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The Importance of Prebiotics in Functional Foods and Clinical Practice

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ABSTRACT

Prebiotics are substances that can promote the growth of beneficial microorganisms, mainly in the intestinal tract, and will modify the colonic microbiota. The following health benefits are attributed to prebiotics: relief from poor digestion of lactose, increased resistance to bacterial infection, better immune response and possible protection against cancer, reduction of the risk of diseases such as intestinal disease, cardiovascular disease, non-insulin dependent diabetes, obesity and osteoporosis. This article presents a discussion of prebiotics, with descriptions of the concepts and its use in clinical practice, and a review of some recent research showing the benefits that these ingredients provide to human health and providing data on the recommended intakes for consumption.

Keywords*: *Prebiotics*, *Oligossaccharides*, *Nutrition*, *Human

1. Introduction

The concept of functional foods was introduced in Japan during the 1980s, and it could be defined as "any food or ingredient that may provide a health benefit beyond the traditional functions hitherto known" [1].

Products claiming to be healthier and to have functional and/or health properties have gained prominence in research, in the development of new products and in supermarkets.

Prebiotics can be defined as non-digestible food ingredients that beneficially affect the body by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon [2-7].

To be considered as having prebiotic action, the compound must reach the colon without degradation or alteration and must be a food substrate that stimulates the existing saprophytic bacterial flora. Food ingredients with prebiotic characteristics generally exhibit certain unique characteristics, such as limited hydrolysis and absorption in the upper gastrointestinal tract, selective stimulation of the multiplication of beneficial bacteria in the colon, potential to suppress pathogens and limit virulence by processes such as immunostimulation and the stimulation of the beneficial microflora, which promote resistance to colonization by pathogens [8].

The consumption of prebiotics has been associated with reduced risks of certain diseases. These include the suppression of diarrhea associated with intestinal infections; reduced risk of osteoporosis because inulin promotes the uptake of calcium and thereby increases bone mass; reduced risk of obesity and of developing Type 2 diabetes; neutralization of toxic products and decreased frequency of colon cancer; stimulation of immunity and protection of the urogenital system [9,10].

2. Prebiotics: Origin and Chemical Nature

The term prebiotic is applied to substances that promote the growth of beneficial microorganisms in the intestine [11].

Prebiotics are substances that will modify the colonic microflora, stimulating the proliferation and growth of non-pathogenic bacteria with health promoting potential, particularly *Lactobacilli* and *Bifidobacteria* [2,6,12]. Some examples of prebiotics include fructooligosaccharides, galactooligosaccharides, arabinose, galactose, inulin, raffinose, mannose, lactulose, stachyose, mannanoligosaccharides, xylooligosaccharides, palatinose, lactosucrose, glycooligosaccharides, isomaltooligosaccharides, soybean oligosaccharides, etc [4,5,13].

The criteria for classifying a food as a prebiotic are:

1) It should not undergo hydrolysis or absorption in the upper gastrointestinal tract;

2) When it reaches the colon, it should be selectively metabolized by a limited number of beneficial bacteria;

3) It should be able to alter the colonic microflora to a healthier bacterial flora;

4) It should be capable of inducing a physiological effect that is beneficial to health [14].

Prebiotics may exhibit the following properties:

- Maintenance of intestinal flora and stimulation of intestinal transit [15];
- Change in colonic microflora, contributing to normal stool consistency, preventing diarrhea and constipation [5,16,17];
- Elimination of excess substances such as glucose and cholesterol, favoring only the absorption of substances needed [2];
- Stimulation of the growth of bifidobacteria [18];
- Stimulation of the absorption and production of B vitamins (B1, B2, B3, B6, B9, B12) [19];
- Support of the immune system [12];
- Contribution to the control of obesity [14];
- Contribution to the decrease of the risk of osteoporosis [2];

Prebiotics can be found in some vegetables, such as leeks, onions, chicory, tomatoes, asparagus, artichokes, bananas, and alfalfa. It can also be added to industrial products such as foods for children, dairy and confectionery products, beverages, light mayonnaise and low-fat cheese, and they can be used as dietary supplements [4,15].

Prebiotics are being used in the food industry as functional ingredients in beverages (fruit juices, coffee, cocoa, tea, soft drinks and alcoholic beverages), milk products (fermented milk, milk powder and ice cream), probiotic yogurts and symbiotic products [20,21]. Other applications include desserts (e.g., jellies, puddings, fruit-flavored ice cream), confectionery items (e.g., sweets), biscuits, breakfast cereals, chocolates, breads and pastas, meat products (e.g., fish paste) and tofu. Prebiotics can also be used in cosmetics, pharmaceuticals and products for people with diabetes [21].

2.1. Fructooligosaccharides (FOS)

Fructooligosaccharides (FOS) belong to the group of oligosaccharides and are isolated from plants. They consist of three to ten monosaccharide units joined by α-glycosidic bonds (1-2) between terminal fructose and glucose [22].

The degree of polymerization (DP), defined by the number of monosaccharide units, is used to define and classify FOS and inulin molecules, with FOS having a DP<10 and inulin a DP between 2 - 60 [23]. The difference between inulin, oligofructose and synthetic fructooligosaccharides is the degree of polymerization, i.e., the number of individual monosaccharide units that make up the molecule [24]. In the extraction commercialization process, FOS can be obtained from inulin by means of the transfructosylation enzymatic reaction in sucrose residues by the action of the β-fructofuranosidase enzyme, with the DP of these products varying between 1 and 7 fructosyl units [25].

Flamm *et al*. [26] have evaluated the caloric value of FOS and found that the energy yield for the host would be in the range of 1.5 kcal/g to 2.0 kcal/g. By using another method based on lipogenesis balance, Roberfroid [27] stated that the caloric value of FOS is around 1.0 kcal/g to 1.5 kcal/g.

FOS are available in some foods such as bananas, garlic, onion, tomato, wheat, asparagus, artichoke, leek, honey, rye, brown sugar, barley, triticale, beer, lettuce, chicory, burdock, beetroot, apples, bulbs like red lilies, yacon and oats, with onion being the food with the highest levels of FOS (**Table 1**).

In Holland, it is estimated that the consumption of FOS is 2 g to 12 g per day. In Japan, the estimate is between 13.7 mg/kg of body weight per day. However, for the approval of FOS, the Japanese law established the amount of 0.8 g/kg of body weight per day as an acceptable daily intake [29,30].

The average *per capita* daily consumption of FOS is 2 - 4 g for North Americans and 2 - 12 g for Europeans [31]. In Brazil, there are no relevant data regarding the amount consumed or the dietary recommendations.

The law considers FOS as ingredients of products, not additives. FOS are considered as dietary fiber, and in the United States, they have a GRAS status (Generally Recognized As Safe). Ingestion may cause flatulence, especially in individuals who have lactose intolerance, but the severity of this symptom is associated with the amount of FOS consumed: the higher the quantity, the greater the symptom [32].

The intake of 20 g to 30 g per day can promote severe discomfort in an individual, and thus, the optimal intake level is 10 g per day [30].

For the promotion of colon floral balance, the amount of FOS needed has been determined to be 2 g to 2.5 g per day [28]. The minimum dose of FOS for the induction of diarrhea is 44 g for men and 49 g for women [23,33].

For enteral nutrition, several clinical studies suggest the amount of 5 - 10 g/day for the maintenance of normal

Table 1. Amount of FOS (%) in some natural foods.

Food	Percentage, %
Onion	2.8
Tomato	1.8
Rye	0.7
Banana	0.3
Garlic	0.2

Source: [28]

flora and from 12.5 g/day to 20.0 g/day for bifidobacteria recovery [34].

In vitro and *in vivo* studies have suggested the lack of genotoxicity and mutagenicity of FOS.

Evaluations conducted in rats showed no adverse effects with quantities lower than 2.17 g/kg/day [23,35].

2.2. Inulin

Inulin is a linear polymer with b-glycosidic bonds (2 → 1) derived from D-fructose, it belongs to the fructan group and is synthesized by a variety of plants [27,36].

Inulin is a reserve carbohydrate found in many plants. Many human foods contain inulin (**Table 2**), and among them, the onion stands out as a food that is highly consumed. The concentration of inulin in each plant depends on the variety, the time between harvesting and its use and storage conditions [37,38].

Functionally and technologically, the inulin extracted from plants passes through a drying process, presenting itself as an amorphous, hygroscopic white powder with neutral odor and taste. Inulin is used to enrich food products with fiber, maintaining the appearance and taste of standard formulations.

The average per capita consumption of inulin in the European diet varies from 2 to 12 g/day; it is around 5-8 g/day in Belgium and 7-12 g/day in Spain [41].

Studies in individuals of different ages have provided results that guarantee the safety of inulin and oligofructose [27]. Inulin has been used to evaluate the glomerular filtration rate by intravenous injection since 1931. This practice has become a standard procedure without toxic effects [42]. Additionally, based on the history of the use of foods containing inulin by humans, there is no evidence of toxic effects [43].

The dose of intolerance is quite high, which allows for a broad therapeutic dose range. Subjective gastrointestinal symptoms are difficult to measure [24,44].

2.3. Polydextrose (PDX)

Polydextrose is a polysaccharide synthesized by the random polymerization of glucose in the presence of minor amounts of sorbitol and an acid catalyst (approximately 90 : 10 : 1, respectively) under high temperature and partial vacuum [45].

The random linkages of the polydextrose polymer prevent digestive enzymes from hydrolyzing the molecule [46]. Thus, polydextrose is hard to digest in the small intestine after oral administration, with approximately 60% being excreted in the feces and 30% being fermented in the large intes tine by intes tinal microflora producing volatile fatty acids and CO_2 [47].

According to Hara *et al.* [48], polydextrose is a dietary fiber that is not easily fermentable at only 1.0 kcal/g; it is very stable, has low viscosity and is widely distributed.

Table 2. Quantity of inulin (%) present in some foods.

Plants	Edible part	Inulin (%)
Onion	Bulb	2-6
Jerusalem Artichoke	Tubercle	16-20
Chicory	Root	15-20
Leek	Bulb	3-10
Garlic	Bulb	9-16
Artichoke	Central leaves	3-10
Banana	Fruit	0.3-0.7
Rye	Cereal	0.5-1.0
Barley	Cereal	0.5-1.5
Dandelion	Leaves	12-15
Yacon	Root	3-19
Goat's beard	Leaves	4-11
Wheat	Cereal	1-4

Source: [39,40]

Polydextrose is partially fermented in the large intestine, increasing the amount of fecal mass, reducing transit time, softening and decreasing the pH of the fecal material. This fermentation leads to the growth of favorable microflora, reduction of putrefactive microflora, increased production of short chain fatty acids and elimination of carcinogenic metabolite production. In a human study, polydextrose increased intestinal function and ease of defecation. Furthermore, it inhibited the excessive absorption of glucose in the small intestine, and fermentation in the large intestine produced short chain fatty acids favoring the reduction of intestinal pH. The daily intake of 4 - 12 g of polydextrose improved physiological function without producing adverse effects [45].

In an animal study, the ingestion of polydextrose (5 g/100 g diet) increased the concentration of calcium in the bones of normal female rats, which may be relevant to decreasing the risk of osteoporosis [48].

A study with Chinese subjects in which the ingestion of polydextrose was administered in the amounts of 4, 8 and 12 g daily, concluded that this ingestion provides effects similar to those of dietary fiber, without causing laxative problems [45].

With the Ordinance N. 29, dated January 13, 1998, the Brazilian National Health Surveillance Agency (ANVISA) determined that products must contain the label "This product may have a laxative effect", if they are products that are expected to be consumed at levels where the resulting daily intake may exceed 90 g of polydextrose [49].

2.4. Galactooligosaccharides (GOS)

Galactooligosaccharides (GOS) are also included among the non-digestible oligosaccharides (NDOs). GOS are

composed of galactose molecules linked to lactose, consisting of tri- to hexasaccharides with 2-5 galactose units joined by β bonds [50]. Its production occurs biochemically, when β-galactosidase acts as a hydrolytic enzyme and also as a condensing enzyme in a reaction called transgalactosylation [51].

GOS are defined as compounds that are not metabolized by the host and that reach the intestine to be metabolized by bifidobacteria. Dietary supplementation of these substances results in an increase in the occurrence and amount of bifidobacteria and inhibits the growth of pathogenic bacteria or putrefactive organisms that cause excessive production of gas [41].

GOS may be formed from lactose, and this is influenced by factors such as the source and concentration of the enzyme, pH, temperature and substrate concentration [52,53]. The higher the amount of lactose, the greater the production of GOS [54,55].

To improve bacterial flora, the recommendation for GOS ingestion is 2 g/day to 3 g/day. For people with diabetes and high content of blood fat (cholesterol and triglycerides), the recommended amounts range from 8 g/day to 20 g/day [24].

Galactooligosaccharides do not present toxicity, and the only known adverse effect is diarrhea when GOS are consumed in excess, with the excess dose estimated at 0.3 g/kg to 0.4 g/kg of body weight [50].

2.5. Xylooligosaccharides (XOS)

The Xylooligosaccharides (XOS) are oligomers of unconventional sugars, formed by xylose units, which are non-caloric and not digestible by humans. They are found in fruits, vegetables, milk and honey.

The production of XOS occurs through the industrial production of lignocellulosic materials (LCMs), obtained from a variety of forest residues (eucalyptus wood) or agro-industries (corn cob, almond, olive, rice hulls, oats, barley) [56-58]. LCMs are composed of three basic polymers: lignin, cellulose and hemicellulose [59].

XOS improves food quality, providing a change in flavor and physico-chemical characteristics and stimulating the activity of *Bifidobacterium* in the intestinal tract [60].

The use of XOS as an ingredient in food products is due to their stability across a wide range of pH (2.5 to 8.0) and temperature, the selective metabolism by bifidobacteria, the increased production of volatile fatty acids, the reduction of stomach ulcer lesions [61] and the acceptable odor [62].

The use of these compounds presents an advantage over inulin in terms of resistance to acids in heating and the resistance to degradation in low pH juices and other carbohydrate beverages [41].

Xylobiose is also considered a Xylooligosaccharides (XOS) with a degree of polymerization of 2 [63]. It presents 30% of the sweetness of sucrose [64].

The recommended daily dose for XOS is 0.7 g [65].

2.6. Lactulose

Lactulose is a synthetic disaccharide composed of fructose and galactose, which is present in milk and dairy products that have undergone heat treatment [66].

During degradation, lactulose produces acidification of the intestinal environment and a decrease in pH, responsible for triggering mechanisms that explain its action in portosystemic encephalopathy and constipation [67].

More recently, lactose has been used as a substrate for the production of bifidogenic factors in the form of lactulose, lactitol or lactosucrose. In general, bifidogenic factors are short-chain oligosaccharides (3 to 10 monosaccharide units) with the unique functional properties of not being digested in the stomach and small intestine. They serve as substrates and stimulate the growth of bifidobacteria and lactobacilli in the large intes tine, and further increas e Ca^{2+} and Mg^{2+} bioavailability, besides delaying or inhibiting certain stages of carcinogenesis [68,69].

Lactulose is predominantly used as a pharmaceutical product that controls constipation [21].

The recommended starting dose is 10 - 30 ml orally or by nasogastric tube (NG) 3-4 times per day until the beginning of the evacuations, and it can be administered as an enema (300 - 500 ml of lactulose in 1 liter of filtered water), after which the dose can be adjusted in order to maintain 2 - 3 pasty stools/day [70].

The results of studies of acute, sub-chronic and chronic toxicity in many species indicate that lactulose has very low toxicity. The observed effects appear to be more related to the volume effect in the gastrointestinal tract than a more specific toxic activity [71].

3. Beneficial Health Effects Related to the Ingestion of Prebiotics

3.1. Fructooligosaccharides (FOS)

Several studies have demonstrated the functional properties of FOS, such as the reduction of cholesterol levels and blood glucose levels, lowering of blood pressure and better absorption of calcium and magnesium [72,73].

FOS are not digested by the human gastrointestinal tract, and when they reach the colon, they beneficially stimulate the growth and strengthening of specific bacteria in the intestine [20]. The bifidobacteria secrete β-fructosidase, which would be the enzyme responsible for FOS hydrolysis [27]. Gibson and Roberfroid [20] found the characteristics of bifidogenic FOS in humans using a

dose of 15 g per day as dietary supplementation. The average counts of bifidobacteria increased, whereas there were significant reductions in Bacteroides, Fusobacterium and *Clostridium* sp. According to the authors, these compounds are utilized better by the bifidobacteria, whereas they cause unfavorable changes for harmful bacteria in the colon.

The mechanism by which the inhibition of pathogens occurs (exogenous or endogenous) can be explained by the lowering of the pH in the intestinal lumen as a consequence of the formation of short chain fatty acids (SCFA) by FOS fermentation [19,27]. The decrease in the number of harmful bacteria (such as *Escherichia coli, Clostridium, Streptococcus faecallis* and *Proteus*) results in the decrease in toxic metabolites, such as ammonia, indoles, phenols and nitrosamines [74].

Modler [41] verified that adding NeosugarR (a trade name of fructooligosaccharides) to the human diet (15 g/day) caused a tenfold increase in the population of bifidobacteria in the large intestine, as well as increasing the occurrence of bifidobacteria from 87% to 100%. Concomitantly, there was a reduction of 0.3 intestinal pH units and a decrease in the enterobacteria count. Hidaka *et al.* [75] found that the administration of 8 g/day of Neosugar in the human diet increased the production of fatty acids. Wang and Gibson [19] found the following benefits could be attributed to bifidobacteria: they are immunomodulatory against malignant cells, produce B vitamins and folic acid, stimulate the production of digestive enzymes and lysozyme and restore normal intestinal biota after antibiotic therapy.

Regarding the bifidogenic dose of FOS, authors like Roberfroid *et al.* [76] established that about 4 g per day would be enough for an adult. Bouhnik [77] demonstrated that FOS ingestion at doses of 12.5 g/day for three days (clinically tolerated dose) produced a decrease in the total count of anaerobes in the feces, in pH, in the activity of nitroreductase, in bile acid concentrations and in serum levels of total cholesterol and lipids.

3.2. Inulin

Experimental studies have shown that the application of inulin and oligofructose acts as bifidogenic factors. As a consequence of its use, there is a stimulation of the immune system of the host, a reduction in the levels of pathogenic bacteria in the intestine, constipation relief and a decreased risk of osteoporosis resulting from the stimulation of mineral absorption, particularly calcium. Thus, there would be a reduced risk of atherosclerosis by decreasing the synthesis of triglycerides and fatty acids in the liver and decreased levels of these compounds in the blood [2].

Studies with rats and hamsters and some with humans have shown that oligofructose and/or inulin increase calcium bioavailability. Increased calcium bioavailability could be due to the transfer of this mineral from the small intestine to the large intestine and the osmotic effect of inulin and oligofructose, which would transfer water into the large intestine, allowing calcium to become more soluble [78]. The improved bioavailability of calcium in the colon could also be derived from the hydrolysis of the calcium phytate complex by the action of bacterial phytases, liberating calcium. The better absorption was associated with a decrease in pH in the contents of the ileum, cecum and colon. This reduction results in increased concentration of ionized minerals, a condition that facilitates passive diffusion, the hypertrophy of the cecum walls and the increased concentration of volatile fatty acids, bile salts, calcium, phosphorus, phosphate and to a lesser degree, magnesium, in the cecum [2].

The hypolipidemic effect of inulin and oligofructose has been observed in some studies with rats, although it is controversial. Experimental data led us to hypothesize that FOS could reduce hepatic lipogenic capacity, through the inhibition of gene expression of lipogenic enzymes, resulting in reduced secretion of very low density lipoproteins (VLDL)-triacylglycerol. This inhibition could be achieved via production of short chain fatty acids or via insulin modulation by mechanisms not yet identified, but which are being investigated [2,78,79]. Future studies on the inulin hypolipidemic effect in humans should take into account the characteristics of the selected individuals, the duration of the study and the background of the subjects in terms of diet because these are important variables that can exert considerable influence on the enzymes [2].

Inulin influences intestinal function by increasing the frequency of bowel movements [75,80], increasing stool weight in bowel movements (about 2 grams per gram of inulin or oligofructose ingested) [81] and reducing fecal pH due to fermentation [20,80].

Research in experimental animal models has shown that inulin has anticarcinogenic properties [82]. In another study, the addition of inulin and oligofructose in the diet of rats reduced the colon carcinogenesis induced by azoxymethane [83].

The effect of a inulin enriched cookie was evaluate in obese patients on cardiovascular risk factors. No changes in anthropometrics parameters and the increase in soluble fiber intake did not produce any gastrointestinal adverse effect. The increase of fiber intake (3 g of inulin) from an enriched cookie reduced LDL cholesterol levels in obese patients [84].

3.3. Polydextrose (PDX)

Polydextrose is partially fermented in the large intestine,

increasing the amount of fecal mass, reducing transit time, softening and decreasing the pH of fecal material. This fermentation leads to the growth of favorable microflora, reduction of putrefactive microflora, increased production of short chain fatty acids and elimination of carcinogenic metabolite production [45]. The large intestine has the capacity to absorb calcium, and the microbial fermentation after ingestion of fermentable material is considered the mechanism responsible for increased calcium absorption in the intestine [48].

In a study with humans, polydextrose favored intestinal function and improved ease of defecation. Furthermore, it inhibited the increased absorption of glucose in the small intestine, and the fermentation in the large intestine produced short chain fatty acids favoring the reduction of gut pH. Therefore, the daily intake of 4 - 12 g of polydextrose improves the physiological function without producing adverse effects [45].

In an animal study, the ingestion of polydextrose (5 g/100 g diet) increased the concentration of calcium in the bones of normal female rats, which may be relevant to the decrease in the risk of osteoporosis [48].

Santos *et al.* [85] showed in a study to verify if polydextrose could stimulate calcium absorption in partially gastrectomized and sham operated rats that the polydextrose feeding (50 g/kg of diet) increased calcium absorption and bone calcium concentration in normal rats and the partially gastrectomy did not affect the bone calcium concentration. In a recent study, Santos *et al.* [86] investigated whether polydextrose stimulates iron absorption in rats submitted to partial gastrectomy and sham operated and the diet with polydextrose reduced the excretion of iron and apparent iron absorption was higher in the polydextrose fed groups than in the control group.

3.4. Galactooligosaccharides (GOS)

The ingestion of GOS promotes the proliferation of bifidobacteria and the reduction of deteriorating bacteria, thus causing beneficial effects to human health, such as liver detoxification by the reduction of toxic metabolites; prevention of pathogenic diarrhea due to short-chain fatty acid production by bifidobacteria; aid in cases of constipation; increased lactose tolerance; increased bone mineralization and fracture resistance caused by the stimulation of calcium absorption, which could possibly reduce the risk of osteoporosis [87]. According to Chonan and Watanuki [88], calcium absorption in rats was stimulated by administering feed containing GOS.

In a study performed with 90 children to determine the effect of a mixture of two prebiotics, fructooligosaccharides (FOS) and galactooligosaccharides (GOS), stool samples were collected for colony forming units (CFU) count and pH influence. Various doses and times were tested, with the objective of verifying the best conditions. The dose of 0.8 g of GOS per kg of body weight was the most suitable when compared with the placebo formula (maltodextrin-based). The mixture of GOS and FOS had a stimulating effect on the growth of Bifidobacterium and Lactobacillus in the intestine and was very promising for the use of supplements to infant formulas [89].

Perez-Conesa *et al.* [90] studied seven groups of rats that were fed for one month, where one group received an infant formula containing *Bifidobacterium bifidum* and *Bifidobacterium longum*, three groups received infant formula containing 4' galactosyl-lactose (GOS) at 1.2%, 5.0% and 10.0%, and three groups received infant formulas containing both ingredients during three periods of observation. Results showed that the proportion of bifidobacteria was greater than the anaerobic bacteria in the 1st period. In the 2nd period, the bifidobacteria decreased significantly, and in the 3rd period, the bifidobacteria count increased, especially in the group fed with a diet containing 1.2% of GOS.

The addition of the inulin/GOS mixture were demonstrated in several studies with infants and children as the increasing of the faecal percentage of Bifidobacteria population in the fecal flora with addition of the inulin/GOS mixture [91], significantly decreased the episodes of gastrointestinal and respiratory tract infections [92], the increasing faecal immunoglobulin levels (Ig) [93], a positive effects indicated by a lower incidence of febrile episodes in infants [94] and a beneficial effect on the immune system of preterm infants after administration of the combination of neutral oligosaccharides with acidic oligosaccharides (maximal dose of 1.5 g/kg/day added to breast milk or preterm formula) [95].

In a study conducted to determine the effect of supplementation with GOS and polydextrose (PDX) in calcium and iron absorption in gastrectomized rats, it was concluded that supplementation with both of the prebiotics increased the serum iron by 15% in non-gastrectomized animals (2.5% GOS + 2.5% PDX) compared with the control non-gastrectomized animals (diet without prebiotic), and 5% PDX increased the apparent absorption in gastrectomized rats. Daily administration of 5% GOS and 5% PDX increased the apparent absorption of calcium in control rats. There is strong evidence that the two prebiotics act synergistically, with increased prebiotic effects [96].

A prebiotic galacto-oligosaccharide mixture (B-GOS) was assessed the effectiveness on the severity and/or incidence of travellers' diarrhoea (TD) in 159 healthy subjects, who travelled for a minimum of 2 weeks to a country of low or high risk for TD. The placebo was maltodextrin. The authors found significant differences between the B-GOS and the placebo group in the inci-

dence, duration, on abdominal pain and the overall quality of life assessment. According to Drakoularakou *et al.* [97], the tested galacto-oligosaccharide mixture showed significant potential in preventing the incidence and symptoms of TD.

3.5. Xylooligosaccharides (XOS)

The health effects of Xylooligosaccharides have mainly focused on the effects on the intestinal flora [98,99].

Results were obtained *in vivo* using rats, with considerable growth of *Bifidobacterium ssp.* in the gastrointestinal tract [100] and the increase of total short chain fatty acids in the rats' cecum [101,102]. Tests in humans showed that the ingestion of XOS benefits the intestinal flora, where the ingested xylobiose (X2) was not excreted in the feces and urine within 24 hours following an oral administration. These compounds are not hydrolyzed by saliva, pancreatic and gastric juice, which suggest the use of XOS by the intestinal bacteria [103].

The digestibility of XOS in the gastrointestinal tract and its effect on the absorption of bile acids are compared to the effects of FOS and isomaltooligosaccharides (IOS). Considering digestibility, HPLC analyses showed the hydrolysis of FOS, IOS and XOS products after 4 hours of digestion *in vitro*; most of the IOS and part of the FOS was digested by the intestinal juice, while XOS was not digested by any digestive enzyme. The delay in the effects of XOS on bile acid absorption compared with IOS and FOS were confirmed in *in vitro* experiments [104].

In vitro assays have shown that *Bifidobacterium spp.* and *B. adolescentis* are active consumers of XOS (x2 and x3); the oral absorption of XOS stimulated the proliferation of *Bifidobacterium bifidum* in the intestine, but not *Staphylococcus, E. coli* and species of *Clostridium spp*. that did not use XOS [100,103].

Most species of *Lactobacillus* used XOS, including *L. fermentum*, which demonstrated this ability, and although *Bacteroides* used XOS, it did so only on a small scale compared with glucose [103].

When prebiotics were compared, the *Bifidobacterium spp.* preferred XOS, raffinose and FOS over hexoses, with XOS being more effective than raffinose and as effective as FOS in *in vitro* growth experiments [105].

Rycroft *et al.* [106] evaluated the fermentative properties of some prebiotics; they found that XOS and lactulose produced the highest increases in the number of bifidobacteria and that while FOS led to the development of lactobacilli, a mixture could increase the functionality.

A study to assess the effects of XOS on the intestinal microbiota, gastrointestinal function and nutritional parameters in elderly patients suggested a XOS daily dose of 4 g in a three-week period. The study concluded that XOS supplementation promoted intestinal health and showed no adverse effects on the nutritional status of the elderly [107].

In a recent study, Makelainen *et al.* [108] realized the fermentation of two new hard-wood derived xylooligosaccharides, xylan and a commercial XOS preparation by human microbiota in a human colon simulator (EnteromixR). The xylooligosaccharides were fermented selectively by *Bifidobacterium lactis* strains and FOS were used as a prebiotic reference. XOS was more efficient than FOS in increasing the numbers of *B. lactis* in the colonic model. The combination of XOS and *B. lactis* might be possible to formulate strain-specific synbiotic product with selective properties on desired probiotics.

3.6. Lactulose

Studies using lactulose have shown that it contributes to the increased population of beneficial bacteria in the intestine at the expense of putrefactive bacteria or other bacteria [77].

In a study of the incorporation of 0.5% of lactulose in formulations for infants, it was found that this quantity promoted changes in the microbiota, predominantly of bifidobacteria, while the control formulas caused the microbiota to consist of coliforms [66].

In patients with long-standing liver cirrhosis, the administration of milk fermented with bifidobacteria and lactulose resulted in the re-stabilization of beneficial microbiota of the intestine, along with the reduction of ammonia and free phenols in the blood [109].

Lactulose has been widely used in the treatment of hepatic encephalopathy, decreasing the concentration of ammonia in the blood and preventing the development of this pathology [110,111].

4. Industrialized Foods with Prebiotic Addition

The health effect of food is a major determining factor in whether to purchase a food item. The food industry has invested in some great innovations, mainly in the formulation of ingredients and additives, functional foods, transgenic foods and packaging [112]. The increased demand for functional foods in recent years is closely related to the growing concern of society with health and quality of life. Moreover, consumers are more informed and aware about the foods that can benefit health.

Several industrial products containing added prebiotics can be found in the consumer market: dairy products, breads, fruit juices, margarine, pasta, dairy desserts, ice creams, cereals, milk, yogurt, biscuits, soft drinks in general, isotonic drinks, liquid sugar and modified sugar, chocolates and candies in general.

5. Conclusion

The various studies in clinical nutrition conducted over the past 20 years have established the indirect role of prebiotic ingredients in promoting healthy and balanced intestinal microbiota.

In addition, the administration of prebiotics reduces blood lipids and blood pressure, increases the synthesis and absorption of nutrients and has anti-carcinogenic action. In addition to its functional properties, prebiotics show interesting properties that have implications for the food processing industry and the content of its end-products.

The proper administration of prebiotics consists of following the recommended daily intake, which should be specific to the pathology indicated and at levels that do not cause side effects.

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