical structures of [G-(F) n -F], composed of fructose units (F), with β (2→1) glycosidic bonds, and glucose terminal unit (G), with α (1→2) glycosidic bond. End products from these enzymatic reactions are fructo-oligosaccharides, and the by-product of free glucose units. Free glucose units in the reaction mixture inhibit the enzymatic process causing low yield of fructo-oligosaccharides. To improve the yield, the released by-product of free glucose is removed by using cross flow ultrafiltration membrane, while retaining the enzyme in the production process.

Fructo-oligosaccharides are also, produced enzymatically at high yield from the polysaccharide inulin as a substrate using the enzyme inulinase (EC 3.2.1.7), derived from *Aspergillus niger*. The substrate inulin is extracted with high yield from higher plants such as, from Jerusalem artichoke, and the enzyme inulinase hydrolyse the long chain inulin of fructose polymer into short chains of fructo-oligosaccharides [11].

There are three major chemical structures of fructo-oligosaccharides that are produced enzymatically as a mixture. These are; kestose (GF2), nystose (GF3), and fructofuranosyl nystose (GF4), of which the fructose units (F) are linked at the ß (2→1) glycosidic bonds, and the terminal glucose unit (G) is linked to fructose unit at the α (1→2) glycosidic bond (Figure 1).

Fructo-oligosaccharides are water soluble with a sweetness about 0.3 to 0.6 time of sucrose depends on the mixture ratio of these three chemical structures of fructo-oligosaccharides, have higher viscosity than sucrose at the same concentration due to, their larger molecular weights, thermostable than sucrose, have PH 4.0 to 7.0 which is the normal PH range to food products, and have estimated caloric values about 1.5 to -2.0 kcal/gram which is about half the caloric values of sucrose. These properties, qualified fructo-oligosaccharides to be used as a substitute for sucrose in foods such as, yogurt, nutrition bars, diet beverages, other food products, and in low-calorie sweetener for diabetes [12]. Fructo-oligosaccharides are also, used as dietary fiber for constipation and traveler's diarrhea. In addition, for the use as prebiotics to enhance the growth of beneficial bacteria in the colon. Fructo-oligosaccharides are being claimed to improve mineral absorption, and decrease phospholipids, serum cholesterol, and triglycerides [13].

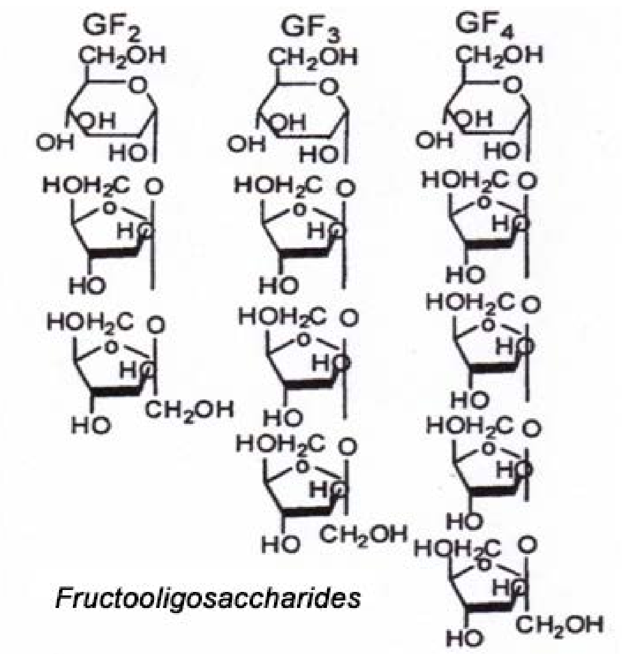

Figure 1: Fructo-oligosaccharides (FOS)
A mixture of Kestose (GF2), Nestose (GF3), and Fructofuranosyl nystose (GF4) which fructose units (F) are bound at β (2→1) glycosidic bonds, and glucose terminal unit (G) is bound at α (1→2) glycosidic bond.

Fructo-oligosaccharides are general recognized as safe (GRAS) by Food and Drug Administration (FDA) [14], and by other worldwide regulatory agencies for food applications and pharmaceutical drugs formulations. In the European Union, fructo-oligosaccharides are approved for use in infant formulas in combination with galacto-oligosaccharide [15] and in the year 2013, Food Standards of Australia, and New Zealand concluded that no adverse effects on healthy infants fed fructo-oligosaccharides up to 3.0 grams per litre for periods ranging from one week to three months [16].

The accepted daily intake of fructo-oligosaccharides should not exceed 20 grams/day for adults, and 4.2 grams/day for infants less than one year old [17]. Higher daily intake of fructo-oligosaccharides could have side effects [18] such as, intestinal gas (flatulence), intestinal noises, bloating, stomach cramps, diarrhea, and may have negative effects on patients suffering from irritable bowel syndrome (IBS) and patients suffering from small intestinal bacteria overgrowth syndrome (SIBO).

Lactose Related Oligosaccharides

Galacto-oligosahharides

Galacto-oligosaccharides (GalOS) are naturally occurred in milk at low concentration [18], and commercially produced enzymatically by trans galactosylation of milk sugar lactose as a substrate, using the microbial enzyme ß-galactosidase (EC 3.2.1.23) derived from the mold *Aspergillus oryzae*. This enzyme uses one of the two disaccharide lactose in the enzymatic reaction as galactose donor, and the second lactose as galactose acceptor for the production of galacto–oligosaccharides (Figure 2).

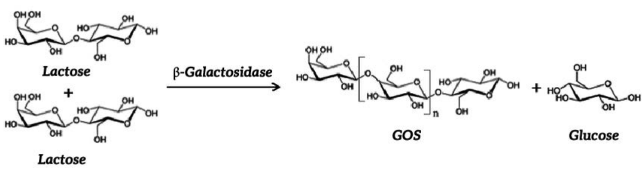

Figure 2: Production of galacto-oligosaccharides from lactose
The enzyme galactosidase catalyzes two reactions; the first reaction is the hydrolysis of lactose into galactose and glucose, the second reaction is trans-galactocylation of galactose as a donor to the second lactose forming galactose-oligosaccharides with different degrees of galactose polymerization.

Chemical structure of galacto-oligosaccharides chain [G-(Gal)2-Gal], are varies in galactose (Gal) unit's length with a terminal of glucose (G) unit. The degree of galactose polymerization is in the range of two to eight galactose units (Figure 3), depends on different factors. These factors are; the enzyme property and activity, lactose concentration as a substrate, enzymatic reaction time, optimum conditions (pH, temperature, etc.), and enzymatic process method (free or immobilized enzymes) [19]. Enzymatic reaction endpoints are galacto-oligosaccharides, free galactose, and free glucose. The presence of free galactose and glucose as by-products inhibit the enzymatic reaction causing low yield of galacto-oligosaccharides. To improve the enzymatic reaction and yield, the released free galactose and glucose units are removed by using cross flow ultrafiltration membrane, while retaining the enzyme in the production process [20].

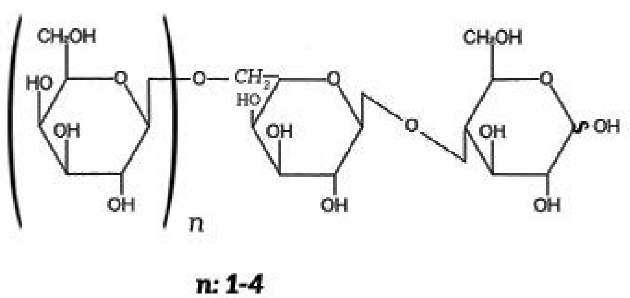

Figure 3: Galacto-oligosaccharides (GOS)
Composed of galactose chain linked together with ß (1-6) glycosidic bonds, and bound to glucose terminal by ß (1-3) or ß (1-4) glycosidic bond.

Galacto-oligosaccharides are commercially available as a polymer of galactose with different degree of polymerization (DP), and are used as low cariogenic as a substitute for high cariogenic sugars such as, sucrose, glucose, or fructose, with properties of having pleasant taste, providing texture, mouth feeling to foods, having moisture retaining capacity, and thermostable during food processing. In addition, to have health benefits such as, neglectable impact on blood glucose level due to, the lack of galacto-oligosaccharides digestible enzymes in the digestive system and having prebiotic properties [21].

Galacto-oligosaccharides are used in wide varieties of adult foods in such as, backed goods, beverages, and in infant formula at the range of 6.0 to 7.2 grams per litre together with fracto-oligosaccharides at the range of 0.6 to 0.7 grams per litre [22]. Other applications are as dietary supplement, in cosmetics, and in pharmaceutical products.

Health benefits of galacto-oligosaccharides as prebiotic stimulate the growth of beneficial bacteria such as, *Bifidobacterium* species, and *lactobacillus* species that inhibit the growth of pathogenic bacteria such as, *Escherichia coli, Salmonella typhimurium, and Clostridium* species via competitive excision, plus, enhance immune response, improve stool frequency, and relieve symptoms of constipation. Constipation symptoms are the main problem for infants, elderly and pregnant women. [23].

Because of the prebiotic property of galacto-oligosaccharides, the application is not limited to human but also, in animal feeds to improve the health and growth of farm animals, and to reduce antibiotics use as a way to minimize the concern of immerging antibiotics resistance against microbial pathogens for both human and animals [24]. Plus, the incorporation of galacto-oligosaccharides in animal feeds have positive impact on environment in reducing methane gas emission from ruminates animals [25], and reducing fecal odor from animals' farms due to, the non-fermentable properties of galacto-oligosaccharides [26].

Galacto-oligosaccharides are generally recognized as safe (GRAS) by FDA in the Unites States, and by other worldwide regulatory agencies due to, their presence naturally in human milk, yogurt, and in both human and animals' intestinal tracks from milk lactose fermentation by resident intestinal microflora. The recommended daily intake of galacto-oligosaccharides is in the range of 0.3 to 0.4 gram/kilogram body weight, and the only known side effect of galacto-oligosaccharides intake is transient osmotic diarrhea that occurred when consumed at higher dose [27].

Lacto-sucrose

Lacto-sucrose is a trisaccharide oligosaccharide composed of galactose, glucose, and fructose (Figure 4), that occurred naturally at low concentration in yogurt when both sucrose and lactose sugars are present together in the milk, and commercially produced enzymatically using both sucrose and lactose sugars as substrates. Microbial enzymes used for this enzymatic process are levansucrase (EC 2.4.1.10), or fructofuranosidase (EC 3.2.1.26). Both enzymes have transfructosylation activities, that transfer fructose unit from sucrose to lactose. The major production method of lacto-sucrose is by enzyme Immobilization technology in a continuous process using both sucrose and lactose sugars as substrates to produce lacto-sucrose at high yield, and lower cost [28].

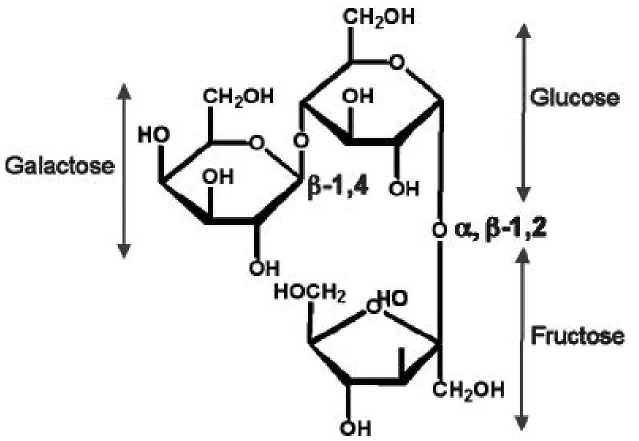

Figure 4: Lacto-sucrose
Trisaccharide composed of galactose, glucose, and fructose linked together with glycosidic bonds.

This rare oligosaccharide of lacto-sucrose is non-digestible, non-cariogenic, promotes intestinal mineral absorption, and is suitable for low-calorie food products, with multiple applications as food additive in both foods and beverages such as, baked goods, ice-creams, candies, infant formula, juices, and mineral water. The degree of lacto-sucrose sweetness is similar to sucrose and its, functional properties are similar to other functional oligosaccharides.

Lacto-sucrose is generally recognized as safe (GRAS) by FDA in United States, and by other worldwide regulatory agencies for the formulation of foods and functional foods products. Plus, it is marketed as prebiotics to regulate intestinal microflora [29] for selectively increase the beneficial bacteria of *Bifidobacterium* species, and inhibit enteric pathogenic bacteria including Enterobacteriaceae family and *Clostridium perfringens* in the colon. The suggested daily intake of lacto-sucrose for adults is in the range of 3.0 grams/day.

In addition, lacto-sucrose, has potential applications as excipient for pharmaceuticals and cosmetics formulations [30]. Some research publications demonstrated that the consumption of lacto-sucrose as prebiotic benefit patients with inflammatory bowel disease (IBD) [31]. Plus, there are several patents highlighted the application of lacto- sucrose as an active ingredient in drugs for the prevention of some skin diseases [32].

Starch Related Oligosaccharides

Starches are homo-polysaccharide of glucose units and are the storage form of carbohydrates in higher plants. [33]. There are two types of starches amylose and amylopectin. Amylose starch is unbranched homo-polysaccharide of hundreds of glucose units linked together by α-(1→4) glycosidic bonds (Figure 5). Amylopectin starch is the branched homo-polysaccharide of thousands of glucose units formed in main

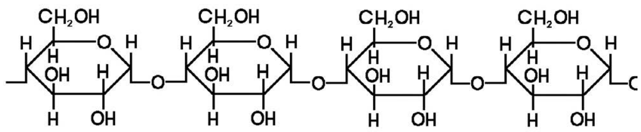

Figure 5: Amylose starch
Leaner homo-polysaccharide composed of hundreds of glucose units linked together by α-(1→4) glycosidic bonds.

chain linked together by-α (1→4) glycosidic bonds, and after every 25-30 glucose units a branch point is formed by a glucose unit joint to the main chain with α-(1→6) glycosidic bonds (Figure 6). These two types of starch are substrates for enzymatic production of starch related oligosaccharides.

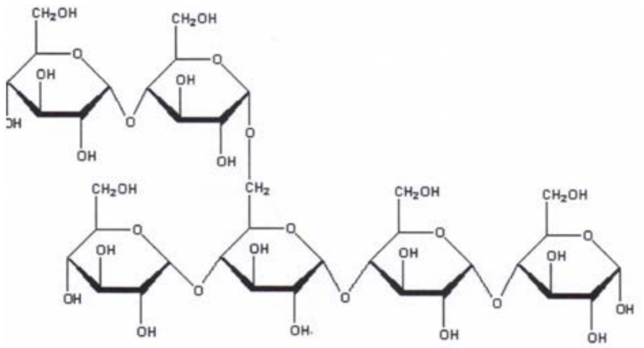

Figure 6: Amylopectin starch
Branched homo-polysaccharide cmposed of thousands glucose units in main chain linked together by-α (1→4) glycosidic bonds, and every 25-30 glucose units a branch point is formed by glucose unit joint to the main chain with by α-(1→6) glycosidic bond. The branched chain of 15 to 25 glucose units linked together by α (1→4) glycosidic bonds.

There are several starch related oligosaccharides that are produced via enzymatic processes from these two types of starch. They are malto-oligosaccharides, trehalose, isomalto-oligosaccharides, cyclodextrins, nigero-oligosaccharides, and gentio-oligosaccharides (Figure 7). From these starch related oligosaccharides malto-oligosaccharides, isomalto-oligosaccharides, trehalose, and cyclodextrins are naturally present and are generally recognized as safe (GRAS) by Food and Drugs Administration (FDA) in United States, and by other worldwide regulatory agencies for safe applications in both foods and pharmaceuticals industries for health benefits.

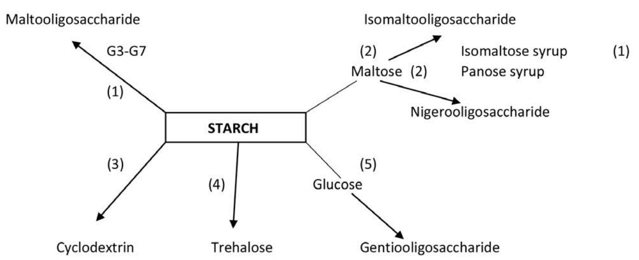

Figure 7: Microbial enzymes for the production of Starch related oligosaccharides
(1). malto-oligosacccharides forming amylase, (2). α-Glucosidase, (3). cyclodextrin glycosyltransferase (CGTase), (4).maltooligosyltrehalose synthase (MTSase) & maltooligosyltrehalose trehalohydrolase (MTHase) (5). ß-Glucosidase

Malto-oligosaccharides

Malto-oligosaccharides are composed of glucose units joined together by α (1→4) glycosidic bonds and are a series of linear oligosaccharides composed of two (G2), three (G3), four (G4), five (G5) and six (G6) of glucose (G) units.

Enzymatic process for the production of malto-oligosaccharides is by adding the enzyme malto-oligosaccharide forming amylase (MFAses) [34] to starch slurry that have been liquefied by the enzyme α-amylase (EC 3.2.1.1), and to improve the production yield of malto-oligosaccharides the second enzyme pullulanase (EC 3.2.1.9) is also, added to the starch slurry to break down α-(1→6) glycosidic bonds in the branched starch of amylopectin. The majority of malto-oligosaccharides produced by these multiple enzymatic processes are a mixture of different degree of glucose (G) polymerization of maltotriose (G3), maltotetraose (G4), maltopentaose (G5), and maltohexaose (G6) (Figure 8).

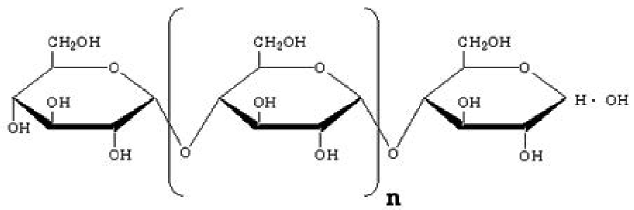

Figure 8: Malto-Oligosaccharides (MOS)
Linear chain of glucose units joined together by α (1→4) glycocidic bonds.

Malto-oligosaccharides are digestible nutrient sweetener, with characteristics of low sweet, low calorie, and have low osmotic pressure, with multiple applications [35] in food processing, beverages, dairy products, juices, jams, cakes, wines, and functional foods.

Malto-oligosaccharides are marketed in the form of syrup or powder. The syrup form is viscous, transparent liquid, and appears as color less or yellowish that has moderate sweet taste, with applications in candies and sweet syrup products. The powder form appears white, and also, has moderate sweet taste, with applications in food products [36] such as, infant's formulas, healthcare products, sport drinks for athletes, backed goods, and lower mellow beers.

Applications of malto-oligosaccharides for health benefits [37] are to relax tiredness, to improve intestines peristalsis/functions, and to prevent constipation. Other application for highly purified malto-oligosaccharides, is in clinical chemistry as a substrate for measuring blood glucose.

Trehalose

Trehalose is non-reducing disaccharide of two glucose units linked together with α (1→1) glycosidic bond (Figure 9). It is naturally present in mushrooms, yeast fermented dough for baked goods, and in other food products [38].

Trehalose is commercially produced from yeast cells by extraction process or produced enzymatically from malto-oligosaccharides as a substrate, using two mutases enzymes in the process. The first mutase enzyme is malto-oligosyltrehalose

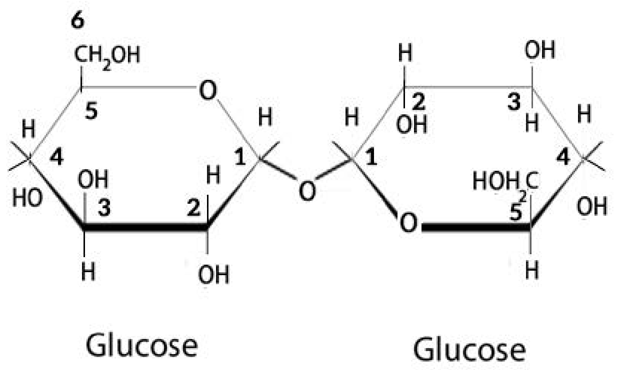

Figure 9: Trehalose
None reducing disaccharide formed from two glucose units joined together by α (1→1) glycosidic bond giving the name α D-glucopyranosyl (1→1)-α D-glucopyranoside.

synthase (MTSase, EC 5.4.99.15) with a mechanism to shift the α (1→4) glycosidic bond from reducing end of the two glucose units into α (1→1) glycosidic bond. The second mutase enzyme is malto-oligosyltrehalose trehalohydrolase (MTHase, EC 3.2.1.141) with a mechanism to cleave the α (1→4) glycosidic bond that is next to the α (1→1) glycosidic bond in the terminal disaccharide of malto-oligosyltrehalose to produce the end product of trehalose, plus, a shorter chain of α (1→4) malto -oligosaccharide [39] (Figure 10).

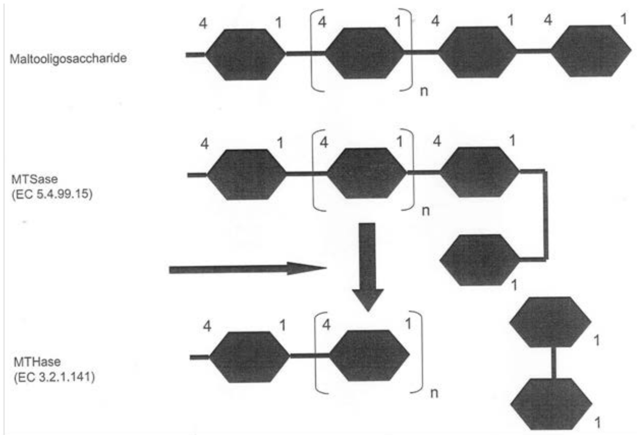

Figure 10: Enzymatic production of Trehalose from Malto-Oligosaccharides
Two mutases enzymatic processes: The first mutase is maltooligosyltrehalose synthase (MTSase) that shift the α (1→4) glycosidic bond from reducing end of two glucose units into α (1→1) glycosidic bond. The second mutase enzyme is maltooligosyltrehalose trehalohydrolase (MTHase) that cleave the α (1→4) glycosidic bond that is next to the α (1→1) glycosidic bond in terminal disaccharide of malto-oligosyltrehalose to yield the end product trehalose, and α (1→4) a shorter chain of malto –oligosaccharide.

This non-reducing disaccharide of trehalose can be hydrolyse (breakdown) by the intestinal lining enzyme trehalase (EC 3.2.1.28) into two glucose units as a source of energy providing 4 calories/gram which, is similar to calorie generated from sucrose intake. Trehalose is 45% sweeter of sucrose, heat stable, and stabilizer. Stabilizer property helps in preserve cell structure, biological materials, and foods after heating or freezing, with applications in food, and pharmaceutical industries.

In food industry, trehalose is used as texturizer, and stabilizer for dried foods, fruit filling, nutrient bars, instant rice, instant noodles, bakery creams, sugar coating, and fruits. In pharmaceutical industry, trehalose is used as cryo-protectant [40] for mammalian cells, and therapeutic proteins to limit the damage after freezing. Trehalose is also, used as an ingredient along with, hyaluronic acid in the artificial tears products for the treatment of tear dry eye.

Health benefits of trehalose includes, low potency in promoting tooth decay, and in triggering a small increase of blood insulin levels. Some research studies demonstrated that daily intake of 10 grams of trehalose improve glucose tolerance and reduce the progress of insulin resistance. Furthermore, researchers suggested that trehalose can potentially reduce the development of metabolic syndrome and reduce other associated lifestyle-related diseases such as, type 2 diabetes [41]. The only, reported disadvantages from trehalose intake is the high glycemic index, and is not suitable for patients with active celiac diseases, or GLUT 1 deficiency syndrome, [42] a disorder affecting the nervous system that can have a variety of neurological signs and symptoms, (GLUT 1, is the protein that transport glucose across brain barrier).

Trehalose, is generally recognized as safe (GRAS) by Food and Drugs Administration (FDA) in United States and is also, approved in the European Union (EU), Australia, and New Zealand, and is commonly used in Japan, Taiwan, and South Korea in food formulations.

Isomalto- oligosaccharides

Isomalto-oligosaccharides (ISMO) are composed of 3 to 6 glucose units linked together with indigestible α (1→6) glycosidic bonds, and present naturally at low concentrations in honey, and in fermented foods such as, in soy sauce. Isomalto-oligosaccharides is manufactured on large scale by enzymatic process [43] using starch as a substrate. This enzymatic process, involves first amylase enzymes for starch liquefaction and scarification, followed by the enzyme transglucosidase (EC 3.2.1.20) to convert glucose glycosidic bonds α (1→4) in starch into indigestible glycosidic bonds of α (1→6) in isomalto-oligosaccharides chemical structure, plus generating some impurities of free glucose, maltose, and maltotriose. These impurities, of glucose, maltose, and maltotriose are fermentable sugars that can be removed by yeast fermentation [44], to produce pure forms of isomalto-oligosaccharides with different degrees of glucose (G) polymerizations composed of isomaltose, panose, isomatotriose, and some other higher oligosaccharides (Figure 11).

Isomalto-oligosaccharides are first produced in Japan for Asian market, and then became more widely commercially available worldwide in the form of syrup or powder. Isomalto-oligosaccharides have low calorie and mild sweet taste, with food applications, as bulking agent, and for adding fibers to food products. In addition, isomalto-oligosaccharides blend with intensive zero calorie sweeteners to mask their unpleasant taste. Intensive zero calorie sweeteners are artificial sweeteners such as saccharine, aspartame, stevia, sucralose, and acesulfame-k that are used as sugar substitute in diet and functional foods. As prebiotic, isomalto-oligosaccharides have

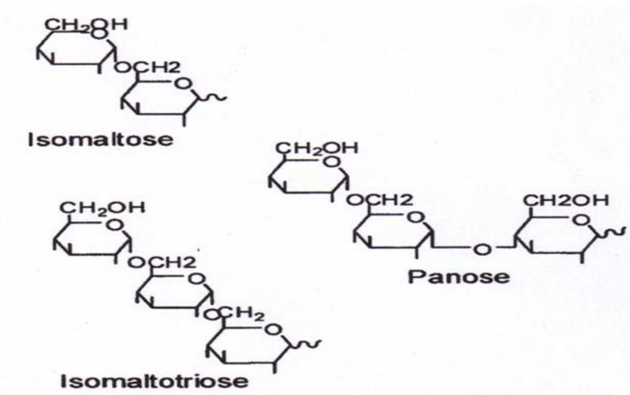

Figure 11: Isomalto-Oligosaccharides (IMO)
Composed of 3 to 6 glucose units linked together with an indigestible α (1→6) glycosidic bonds. The mixture is mainly composed of:
Isomaltose a disaccharide O-α-glucopyranosyl α (1→6) α-D-glucopyranoside.
Isomaltotriose a trisaccharide of α-D- glucosyl- (1→6) α-D-glucosyl (1→6) α-D-glucose.
Panose a trisaccharide of α-D-glucopyranosyl (1→6) α-D-glucopyranosyl (1→4)-D-glucose.

been shown to promote the growth of beneficial bacteria in the colon. Other, health benefits of isomalto-oligosaccharides are in reducing blood cholesterol and triglycerides, plus assist the body to absorb minerals from foods [45].

Isomalto-oligosaccharides are general recognized as safe (GRAS) by FDA in United States and are approved by other worldwide regulatory agencies with maximum daily intake of 30.0 grams/day. Over consumption of isomalto-oligosaccharides may cause gastrointestinal symptoms like flatulence, bloating, soft stool, or diarrhea.

Cyclodextrins

Cyclodextrins (CD) also known by the name cyclo-amyloses are family of cyclic oligosaccharides made of glucose molecules linked together with α (1→4) glycosidic bonds in a ring forming shape of glucopyranose molecules. Typical chemical structure of cyclodextrins contain a number of glucose units ranging from six to eight units, creating a cone shapes of α-cyclodextrin a six glucose units (G6) ring molecule, β-cyclodextrin a seven glucose units (G7) ring molecule, and γ-cyclodextrin an eight glucose units (G8) ring molecule (Figure 12)

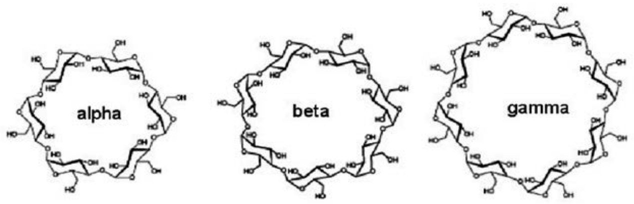

Figure 12: Cyclodextrins (CD)
Three oligosaccharides are made up of glucose molecules linked together with α (1→4) glycosidic bonds in a ring forming cyclic oligosaccharides of:
α-Cyclodextrin: Sex glucose unites ring molecule.
ß-Cyclodextrin: Seven glucose unites ring molecule.
γ -Cyclodextrin: Eight glucose units ring molecules.

Cyclodextrins are produced enzymatically [46] using starch as a substrate, first the starch is liquefied by heat treatment or by the enzyme α-amylase, then the liquefied starch is treated with the microbial enzyme of cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) to synthesize a mixture of all forms of cyclodextrins in a ratio that is vary depends on the property of the CGTase enzyme used. Separation of these three types of cyclodextrins individually is performed by column chromatography, or by taken advantage of the different water solubility of these three types of cyclodextrins.

The ring-shape of the three types (α, ß, and γ) of cyclodextrins have hydroxyl (hydrophilic) groups outside the ring and hydrophobic groups of glyosidic oxygen bonds in the cavity (inner circle). This confirmation of glucopyranose molecules makes cyclodextrins hydrophilic and shaped like truncated cone rather than perfect cylinder (Figure13) and enables cyclodextrins to insert hydrophilic compounds as guests inside the cavity, forming what is so-called host/guest inclusion complexes [47]. The cavity size of cyclodextrins is the smallest for α-cyclodextrin, and the largest for γ- cyclodextrin.

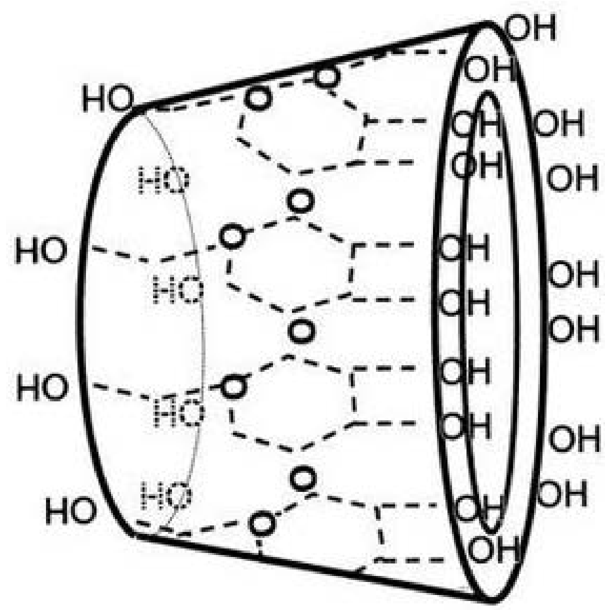

Figure 13: Cylodextrin cone shape
The Cyclodextrin exterior is water soluble (hydrophilic groups) and the interior is lipophilic (hydrophobic groups), therefore cyclodextrins are water soluble.

Cyclodextrins, α, ß, and γ are generally recognized as safe by FDA in United stated and are approved by other worldwide organizations for the application in both food, and pharmaceutical formulations [48] in addition, to the application of cyclodextrins for drugs delivery system by encapsulating in cyclodextrins cavities active drug ingredients (ADI) for target delivery in human body (Figure 14). Some examples, for cyclodextrins in pharmaceutical industry are used as, complexing agents to increase the aqueous solubility of poorly water-soluble drugs, to increase both drugs bioavailability and stability, to reduce or prevent gastrointestinal irritation, to reduce or eliminate unpleasant smells or tastes, to prevent drug to drug interactions, and to convert oils and liquid drugs into microcrystalline or amorphous powders [49].

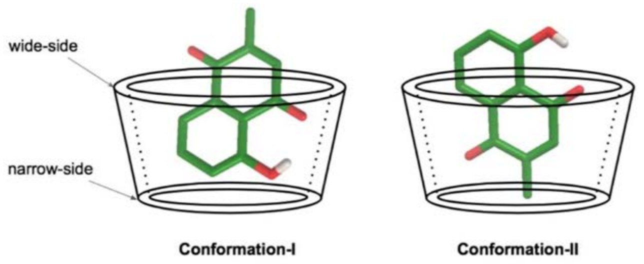

Figure 14: Cyclodextrins drugs delivery system
The Cyclodextrin exterior is water soluble (hydrophilic groups) and the The genomic shape of cyclodextrins creates cavity that allow cyclodextrins to engulf smaller molecules. The size of the cavity dictated the size molecule that may be encapsulated. γ-cyclodextrin encapsulate a larger molecule than α-cyclodextrin. The modified natural cyclodextrin known by the names cyclodextrin derivatives are developed to improve binding affinity and engulfed drug's efficacy.

Unlike γ -cyclodextrin, both α-and β-cyclodextrin, cannot hydrolyse by human saliva, and by pancreatic amylases [50], and are described as non-digestible prebiotic carbohydrates, which support the beneficial intestinal microflora in the colon.

In the United States' cyclodextrins applications in food industry are regulated as food additives, and in the European Union cyclodextrins applications in food industry are regulated as novel foods. General application of cyclodextrins in foods are in such as, stabilization of flavors, flavor delivery, the eliminations of undesired tastes, the eliminations of microbiological contaminations, and developing browning reaction in foods and baked goods. Other examples for the application of ß-cyclodextrin is to remove cholesterol from milk, butter, eggs, and its use in food preservation [51].

In addition, to the application of cyclodextrins in foods, and pharmaceutical industries, cyclodextrins have other industrial applications such as, in cosmetics, and in agriculture chemicals formulation. In cosmetic industry, cyclodextrins are used to control release fragrances from inclusion products such as, from detergents, perfumes and room fresheners [52]. In agriculture industry, cyclodextrins are used in manufacturing complex formulas of pesticides, herbicides, and insects' repellents for the production of safety products to consumers, and to be environmentally friendly [53].

In general, the three types of cyclodextrins have wide range of applications, and ß-cyclodextrin seems to be the more used especially in pharmaceutical industry due to, the size of its cavity, its efficacy, and for its lower production cost [54].

Other Oligosaccharides

Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are polymer of five carbons (C5) of the pentose sugar xylose which, makes xylo-oligosaccharides are different from other oligosaccharides that are polymers of six carbon (C6) of hexose sugars. The number of xylose units in xylo-oligosaccharides chemical structure that are linked together by ß (1→4) glycosidic bonds are vary from tow to ten units (Figure 15).

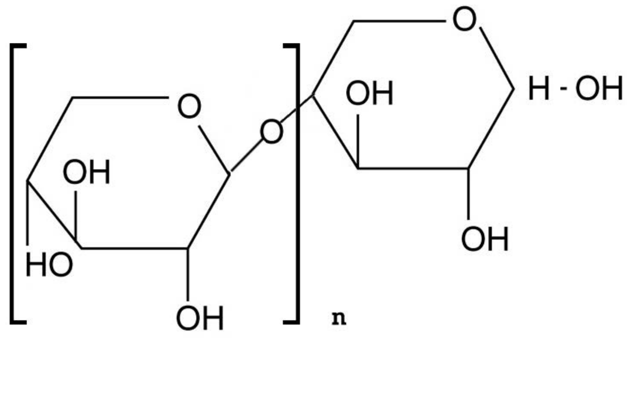

Figure 15: Xylo-oligosaccharodes
Composed of Xylose residues linked together by ß (1→4) glycosidic bonds. (n) is the variable number of xylose units linked together.

Xylo-oligosaccharides are produced enzymatically using xylan as a substrate. Xylan is a pentose polymer of sugar xylose present in hemicellulose structure of plant cells and can be extracted from agriculture residue [55] by using alkali chemicals such as, potassium or sodium hydroxide (KOH or NaOH), or by using dimethyl sulfoxide (DMSO).

Enzymatic process for the production of xylo-oligosaccharides from xylan as a substrate, is by using the microbial enzyme xylanase (EC 3.2.1.8). This enzyme hydrolysis ß (1→4) glycosidic bonds that linked xylose units in xylan chemical structure. This enzymatic process was originally developed and manufactured in Japan, and is recently became commercially available worldwide due to, the improved production yield that reduced the production cost [56].

Xylo-oligosaccharides are naturally present at low concentration in fruits, vegetables, bamboo, honey, and milk. And are generally recognized as safe (GRAS) by FDA in U.S. and are approved by other worldwide organizations for the application in foods, and pharmaceutical products formulation.

Xylo-oligosaccharides are water soluble, have low caloric value with sweet taste that about 40% sweetness comparing to sugar sucrose, have humectant, and antimicrobial properties. In food industry, xylo-oligosaccharides are incorporated in a wide variety of food products, such as, beverages, dairy products, acid products, salad dressings, alcohol beverages, functional foods, and sugarless or low sugar confections. In pharmaceutical industry, several clinical trials have been demonstrated a variety of health benefits from xylo-oligosaccharides consumption such as, reducing blood sugar and lipid, laxation, and showed beneficial changes in immune response. Xylo-oligosaccharides are also, used as prebiotic [57] and as, soluble fiber in dietary supplement products. These health benefits have been observed at low daily intake of 1.0 to 4.0 grams/day [58].

Soy-oligosaccharides

Soy beans are rich in oligosaccharides, with small amounts of fructose, rhamnose, and arabinose, in addition, to non-starch polysaccharides (NSP) that are divided into polymers of insoluble cellulose, and soluble pectin.

Soy-oligosaccharides are easily extracted from defatted soy meal (DSM) in 50 ^{0}C water containing 10% ethanol. The insoluble extract of proteins and cellulose are removed by ultrafiltration, and the soluble fraction that contain the soy-oligosaccharides is concentrated. The extracted soy-oligosaccharides from soy beans is a mixture of raffinose, stachyose, and verbascose. This mixture is also known by the name Raffinose Series Oligosaccharides (RSO).

Chemical structures of soy-oligosaccharides (Figure 16) are:

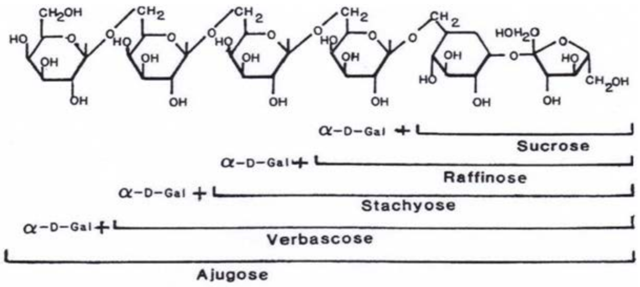

Figure 16: Extraction of Soy –oligosaccharide
Soy- oligosaccharides are namely, raffinose, stachyose, and verbascose. Raffinose is a trisaccharide containing galactose linked by α (1→6) bond to the glucose unit of sucrose. Stachyose is a tetrasaccharide containing extra galactose unit linked to raffinose by α (1→6) bond. Verbascose is pentasaccharide containing extra galactose unit linked to stachyose by α (1→6) bond.

• Raffinose (C_{18} H_{32} O_{16}) is a trisaccharide of galactose polymer linked by α (1→6) bond to the glucose unit in the disaccharide sucrose.

• Stachyose (C_{24} H_{42} O_{21}) is a tetra-saccharide of one galactose unit linked by α (1→6) bond to the trisaccharide raffinose.

• Verbascose (C_{30} H_{52} O_{26}) is penta-saccharide of one galactose unit linked by α (1→6) bond to the tetra-saccharide stachyose.

Soy-oligosaccharides are low calorie sweeteners and soluble fiber that, can make the stools softer. In addition, to have common health benefits such as, preventing constipation, improve absorption of calcium and other minerals, increase microbial metabolites of short chain fatty acid that help reducing the risk of colon cancer, and minimize toxic metabolites production that effect liver. The recommended daily intake of soy-oligosaccharides is 4.0 grams/day, and It is important to highlight that over consumption of soy-oligosaccharides may trigger abdominal bloating, excessive gas, and diarrhea [59].

The characteristic of α (1→6) linked bonds in soy-oligosaccharides chemical structures is unbreakable due to, the lack of α- galactosidase enzyme in human digestive system. The undigested soy-oligosaccharides reached the colon intact where fermented by intestinal microflora that are producing the enzyme α- galactosidase enzyme capable to break down (hydrolyze) soy-oligosaccharides into fermentable sugars. These fermentable sugars enhance the growth of enteric bacteria in the colon. Currently, some scientists did not consider soy-oligosaccharides as prebiotic due to, conflict in published research reports on the property of Soy-oligosaccharides as prebiotic. Some researchers observed a significant increase in only the beneficial bacteria of *Bifidobacterium* species [60], while others, observed significant increase in both beneficial bacteria of *Bifidobacterium* species and the pathogenic bacteria of *Clostridium* species. This disputed research reports on the prebiotic property of soy-oligosaccharides still needs further investigation.

Discussion

Functional oligosaccharides are low molecular weight carbohydrates with different degrees of polymerization (DP) of monosaccharides in the range of two to ten units. They are intermediate chemical structures in molecular weight between monosaccharides, and polysaccharides. They are present naturally in small quantities in different natural sources such as higher plants, algae, bacteria, yeast, and fungi.

Due to, increasing demands of these functional oligosaccharides for health benefits, functional oligosaccharides are produced commercially by enzymatic processes at higher yield and lower costs comparing to extraction methods from natural sources. Carbohydrates are the main substrates for microbial enzymes in the production of functional oligosaccharides enzymatically at large scale operations.

With the exception of malto-oligosaccharides and trehalose, all known functional oligosaccharides are non-digestible dietary fibers with prebiotic properties, that are gaining consumers demand since 1980s for health benefits. The definition of prebiotics are non-digestible compounds that promote in the colon the growth of healthy endogenous or oral intake live beneficial bacteria. These healthy bacteria (probiotics) with the help of functional oligosaccharides as prebiotics multiply in the colon with high growth rate that inhibit the growth of harmful pathogenic bacteria via competitive exclusion plus, producing metabolites and short chain fatty acids that help maintaining healthy colon. These metabolites, and short chain fatty acids are generated by the microbial fermentation of functional oligosaccharides in the colon. This relationship between probiotics (beneficial bacteria), and prebiotics (functional oligosaccharides) is referred to synbiotics.

Furthermore, the low sweetness attribute and low caloric values of these functional oligosaccharides makes them useful as bulking agents in low calories food formulations. This bulking property of these functional oligosaccharides, stimulates healthy bowel movements, preventing constipation, and diarrhea symptoms. In addition, these functional oligosaccharides are humectants due to, their high moisture retaining capacity without increasing foods water activity. This humectants property of functional oligosaccharides has multiple applications in foods, pharmaceuticals, and in cosmetics formulations.

The major known functional oligosaccharides are fructo-oligosaccharides, galacto-oligosaccharides, lacto-sucrose, isomalto-oligosaccharides, malto-oligosaccharides. trehalose, cyclodextrins, xylo-oligosaccharides, and soy-oligosaccharides. These types of oligosaccharides are present naturally at low concentrations in higher plants, honey, milk, dairy products, algae, bacteria, yeast, and fungi. Low concentrations of these functional oligosaccharides in natural sources makes extraction processes of these types of oligosaccharides from their natural sources are very costly. Currently, commercial production of these types of functional oligosaccharides are by enzymatic processes using carbohydrates as substrates for specific microbial enzymes. Carbohydrates used as substrates for these enzymatic processes are sucrose from sugar cane or beats, lactose from milk or cheese whey, starch from corn, wheat, or rice, xylose from wheat bran, barley hulls, or brewery spent grain, inulin from Jerusalem artichoke, and pentose polymers from soy beans.

These enzymatically producing functional oligosaccharides are generally recognized as safe (GRAS) in United States and also, granted safety status in both European union and Asia due to, the fact that they are naturally present at low concentrations in our daily diet intake, and because they are commercially manufactured from natural carbohydrates as substrates and by using natural microbial enzymes in production processes. The only, two starch related oligosaccharides that are not generally recognized as safe (GRAS) in United States and in other parts of the world are nigero-oligosaccharides and gentio-oligosaccharides. These two oligosaccharides are not naturally present in plants or in any other natural sources and are manufactured from starch using genetically engineered enzymes that are not exist in nature. These two starch related oligosaccharides (nigero-oligosaccharides and gentio-oligosaccharides) are not approved for food or pharmaceutical applications, but they might have other industrial applications.

Functional oligosaccharides with GRAS statutes have mildly sweet taste and have certain other characteristics such as, mouth feeling and texture. These properties, drawn the interest of foods industry for the application of these functional oligosaccharides as partial substitute for fats and sugars in food formulations. These functional oligosaccharides, with the exception of malto-oligosaccharides and trehalose have prebiotics property, because they are indigestible and stimulate the growth of beneficial bacteria in the colon. In addition, they have multiple health benefits such as, increase the digestion of lactose metabolism, increase calcium and other minerals absorption, decrease serum lipids, decrease blood cholesterol, improve HDI/LDL ratio, improve blood pressure, enhance immune response, trigger anti-inflammatory response, decrease fecal PH, and eliminate the effect of toxic, or carcinogenic chemicals. These functional oligosaccharides as dietary supplements, stimulate healthy bowel movements, and overcome diarrhea or constipation. Recently, some researchers suggested that the intake of these functional oligosaccharides as dietary supplements promote the secretion of substances that influence, satiety and weight loss. The only reported common side effects are bloating and gas when the consumption of these functional oligosaccharides exceeded the recommended daily intake.

These properties and major health benefits lend the functional oligosaccharides to be added in infant formulas, regular foods, and in the production of functional foods. Functional foods concept was first introduced in the Japan for health and wellness and are expanded globally with total global market over $90 billion per year.

The use of functional oligosaccharides in pharmaceuticals industry as excipients in pharmaceutical drugs formulations have been documented, and the most common functional oligosaccharides used in pharmaceuticals industry are cyclodextrins and trehalose, not only as excipients in drugs formulations such as, the application of cyclodextrins in nanoparticles, liposomes, nasal, ophthalmic, and rectal formulations, but for the application in drugs delivery systems. The concept of drugs delivery system is to deliver the required dose of active pharmaceutical ingredients (drugs) to the target site with efficiency and precisely at the necessary time period. Cyclodextrins and cyclodextrin derivatives, have been found to be the best candidates for this concept of drugs delivery systems due to, their capabilities at lower cost to alter physical, chemical, and biological properties of pharmaceutical drugs chemical structure, as potential guest molecule through the formation of inclusion complex (encapsulation). This drug delivery system leads to the production of cyclodextrins and cyclodextrin derivatives at higher purity for the safety of pharmaceutical drugs delivery. These cyclodextrin derivatives are developed to enhance the bioavailability of insoluble drugs by increasing their solubility, and to improve the stability of labile pharmaceutical drugs against dehydration, hydrolysis, oxidation, or photodecomposition. Cyclodextrins and derivatives also, have industrial applications such as, the encapsulations of foods, cosmetics, and agriculture chemicals. The application of cyclodextrins and derivatives in agriculture chemicals are in pesticides and fertilizers industry.

In addition, to multiple applications of trehalose in foods, pharmaceuticals, and cosmetics formulations, trehalose has a unique important biopharmaceutical application as preservative, and stabilizer for labile therapeutic proteins and as cryoprotectant for the maintenance of mammalian cells, and other biological materials. These unique properties of trehalose is recently extended into ophthalmology products for the treatment of dry eye syndrome and other eye diseases.

Conclusion

Functional oligosaccharides are commercially manufactured enzymatically from natural sources of carbohydrates. They have unique properties, and health benefits, with wide varieties of applications such as, in food industry as food additives as being regulated in United States, and in pharmaceutical industry as being regulated as expedients for drugs formulation. In addition, these functional oligosaccharides have other applications in cosmetics, and agriculture chemicals.

Cyclodextrins and cyclodextrin derivatives have extra unique application in drugs delivery system, and trehalose has extra unique application as preservative, as stabilizer for labile therapeutic proteins and as cryoprotectant for the maintenance of mammalian cells, and sensitive biological materials.

References

1. Cheng W, Lu J, Li B, Lin W, Zhang Z, et al. 2017. Effect of functional oligosaccharides and ordinary dietary fibers on intestinal microbiota diversity. *Front Microbiol* 8: 1750. https://doi.org/10.3389/fmicb.2017.01750
2. Rudd PM, Wormald MR, Stanfield RL, Huang M, Mattsson N, et al. 1999. Roles for glycosylation of cell surface receptors involved in cellular immune recognition. *J Mol Biol* 293(2): 351-366. https://doi.org/10.1006/jmbi.1999.3104
3. Lingyun W, Jianhua W, Xiaodong Z, Da T, Yalin Y, et al. 2007. Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. *J Food Eng* 79(3): 1087-1093. https://doi.org/10.1016/j.jfoodeng.2006.03.028
4. Scheppach W, Bartram HP, Richter F. 1995. Role of short-chain fatty acids in the prevention of colorectal cancer. *Eur J Cancer* 31(7-8): 1077-1080. https://doi.org/10.1016/0959-8049(95)00165-F
5. Slavin J. 2013. Fiber and prebiotics: mechanisms and health benefits. *Nutrients* 5(4): 1417-1435. https://doi.org/10.3390/nu5041417
6. Crittenden RG, Plaync MJ. 1996. Production, properties, and applications of food grade oligosaccharides. *Trends Food Sci Technol* 7(11): 353-361. https://doi.org/10.1016/S0924-2244(96)10038-8
7. Morris C, Morris GA. 2012. The effect of inulin and fructo-oligosaccharides supplementation on the textural rheological and sensory properties of bread and their role in weight management: a review. *Food Chem* 133(2): 237-248. https://doi.org/10.1016/j.foodchem.2012.01.027
8. Loftsson T, Duchêne D. 2007. Cyclodextrins and their pharmaceutical applications. *Int J Pharm* 329(1-2): 1-11. https://doi.org/10.1016/j.ijpharm.2006.10.044
9. Muir JG, Rose R, Rosella O, Liels K, Barrett JS, et al. 2009. Measurement of short chain carbohydrates in common Australian vegetables and fruits by high-performance chromatography (HPLC). *J Agric Food Chem* 57(2): 554-565. https://doi.org/10.1021/jf802700e
10. Duan KJ, Chen JS, Sheu C. 1994. Kinetic studies and mathematical model for enzymatic production of fructo-oligosaccharides. *Enzyme Microb Technol* 16(4): 334-339. https://doi.org/10.1016/0141-0229(94)90176-7
11. Sing RS, Singh RP. 2010. Production of fructo-oligosaccharides from inulin by endo-inulinases and their prebiotic properties. *Food Technol Biotechnol* 48(4): 435-450.
12. Mabel MJ, Sangeetha PT, Platel K, Srinivasan K, Prapulla SG. 2008. Physicochemical characterization of fructo-oligosaccharides and evaluation of their stability as a potential sweetener for diabetics. *Carbohydr Res* 343(1): 56-66. https://doi.org/10.1016/j.carres.2007.10.012
13. Costa GT, Abreu GC, Guimaraes AB, Vasconcelos PR, Guimaraes SB. 2015. Fructo-oligosaccharide effects on serum cholesterol levels. An overview. *Acta Cir Bras* 30(50): 366-370. https://doi.org/10.1590/S0102-865020150050000009
14. Generally recognized as safe for short chain fructo-oligosaccharides. US Food and Drug Administration. According with 21 CFR 170.30.
15. Abrahamse-Berkeveld M, Alles M, Franke-Beckmann E, Helm K, Knecht R, et al. 2016. Infant formula containing galacto and fructo-oligosaccharides and Bifidobacterium breve M-16v supports adequate growth and tolerance in healthy infants in a randomized, controlled, double-blind, prospective, multicenter study. *J Nutr Sci* 5: e42. https://doi.org/10.1017/jns.2016.35
16. Jain M, Gote M, Dubey AK, Narayanan S, Krishnappa H, et al. 2018. Safety evaluation of fructo-oligosaccharides (FOSSENCE TM): acute,14 days, and sub-chronic oral toxicity study in Wistar rats. *Toxicology Research and Application* 2: 1-4. https://doi.org/10.1177/2397847318787750
17. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Katan MB, van der Meer R. 2006. Dietary fructo-oligosaccharides affect intestinal Barrier-function in healthy men. *J Nutr* 136(1): 70-74. https://doi.org/10.1093/jn/136.1.70
18. Sairanen U, Pirained L, Nevata R, Korpela R. 2007. Yoghurt containing gala-oligosaccharides, prunes and linseed reduces the severity of mild constipation in elderly subjects. *Eur J Clin Nutr* 61(12): 1423-1428. https://doi.org/10.1038/sj.ejcn.1602670
19. Matella NJ, Dolan KD, Lee YS. 2006. Comparison of galacto-oligosaccharides production in free enzyme ultrafiltration and immobilized enzyme systems. *Food Sci* 71(7): 363-368. https://doi.org/10.1111/j.1750-3841.2006.00086.x
20. Chockchaisawasdee S, Athanasopoulos VI, Niranjan K, Rastall RA 2005. Synthesis of galcto-oligosaccharides from lactose using ß from Kluyveromyces lactis: studies on batch and continuous UF membrane filtered bioreactor. *Biotechnol Bioeng* 89(4): 434-443. https://doi.org/10.1002/bit.20357
21. Lamsal BP. 2012. Production, health aspects, and potential food uses of dairy prebiotic galacto-oligosaccharides. *J Sci Food Agric* 92(10): 2020-2028. https://doi.org/10.1002/jsfa.5712
22. Bakker-Zierikzee AM, Alles MS, Knol J, Kok FJ, Tolboom JJ et al. 2005. Effect of infant formula containing a mixture of galacto-oligosaccharides and fructo-oligosaccharides or viable *Bifidobacterium* animalis on the intestinal microflora during the first 4 months of life. *Br J Nutr* 94(5): 783-790. https://doi.org/10.1079/BJN20051451
23. Macfarlane FT, Steed H, Macfarlane S. 2008. Bacterial metabolism and health related effects of galacto-oligosaccharides and other prebiotics. *J Appl Microbiol* 104(2): 305-344. https://doi.org/10.1111/j.1365-2672.2007.03520.x
24. Huyghebaert G, Ducatelle R, Van Immerseel F. 2011. An update on alternative to antimicrobial growth promoters for boilers. *Vet J* 187(2): 182-188. https://doi.org/10.1016/j.tvjl.2010.03.003
25. Iqbal MF, Cheng YF, Zhu WY, Zeshan B. 2008. Mitigation of ruminant methane production: current strategies, constraints and future options. *World J Microbiol Biotechnol* 24(12): 2747-2755. https://doi.org/10.1007/s11274-008-9819-y
26. Yang GQ, Yin Y, Liu HY, Liu GH. 2016. Effects of dietary oligosaccharide supplementation on growth performance, concentrations of the major odor-causing compounds in excreta, and the cecal microflora of broilers. *Poult Sci* 95(10): 2342-2351. https://doi.org/10.3382/ps/pew124
27. Niittynen L, Kajander K, Korpela R. 2007. Galacto-oligosaccharides and bowel function. *Scand J Food Nutr* 51(2): 62-66. https://doi.org/10.1080/17482970701414596
28. Choi HJ, Kim CS, Kim P, Jung HC, Oh DK. 2004. Lacto-sucrose bioconversion from lactose and sucrose by whole cells of *Paenibacillus polymyxa* harboring levansucrase activity. *Biotechnol Prog* 20(6): 1876-1879. https://doi.org/10.1021/bp049770v
29. Hara H, Shou-Tou LI, Sasaki M, Maruyama T, Twrada A, et al. 1994. Effective dose of lacto-sucrose on fecal flora and fecal metabolites of humans. *Bifidobacteria and Microflora* 13(2): 8-63. https://doi.org/10.12938/bifidus1982.13.2_8
30. Bassarb S, Bechtold-peters K, Fuhrherr R, Friess W, Gardiel P, et al. 2005. Powder comprising new compositions of oligosaccharides and methods for their preparations. US20050250704A1.
31. Teramoto F, Rokutan K, Kawakami Y, Fujimura Y, Uchidac J, et al. 1996. Effect of 4G-β-D-galactosylsucrose (lacto-sucrose) on fecal microflora in patients with chronic inflammatory bowel disease. *J Gastroenterol* 31(1): 33-39. https://doi.org/10.1007/BF01211184

32. Nobuaki I. 1998. Antiathlete's foot Composition. WO1998057650A1.

33. Ball SG, Morell MK. 2003. From bacterial glycogen to starch: Understanding the biogenesis of the plant starch granules. *Annu Rev Plant Biol* 54: 207-233. https://doi.org/10.1146/annurev.arplant.54.031902.134927

34. Dey G, Palit S, Banerjee R, Maiti BR. 2002. Purification and characterization of maltooligosaccharide-forming amylase from *Bacillus circulans* GRS 313. *J Ind Microbiol Biotechnol* 28(4): 193-200. https://doi.org/10.1038/sj/jim/7000220

35. Smits ALM, Kruiskamp PH, Van Soest JJG, Vliegenthart JFG. 2003. The influence of various small plasticizers and malto-oligosachharides on the retrogradation of (partly) gelatinized starch. *Carbohydr Polym* 51(4): 417-424. https://doi.org/10.1016/S0144-8617(02)00206-0

36. Grittenden RG, Playne MJ. 1996. Production, properties, and applications of food grade-oligosaccharides. *Trends Food Sci Technol* 7(11): 353-361. https://doi.org/10.1016/S0924-2244(96)10038-8

37. Qiang X, YongLie C, QianBig W. 2009. Health benefit applications of functional oligosaccharides. *Carbohydr Polym* 77(3): 435-441. https://doi.org/10.1016/j.carbpol.2009.03.016

38. Elbein AD, Pan YT, Pastuszak I, Carroll D. 2003. New insights on trehalose: a multifunctional molecule. *Glycobiology* 13(4): 17R-27R. https://doi.org/10.1093/glycob/cwg047

39. Kobayashi K, Komeda T, Miura Y, Kettoku M, Kato M. 1997. Production of trehalose from starch by novel trehalose-producing enzymes from sulfolobus solfataricus KM1. *J Ferment Bioeng* 83(3): 296-298. https://doi.org/10.1016/S0922-338X(97)80996-8

40. Rodrigues JP, Paraguassú-Braga FH, Carvalho L, Abdelhay E, Bouzas LF, et al. 2008. Evaluation of trehalose and sucrose as cryoprotectants for hematopoietic stem cells of umbilical cord blood. *Cryobiology* 56(2): 144-151. https://doi.org/10.1016/j.cryobiol.2008.01.003

41. Mizote A, Yamada M, Yoshizane C, Arai N, Maruta K, et al. 2016. Daily intake of trehalose is effective in the prevention of lifestyle-related diseases in individuals with risk factors for metabolic syndrome. *J Nutr Sci Vitaminol* 62(6): 380-387. https://doi.org/10.3177/jnsv.62.380

42. Casarejos MJ, Solano RM, Gómez A, Perucho J, de Yébenes JG, et al. 2011. The accumulation of neurotoxic proteins, induced by proteasome inhibition, is reverted by trehalose, an enhancer of autophagy, in human neuroblastoma cells. *Neurochem Int* 58(4): 512-520. https://doi.org/10.1016/j.neuint.2011.01.008

43. Kuriki T, Yanase M, Takata H, Takesada Y, Imanaka T, et al. 1993. A new way of producing isomalto-oligosaccharide syrup by using the transglycosylation reaction of neopullulanase. *Appl Environ Microbiol* 59(4): 953-959.

44. Pan YC, Lee WC. 2005. Production of high purity isomalto-oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. *Biotechnol Bioeng* 89(7): 797-804. https://doi.org/10.1002/bit.20402

45. Yen CH, Tseng YH, Kuo YW, Lee MC, Chen HL. 2011. Long-term supplementation of isomalto-oligosaccharides improved colonic microflora profile, bowel function, and blood cholesterol levels in constipated elderly people--a placebo-controlled, diet-controlled trial. *Nutrition* 27(4): 445-450. https://doi.org/10.1016/j.nut.2010.05.012

46. Biwer A, Antranikian G, Heinzle E. 2002. Enzymatic production of cyclodextrins. *Appl Microbiol Biotechnol* 59(6): 609-617. https://doi.org/10.1007/s00253-002-1057-x

47. Morrison PW, Connon CJ, Khutoryanskiy VV. 2013. Cyclodextrin mediated enhancement of riboflavin solubility and corneal permeability. *Mol Pharm* 10(2): 756-762. https://doi.org/10.1021/mp3005963

48. Tiwari G, Tiwari R, Rai AK. 2010. Cyclodextrins in delivery systems: Applications. *J Pharm Bioallied Sci* 2(2): 72-79. https://doi.org/10.4103/0975-7406.67003

49. Shimpi S, Chauhan B, Shimpi P. 2005. Cyclodexrins Applications in different routes of drugs administrations. *Acta Pharm* 55(2): 139-156.

50. Reineccius TA, Reineccius GA, Peppard TL. 2002. Encapsulation of flavors using cyclodextrins. Comparison of flavor retention in alpha, beta, and gama types. *J Food Sci* 67(9): 3271-3279. https://doi.org/10.1111/j.1365-2621.2002.tb09577.x

51. Jeong HJ, Sun H, Chogsom C, Kwak HS. 2014. Cholesterol removal from whole eggs by crosslinked ß-Cyclodextrin. *Asian-Australas J Anim Sci* 27(4): 537-542.

52. Buschmann HJ, Schollmeyer E. 2002. Applications of Cyclodextrins in cosmetic products. A review. *J Cosmet Sci* 53(3): 185-191.

53. Szejtli J. 1998. Cyclodextrins in pesticides. In: Szejtli J (ed) Cyclodextrins technology. Springer, Dordrecht, pp 335-364.

54. Figueiras A, Carvalho RA, Ribeiro L, Torres-Labandeira JJ, Veiga FJ. 2007. Solid-state characterization and dissolution profile inclusion complexes of omeprazole with native and chemically modified ß-Cyclodextrin. *Eur J Pharm Biopharm* 67(2): 531-539. https://doi.org/10.1016/j.ejpb.2007.03.005

55. Rowley J, Decker SR, Michener W, Black S. 2013. Efficient extraction of xylan from delignified corn stover using dimethyl sulfoxide. *3 Biotech* 3(5): 433-438. https://doi.org/10.1007/s13205-013-0159-8

56. Samanta AK, Jayapal N, Kotte AP, Senani S, Sridhar M, et al. 2015. Process for enzymatic production of xylo-oligosaccharides from the xylan of corn cobs. *J Food Process Preserv* 39(6): 729-737. https://doi.org/10.1111/jfpp.12282

57. Chung YC, Hsu CK, Ko CY, Chang YC. 2007. Dietary intake of xylo-oligosaccharides improves the intestinal microbiota, fecal moisture, and pH value in the elderly. *Nutr Res* 27(12): 756-761. https://doi.org/10.1016/j.nutres.2007.09.014

58. Jain I, Kumar V, Satyanarayana T. 2015. Xylo-oligosaccharides an economical prebiotic from agroresidues and their health benefits. *Indian J Exp Biol* 53(3): 131-142.

59. Suarez FL, Springfield J, Furne JK, Lohrmann TT, Kerr PS, et al. 1999. Gas production in human ingesting soy bean flour derived from bean naturally low in oligosaccharides. *Am J Clin Nutr* 69(1): 135-139. https://doi.org/10.1093/ajcn/69.1.135

60. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, et al. 2004. The capacity of non-digestible carbohydrates to stimulate fecal bifidibacteria to healthy humans: a double-blind randomized placebo-controlled parallel, dose response relation study. *Am J Clin Nutr* 80(6): 1658-1664. https://doi.org/10.1093/ajcn/80.6.1658