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Prebiotics—Clinical relevance

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Prebiotics are dietary substances that mostly consist of non-starch polysaccharides and oligosaccharides that nurture a selected group of microorganisms residing in the human intestine. These components are poorly hydrolyzed by our digestive enzymes. Prebiotics preferentially favour the growth of probiotic organisms like *Lactobacillus* and *Bifidobacterium* that are helpful in gut health maintenance, colitis prevention, cancer inhibition, immunopotentiation, cholesterol removal, reduction of cardiovascular disease, prevention of obesity and constipation. The natural sources of prebiotics are certain fruits and vegetables like asparagus, banana, chicory, garlic, onion, wheat and tomato. Considering increasing market demand of prebiotics, commercial production of them is standardized from wastes of food industries as well as from other sources. Due to their therapeutic support and history of safe use, fructo-oligosaccharides and galacto-oligosaccharides are now widely used for pharmaceutical formulations, combined with probiotics.

Keywords: Prebiotics, Non digestible oligosaccharides, Fructo-oligosaccharides, Galacto-oligosaccharides, Lactic acid bacteria.

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Introduction

The term prebiotics was first defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and / or activity of one or limited number of bacteria in colon thus improving the host health"[1]. This definition was later refined in 2004 to include other areas that may benefit from selective targeting of particular microorganisms: "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora which confers benefit"[2]. These food ingredients have attracted a tremendous interest in human nutrition both in the field of clinical research and food applications. A 2007 report on the world probiotic market states that there are over 400 prebiotic food products and more than 20 companies producing oligosaccharides and fibres used as prebiotics. European prebiotic market is currently worth € 87 million and according to a new report from Global Industry of Analysis it is projected to reach nearly $ 1.2 billion by the year 2015[3].

Among the large number of different bacteria present in the colon, three groups are most prominent. The first group comprising bifidobacteria, lactobacilli and other genera of lactic acid bacteria (LAB), is the beneficial group responsible for improvement of health[4]. The second group consists of the bacteria from the family Enterobacteriaceae and species from the genus *Clostridium*. This group is considered as negative for good health. The third group comprises of all the other bacteria and considered as neutral. The proliferation of bifidobacteria and lactobacilli in presence of prebiotics is well established in *in vitro* studies[5-7]. Increase in number of bifidobacteria and lactobacilli have also been reported in gut mucosa of patients waiting for colonoscopy with the ingestion of 15 g/day for 2 weeks of fructooligosaccharides-enriched inulin[8]. Feeding with 5 % (w/v) galacto-oligosaccharides to human microflora associated rats, has been shown to significantly increase population of *Bifidobacterium* and lactobacilli while decreasing the number of enterobacteria[9].

Prebiotics that are described and commercially available are carbohydrate in nature. Prebiotics are composed of 2-20 monosaccharide units. The criteria for food ingredient as prebiotics are[1] (i) resistant to gastric acidity, resistant to hydrolysis by mammalian enzymes and can be absorbed in the upper gastrointestinal tract, (ii) should be fermented by the intestinal microflora and (iii) selectively stimulates

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the growth and/or activity of intestinal bacteria potentially associated with health and well-being.

Properties of prebiotic[10]

Prebiotic is not an organism or drug. It is a substance that can be characterized chemically. Most of the prebiotics are food grade component. Health benefits of prebiotics are measurable and benefits are not due to absorption of the component into the blood stream or due to the component acting alone and over-riding any adverse effects. The sole presence of the prebiotic component and formulation in the host can derive changes in the composition or activities of the microbiota. Mechanism might include fermentation, receptor blockage or others.

Commercially available prebiotics

The prebiotics are low-molecular weight, non-digestible oligosaccharides (NDO) with a low degree of polymerization (DP). After reaching colon, these NDOs (inulin, lactulose, fructo-oligosaccharides and galacto-oligosaccharides) are utilized by the resident bacteria (i.e. bifidobacteria and lactobacilli). This type of fermentation produces short chain fatty acids (SCFA) and gases as well as results in an increase of metabolic energy, growth and proliferation of these bacteria[11]. Among the different NDOs which are produced enzymetically from various raw materials[12], fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) have the most scientific support to date.

Fructo-oligosaccharides

FOS are food ingredients which can be classified as prebiotics. These are calorie-free, non carcinogenic and non mutagenic. FOS are present in wide variety of foods like onion (2.8 %), banana (0.3 %), garlic (0.2 %), tomato (1.8 %), rye (0.7 %), etc[13]. Average daily consumption of FOS's is estimated to be 1-4 g in the USA and 3-11g in Europe[14].

FOS are oligosaccharides of D-fructose attached by β-(2→1) linkages that carry a D-glucosyl residue at the end of the chain. They constitute a series of homologous oligosaccharides derived from sucrose usually represented by the formula GF_n[15] (Where, G= Glucose, F= Fructose and n= 2~10). Inulin (Fig. 1) and oligofructose are common forms of FOS that are widely found in nature. Theoretically, oligosaccharides have a DP lower than 9. Inulin is commonly referred to as a FOS, despite the fact that it contains polymers with a DP of up to 60[(Ref.16)]. Inulin contains polymers with average DP of 10 and exhibit neutral taste. Standard inulin is slightly sweet (10 % sweetners compared with sugar), whereas high performance inulin (DP~25) does not have a sweet taste. Inulin is moderately soluble in water (10 g/100 mL, maximum at room temperature) and has a low viscosity[17]. In terms of sweetness profile, the oligofructose provide functional qualities similar to sugars or glucose syrups[1]. Oligofructose is much more soluble than inulin (80 % at room temperature). Using animal model or volunteers fed with inulin or FOS, *in vivo* experiments support the bifidogenic effect of FOS[18]. Scientists also reported that after the prebiotic treatment beneficial colonic microflora increased considerably[19].

Production of FOS

The majority of commercially produced fructans are inulin type. The production of naturally occurring inulin involves extraction from chicory roots (*Cichorium intybus* L.) followed by refining and drying[20] (Fig. 2). The FOS can be manufactured by two different enzymes. The oligofructose can be produced by partial enzyme hydrolysis of inulin (extracted from chicory roots) using endo-inulinase, followed by spray drying. In another process short chain FOS (scFOS) is produced on a commercial scale by transfructosylation of sucrose using β-fructosyltransferase enzyme derived from microorganism or plants. The plants like asparagus, sugarbeet, onion, jerusalem artichoke, etc. are the source of β-fructosyltransferase enzyme. On the other hand *Aspergillus sp*, *Aureobasidium sp*, *Arthrobacter sp* and *Fusarium sp* are the microbial source of the said enzyme[15].

Meiji Seika Co (Japan) in 1984 first succeeded in the commercial production of FOSs (Neo sugar) by

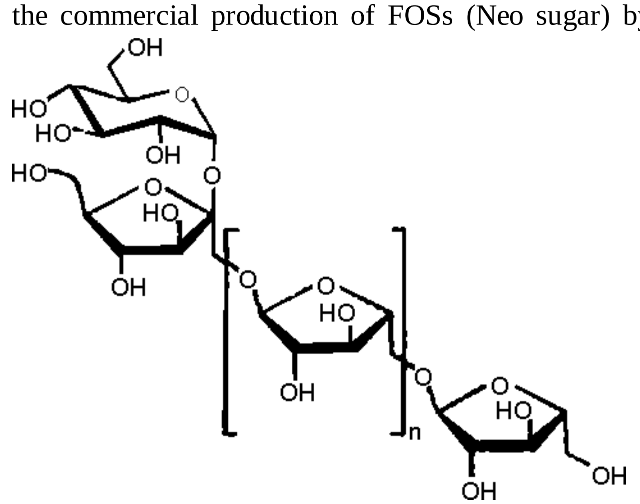

Fig. 1.− Structure of Inulin

using *A. niger*. It was found to have excellent functional properties[21]. In industry the production of FOSs are carried out by two types of processing methods. The first is the batch system using soluble enzyme and the second is the continuous one using immobilized enzyme or whole cells[20, 22] (Fig. 3).

Potential benefits of FOS

The FOS or inulin type fructans play important role in (i) Enhancement of the bio-availability and absorption of minerals and vitamins, (ii) Production and absorption of fermentation endproducts like SCFA and lactate, (iii) Protection of the body against translocation of bacteria, (iv) Protection of the body against the *in situ* proliferation of pathogen, (v) Endocrine function (via gastrointestinal peptides), (vi) Regulation of intestinal epithelial cell growth & proliferation and (vii) Boosting of immune system[23-25].

Galacto-oligosaccharides

GOS (Fig. 4) are NDO derived from lactose. These disaccharides are present in low concentrations in human milk, cow milk and yoghurt. Human milk GOS are reported to have a protective action against infection of the gastrointestinal tract during first year of life and it is thought that they may bear structural homology to cell surface glycoconjugates used as receptors by pathogens[26-28].

The chemical structure of GOS is composed of a number of β-(1-6) and β-(1-4) galactopyranosyl units linked to a terminal glucopyranosyl residue through an -(1-4) glycosidic bond. In general GOS have a DP of 2-10. Because of their β configuration, GOS are believed to escape digestion in the upper gastrointestinal tract[29]. These compounds are rapidly fermented in the proximal colon, particularly by bifidobacteria[30] and also by some strains of *Lactobacillus*, Bacteroides and *Clostridium*. GOS are highly soluble, relative sweetness is about 35 % that of sucrose can decrease the water activity and also have remarkable stability at high temperature and variable pH levels[11].

Production of GOS

GOS are produced commercially by utilizing highly concentrated whey-derived lactose[31]. This concentrated lactose solution is converted by the enzyme reaction using β-galactosidase enzymes (the glycosyltransferases and glycohydrolases). The type

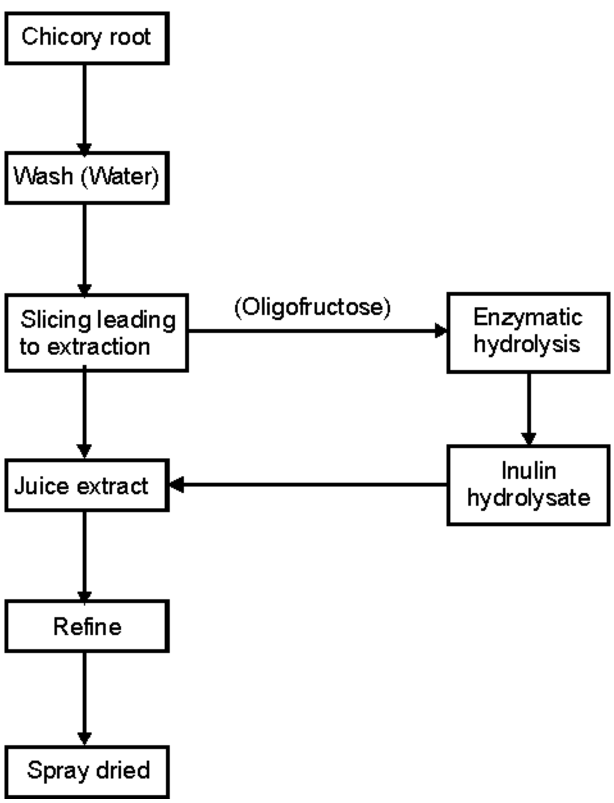

Fig. 2. − Inulin and oligofructose manufacturing process

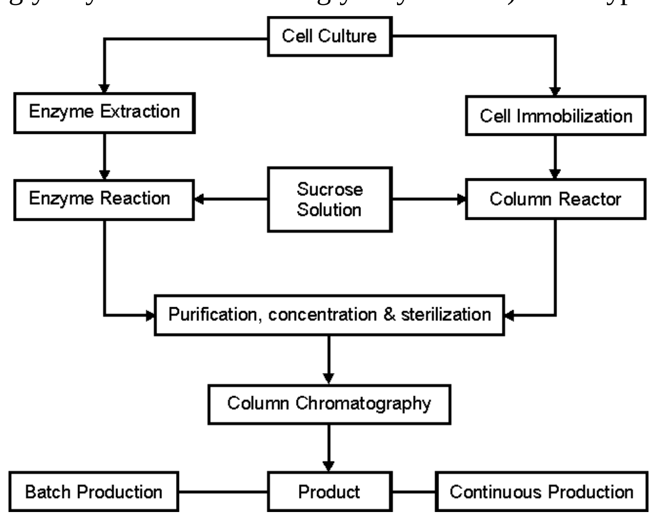

Fig. 3. − Industrial process for the production of fructo-oligosaccharide

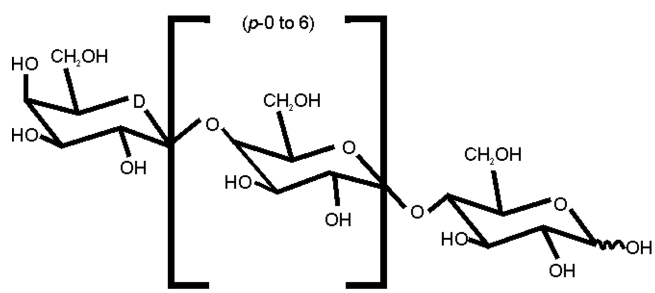

Fig. 4. − Structure of galacto-oligosaccharide

of GOS produced depends on the type of β-galactosidases used and the processing conditions[32].

Glycosyltransferases move a sugar unit from the donor to the receptor molecules, forming a glycosidic bond[33]. During this manufacturing process, generally the main product produced is trisaccharides, namely 4´- or 6´- galactosyllactose and longer oligosaccharide consisting of four or more monosaccharide units are also produced[34]. This mixture following subsequent purification is collected into syrup or powder form as per industrial demand[35]. Commercially available GOS are generally mixture of lactose, glucose, galactose and usually more than 55 % of oligosaccharides[11]. Generally 80 % of the oligosaccharides formed are trisaccharides[36].

Generally GOS are commercially produced by batch system or continuous system. In batch system, the method is simple but least efficient. In this process most of the enzyme added to the initial reaction is lost. But in case of continuous system, the production cost is lower as ultrafiltration is used to retain soluble enzymes via enzyme immobilization[33]. A highly superior quality of product can be manufactured by maintaining strict production condition, although all final products are mixtures of various GOS products[36].

Potential benefits of GOS[11]

(i) Stimulation of carbohydrate metabolism in colonic bacteria, (ii) production of enhanced bacterial biomass, (iii) SCFA and gas production, (iv) source of low-calorific sweetener, (v) bifidogenic effect, (vi) stool bulking agent thus helping in alleviation of constipation and (vii) improved calcium bioavailability.

Other prebiotics

Lactulose

Lactulose [galactosyl β-(1-4) fructose] is disaccharide of D-galactose linked β (1-4) fructose (Fig. 5). It is formed through alkali isomerisation of the glucose moiety of lactose to fructose, thereby making it a combination of fructose and galactose[37]. This disaccharide with β-1, 4 linkage is resistant to hydrolysis by human digestive enzymes. Animal and human studies indicate that lactulose is fermented in colon by bifidobacteria and lactobacilli and thus selectively stimulate their population.

Soybean oligosaccharides

Soybean whey is a by-product during production of soy protein. It is composed of oligosaccharides raffinose, stachyose with glucose, sucrose and fructose[38]. Raffinose is comprised of one molecule each of D-glucose, D-galactose and D-fructose. Stachyose is comprised of two molecules of D-galactose, one molecule of D-glucose and one molecule of D-fructose. As there is no α- galactosidase activity in the human small intestine to digest α-(1-6) linkages present in raffinose and stachyose, soyabean oligosaccharides may be able to reach the colon intact[39-41]. Soy oligosaccharides act to stimulate the growth of *Bifidobacterium* species in large intestine.

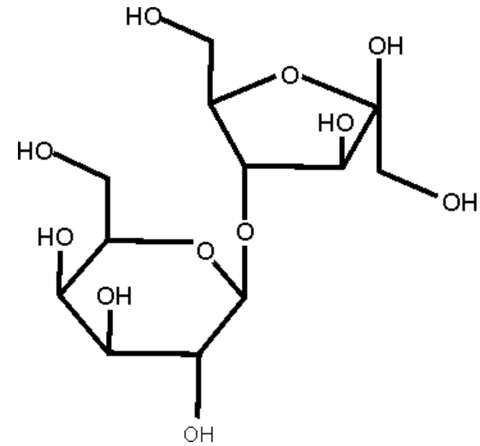

Fig. 5. − Structure of lactulose

Pharmacokinetics

Pharmacokinetics deals with the drug concentration in the product and changes of concentration of a drug or its metabolites in human or animal body following administration. It is very much important to study the bioavailability of any drug molecule used for clinical purpose and its dose or time dependent pharmacokinetics.

Prebiotics supports the proliferation of beneficial microbial population (probiotics) and also influence their metabolic activity. Usually after ingestion, the prebiotic compounds reach colon as there is very little scope of digestion in the upper part of the GI tract. In colon these compounds are utilized by probiotic organisms (*Bifidobacterium*, *Lactobacillus*) for their growth and as a result of this metabolic activity some value added compounds such as SCFAs, gases (hydrogen, hydrogen sulfide, carbon dioxide, methane), lactate, pyruvate, succinate and formate are also produced[42]. The SCFAs and lactate are responsible for determining the pH of the colonic lumen[43]. Acetate, propionate and butyrate are absorbed in colon and transported to liver through portal circulation. These SCFAs are maximally metabolized in hepatocytes and unmetabolised part transported to various tissues via circulation. Acetate and propionate are gluconeogenic and influence cholesterol production[44]. Butyrate is a major source of energy for colonic epithelial cells[45].

Safety issues of prebiotics

Foods containing prebiotics have been consumed since prehistoric times. Archaeological evidence from dry cave deposits in the northern Chihuahuan desert show intensive utilization of desert plants that were high in inulin[46]. Analysis of well-preserved coprolites suggest that dietary intake of inulin was about 135 g/day for the typical adult male hunter-forager[47]. Inulin type fructans FOS and GOS are naturally present in many daily used food products. By virtue of their potential as prebiotic, which is used in therapeutic category, the safety aspects of its application is a necessity. Thus the results obtained from traditional methods of toxicology for evaluation of these products are insufficient for complete safety assessment which is needed in therapeutic class of compounds. So it is obligatory to perform specific approaches which are more suitable for safety evaluation of food or food ingredients that have history of safe use (GRAS).

The consumption of fructan-containing (mostly inulin) plants seems to be quite old. Onion has been found to be the most commonly used vegetable in ancient times (at least 5000 yrs). Consumption of chicory dates back at least 2000 years[48]. Leach hypothesizes that the ancient human diet may contain up to 50 g/day of inulin and approximately 200 g/day of dietary fiber[49].

Throughout the World inulin type fructans are classified as food ingredients. EU Directive EC 95/2 explicitly lists inulin as a food substance. The EU Standing Committee meeting of June 1995 confirmed that oligofructose is a food ingredient[50]. In United States, a panel of experts convened by Orafti declared inulin and oligofructose as GRAS in 1992[(Ref. 51)].

In a subacute toxicity study, GOS syrup is administered for 90 days at the rate of 2.5 and 5 g/kg body weight (bw) compared to control group receiving only deionized water. There is no toxicological effect found for GOS syrup up to 5 g/kg bw/day when administered for 90 days[50]. Acute toxicity of FOS is evaluated in male and female rats and mice. They are administered FOS by gavages at the dose of 0, 3, 6 and 9 g/kg bw. It is found that dose up to 9 g/kg bw does not show any kind of adverse effect like mortality or general state of health and bw of the experimental animals. The LD_{50} for FOS/oligofructose is estimated to be greater than 9 g/kg bw[52].

The chronic toxicity and carcinogenicity studies have been performed with FOS/oligofructose in 344 male and female Fisher rats. It was found that the incidence of neoplasms is not influenced by FOS/oligofructose administration and they do not show any carcinogenic potential[53]. It is also found that allergic reaction to inulin is extremely rare and affects patients often with a history of allergy contracted by occupational contact[50].

Conclusion

Though human intestinal tracts are shelved with an array of friendly bacteria, the maintenance and proliferation of those bacteria are turned out to be a major concern in terms of the longevity of that friendship. It becomes a serious task to provide them with right kind of supplements for their ease of growth. Prebiotics has widened the scope in finding those supplements with easier and cheaper options. It is true that only a number of them have been discovered but intensive researches are ongoing for its advancement. The limitless opportunities of harvesting those prebiotic raw materials, their hustle free and low-cost processing have also signaled the modern industries for a promising profit. As a result various therapeutic product containing probiotic organisms along with FOS are available in the pharmaceutical market. Thus, if taken care of, prebiotics from its debutant status in food and pharmaceutical industries will leap forward into an obligatory part of other formulations, as many as possible, in near future.

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