

## Health Benefits and Application of Prebiotics in Foods

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### Abstract

Prebiotics are supplements or foods that contain nondigestible food ingredients that selectively stimulate the favourable growth and/or enhance the activities of indigenous probiotic bacteria. Prebiotic therapies have been found to cure gut related diseases such as constipation relief, suppression of diarrhoea, reduction of the risks of osteoporosis, atherosclerotic cardiovascular disease associated with dyslipidemia, insulin resistance, obesity, and possibly type 2 diabetes. Fast life style of humans has changed the regular diets which were sufficiently enriched with fibers needed for proper gut work. Prebiotics are special fibers having selective fermenting ingredients that are needed by microbiota of human gut. Human colon is one of the body's most metabolically active organs because of the presence of microbiota. Lot of work has been done to overcome the problems related to the market foods having low fibre. Thus, this review will focus on the health benefits of prebiotics and their application in foods.

**Keywords:** Prebiotic; Probiotic; Microbiota; Nondigestible ingredients; Therapies

### Introduction

The prebiotic word was first introduced by Gibson and Roberfroid in 1995 and is defined as “a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [1]. In common terms, bacterial species that are believed to be beneficial for health and well-being of humans use prebiotics as food. The fermentation features selecting a substance as prebiotic should include nutraceutical effects that extend beyond those of daily nutrition. The greater number of these effects have dealt with optimized colonic function and metabolism, such as increase in the expression or change in the composition of short-chain fatty acids, increased faecal weight, a mild decrease in luminal colon pH, a decrease in nitrogenous end-products and reductive enzymes, an increased expression of the binding proteins or active carriers associated with mineral absorption, and immune system regulation [2,3]. On the basis of fermentability alone many substances and foods are being considered prebiotic [4,5]. Various whole foods are also being considered as prebiotics because of having different types of fibers, some of which are fermentable [6,7]. This information is confusing and lacks accurateness. Because foods consist of many different items with broad metabolic and nutritional effects, they should not be classified as narrowly as the prebiotic definition demands [8]. Besides, all macronutrients and their partially digested remnants reaching in the colon are able to be used as microbial substrate and produce a variety of fermentation products of a highly mixed nature, which may be either potentially beneficial or detrimental to health [9]. Consequently, the word prebiotic should be specific always: defined substances that exhibit a particular scientifically observed effect as stated by the prebiotic definition. Fermentable fibers in particular to other fibers are crucial for good health. But prebiotics are specified ingredients which are aimed to affect the specific bacteria, end-products of their fermentation, and possible health benefits on the host. So, prebiotics are typically oligosaccharides or more complex saccharides that are selectively used by commensal bacteria, including species considered to be beneficial for the human host. Prebiotics must resist host digestion, absorption, and adsorption before fermentation by one species of the resident microbiota [10]. The most common prebiotics are inulin, its derivatives fructooligosaccharides (FOSs) and galactooligosaccharides (GOSs), however, other complex saccharides and fibers have been recently considered as prebiotics. Poly-

oligosaccharides are commonly available in the usually taken diets [11]. They are mainly found in vegetables, as a reserve of energy to be used during germination. In the milk of other mammals some oligosaccharides with prebiotic activity are also found. The most commonly used prebiotics to develop the foodstuffs, including baby foods, are fructans (inulin and fructo-oligosaccharides, FOS) and galacto-oligosaccharides (GOS) [12,13]. They are believed as GRAS and their health-benefiting effects have been widely studied. For example, Inulin is a mixture of polymers composed of fructose units forming linear chains having variable degrees of polymerization, bound to a single glucose moiety. Chicory is the source of inulin which is isolated and purified from it after energy density [14]. On the other hand, various new prebiotics are having either natural origin (soy oligosaccharides, resistant starch, etc.) or synthetic origin (xylo-oligosaccharides, pyrodextrin, isomalte-oligosaccharides, lactosucrose, polydextrose, lactulose) are entering in the world market. In Japan many of these new products have been developed and some of them have been currently commercialized. However, these new compounds have been studied mainly *in vitro* and animal models. It is important to note that more clinical trials are needed to confirm their safety as well as their healthy effects in humans. It is becoming more and more acceptable that the specific health effects are due to specific bacterial strains, thanks to double blind placebo controlled clinical studies for providing an increasing number of citations [15]. It is now well-known that specific prebiotics encourage specific colon microbial populations both *in vitro* and *in vivo* [1]. For example, it has been found that amylo maize starch selectively stimulates the growth of (bifidobacteria) at the cost of (bacteroides and coliforms) [16]. Likewise, lactulose has been reported to support the growth of bifidobacteria and decrease the number of bacteroides in a continuous culture model [17] as well as in the human colon [18]. Additionally it has been found that the

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faecal levels of *B. lactis* increases by oral administration of galactooligosaccharides [19]. On the contrary, b-glucans support the growth of *lactobacilli* more than the bifidobacteria [20]. So prebiotics support a specific microorganism for specific function.

## Functions

Prebiotics reach the colon without being digested because of their chemical nature. A part of the material is not digested by pancreatic and small-bowel enzymes in the human gut and therefore, reaches the large bowel. The whole length of human gut is occupied by microorganisms with population numbers and species distribution characteristics of specific region of guts [21]. In the gut the relatively more stable colonies are in large intestines than smaller intestines because the transit time in small intestines is faster (4-6 h) than large intestines (48-70 h) in adults [22]. The pH and relatively low absorptive state of colon further supports large microbial colonisation and growth [23]. Due to this microflora, the colon has the ability to undertake complex hydrolytic digestive functions [24]. This involves breakdown of dietary components, complex carbohydrates and some proteins that are not hydrolysed or absorbed in the upper digestion tract. The colonic microflora derives their food from the undigested food e.g., nondigestible oligosaccharides, dietary fibre, and undigested protein. They also get their substrates from the mucin: the main glycoprotein constituent of the mucus. Therefore, any undigested food that reaches the colon e.g., nondigested carbohydrates, some peptides and proteins, certain lipids is a source of prebiotics [25]. Prebiotics have been suggested of having various health benefits on humans. It acts as a substrate for the useful gut microorganisms. The various benefits are:

### Gastroenteritis

This is a common disease usually occurs due to ingestion of food or water contaminated with pathogenic microorganisms and/or their toxins. The main pathogenic organisms responsible for gastroenteritis are *Shigellae*, *Salmonellae*, *Yersina enterocolitica*, *Campylobacter jejuni*, *Escherichia coli*, *Vibrio cholera* and *Clostridium perfringens*. These pathogens grow and colonise within the gastrointestinal tract and invade the host tissue or they secrete toxins in the foods before ingestion. These toxins disrupt the functions of the intestinal mucosa, causing nausea, vomiting and diarrhoea [26]. Therefore, prebiotics help to increase the population of useful organisms in large intestines that may possibly help in preventing gastroenteritis.

### Inflammatory bowel disease

Inflammatory bowel disease has been related to the intestinal microbiota pathogenesis. One of the understandable ways for therapeutic intervention is probiotic treatment. Administration of prebiotics is an additional treatment [27,28]. Germinated barley foods are high in glutamine rich protein and hemicelluloses rich dietary fibre showing the prebiotic characteristics in the dextran sulfate sodium model of rat colitis as it has reduced the incidence of bloody diarrhoea and mucosal injury [29]. Germinated barley foods also displayed a greater ability to decrease the symptoms of dextran sulfate sodium colitis than that of a probiotic mixture of *lactobacilli* and *C. butyricum* [30]. A mixture of long chain inulin and oligosaccharide has shown the ability to decrease the inflammatory histological and gross cecal scores in the cecum and colon. It also decreased the levels of the pro-inflammatory cytokine IL-1 $\beta$  and increased the anti-inflammatory TGF- $\beta$ , at the same time it increases cecal lactobacillus and bifidobacterium levels [31]. Two other prebiotics lactulose and goat's milk oligosaccharides have shown an ability to reduce dextran sulfate sodium colitis in rats [32,33]. Despite having definitive proof

on the therapeutic value of prebiotic in the treatment of inflammatory bowel disease from animal models. However, all prebiotics are not beneficial. As in the case of probiotics, not every prebiotics have anti-inflammatory effects in curing of inflammatory bowel disease, instead of that the severity of damage increases by some prebiotics. For example, fructooligosaccharides (FOS) has demonstrated antagonistic effects in the intestine. Administering of FOS as a dietary supplement (6% w/w of total diet), stimulate *lactobacilli* and bifidobacteria [34,35], and increase SCFAs in the large intestines [36] (a result that has been repeated in humans with ulcerative colitis [37,38]. Due to the rapid fermentation of elevated FOS levels in the cecum by cecal bacteria causing elevated levels of organic acids, which damage the mucosa of the cecum and colon [39]. Interestingly, however, oral gavage of FOS in the trinitrobenzene sulfonic acid (TNBS) rat model of colitis showed the decrease of severity of damage, which means by increasing lactic acid bacteria, lactate and butyrate causes to decrease inflammation scores and myeloperoxidase activity [40]. On the contrary, the effects of FOS in dextran sulfate sodium colitis showed that FOS treated rats had no shield from inflammation [41]. These contradictory conclusions may be due to differences in the model of colitis or may be a result of change in the delivery of FOS, leading to change the rates of fermentation, and consecutively SCFA production.

### Reduction of cancer risk

Upon the administration of prebiotics it has been found that the activity of genotoxic enzyme decreases. A study carried on feeding galactooligosaccharides to humans showed decrease in nitroreductase (a mutagenic/carcinogenic substances) and also decreased levels of indole and isovaleric acid (produced due to proteolysis and deamination and markers of putrefaction) [42]. It has been shown that genotoxic enzymes b-glucosidase, b-glucuronidase, and arylsulphatase were strongly inhibited when galactooligosaccharides was applied on model system of the human gut [43]. As these effects happened quickly upon the addition of GOS to the system, changes attributable to population levels can be ruled out and it is more possible that direct inhibition by GOS or the production of repressors by bacteria was responsible. However, the proportion of bifidobacteria and *lactobacilli* increasing at the expense of bacteroides and clostridia may too decrease genotoxic enzyme production, as the former produces lower levels of such enzymes than the latter [44]. Further study was carried to see the effects of resistant starch administration to human flora-associated rats. Even though b-glucosidase increased while as b-glucuronidase and ammonia levels got decreased [45]. Another observation a high level of caecal butyrate important to the reduction of cancer is not only the major source of energy for colonocytes but also helps to maintain a healthy epithelium. It can also play an important role in preventing cancer [46]. These interactions include apoptosis induction, a process which is inactivated in cancer cells which would generally lead to their elimination and an increase in the immunogenicity of cancer cells due to an increase in cell surface proteins expression. However, the usual target bacteria for prebiotic use are bifidobacteria and *lactobacilli* but not butyrate producers. Therefore, other gut flora components (e.g. Eubacteria) should be fortified.

### Prevention and treatment of allergy

The series of bacterial intestinal colonization is possibly important for the development of the immune response of neonates and young infants [47]. In pregnancy, the cell mediated immunity (T-helper 1 [Th1] type) is sidetracked away towards the humoral immunity (Th2 type) due to the cytokine inflammatory response profile of the fetus. Therefore, the general type of immune response in early

babyhood is Th2. The lack or delay in the eventual shift of the main Th2 type of response to more of a balance between Th1 and Th2 type responses could well be the result of the risk of allergic disease [48]. In a review it was concluded that there was no firm conclusive evidence for giving prebiotics to prevent allergic disorders in infants [49]. However, a report showed that 132 infants were at risk of atopy because of parental atopy, based on the 2 year follow-up of randomized controlled trials. FOS and GOS or maltodextrin placebo were fed to infants with partially hydrolyzed formula in first 6 months of life. There was a reduced incidence of atopic disease in those which was given the prebiotic mixture of FOS and GOS. Increasing rates of atopic eczema, recurrent wheezing, and allergic urticaria were higher in the maltodextrin placebo group (27.9%, 20.6%, and 10.3%, respectively) than in the intervention group (13.6%, 7.6%, and 1.5%) ( $P=0.05$ ) [50]. After analyzed the relevant publications it was concluded that there is no sufficient proof currently available to support the use of probiotics, prebiotics, or synbiotics for the prevention or treatment of allergic dermatitis in children. Studies supporting the benefits of prebiotics, particularly for children fed formula either partially hydrolyzed or not partially hydrolyzed formula, which are previously being promoted to decrease the prevalence of atopic disease, are needed to continue till other recommendations can be made for the use of prebiotics in infants and toddlers to stop infection or atopic disease [51].

### Effects of prebiotics on bone mineralization

Bone mass of an adult is dependent on supply as well as bioavailability of calcium. In either cases of deficiency osteoporosis is prone to occur and in addition to this it is highly associated with increasing age and postmenopausal conditions. Presently, osteoporosis treatment and prevention is restricted to increasing calcium uptake, or by stimulating bone formation. Even though, a lot of studies have been carried out on calcium metabolism using rats, results depicted that prebiotics play a role in escalating the bioavailability of calcium. But so far, only a few human trials have been translated from these results. Prebiotic consumption increasing calcium absorption has the majority supporting data from the rat studies where as human trials have had diverse results [52].

### Cardiovascular effects of prebiotics

Obesity and diet have direct link with atherosclerosis, cardiovascular disease and type 2 diabetes [53,54], and there are number of animal experiments signifying the ability of prebiotics to influence the serum lipid levels [55,56]. Double-blind randomized controlled trials were carried on 12 humans using inulin, FOS and galacto-oligosaccharides (GOS) of the dosage of 15g per day. It was shown that higher concentrations of faecal acetate in inulin and GOS treatment periods ( $P<0.05$ ) lowered  $\beta$ -glucuronidase activity in inulin and GOS periods [57]. Alles et al. [58] investigates the effect of FOS (20 days) in diabetics on cholesterol and blood glucose using FOS 15 g per day. It was seen that there has been no changes in blood glucose. No effects on total, HDL, LDL cholesterol or serum triacylglycerol. A study on inulin supplement of 10g per day was carried on 54 healthy middle aged volunteers using fasting blood samples to analyze lipids and glucose. It was seen that insulin concentrations got significantly lower in inulin group ( $P<0.01$ ). Trends for lower triacylglycerol levels in inulin group ( $P<0.08$ ) returned to baseline 4 weeks after end of study [59]. Similarly inulin supplement of 18g per day for 6 weeks of human trails having hyperlipidaemic showed reduction in LDL as compare to those having normal diets [60]. A significant reduction in serum triglycerides was seen in hyperlipidaemic patients after 21 days administration of ice cream supplemented with inulin [61]. FOS in

combination of inulin when used for 4 weeks on normal lipidaemic persons showed significant reduction in triacylglycerol [62].

### Food Applications of Prebiotics

Both important technological characteristics and interesting nutritional properties have been shown by Prebiotics [63,64]. Some of them are found in fruits and vegetables and some can be processed industrially from renewable materials. In foods they affect considerably by improving organoleptic characteristics, enhancing both taste and mouth feel. As functional food ingredients prebiotics must be chemically stable to processing treatments of food, such as high temperature, low pH, and Maillard reaction conditions. Therefore, if the prebiotic was degraded to its component mono- and disaccharides or chemically altered, a prebiotic would no longer provide selective stimulation of beneficial microorganisms and it was unavailable for bacterial metabolism [65]. A study ascertained the effect on the prebiotic activity by processing conditions of commercial prebiotics using a prebiotic activity assay. The results showed that a significant decrease in prebiotic activity was only seen when heating at low pH, with one of the fructooligosaccharides (FOS) products being the least stable. Little change in activity was caused by other conditions [66-68]. These results help us in selecting of prebiotics as functional food ingredients and predicting the extent to which processing affects prebiotic activity. Presently known prebiotics and prebiotic candidates are nondigestible oligosaccharides. They are acquired either by extraction from plants (e.g., chicory inulin), enzymatic hydrolysis (e.g., oligofructose from inulin) or by synthesis (by trans-glycosylation reactions) from mono- or disaccharides such as sucrose (fructooligosaccharides) or lactose (trans-galactosylated oligosaccharides or galactooligosaccharides) [69]. Among these prebiotics, inulin and oligosaccharides have been most studied prebiotics and recognized as dietary fibers in most countries [70]. Prebiotics can be used to offer a double benefit such as an improved organoleptic quality and a better-balanced nutritional composition [71]. As fiber ingredients the use of inulin and nondigestible oligosaccharides is simple and often leads to enhance taste and texture. Specific colonic bacteria, such as bifidobacteria and *lactobacilli* species readily ferment these particular forms of dietary fibre and by raising their cell population with the parallel production of short-chain fatty acids [72]. These acids, mainly butyrate, acetate, and propionate provide acidification of the bowel as well as metabolic energy for the host [73]. Lactose occurs wholly in the milk of mammals and one might observe from a teleological point of view that lactose ingestion provides specific health benefits beyond just being a source of energy to the suckling animal [74]. In fact, it is used in different foods such as in baby foods, cakes, biscuits, chocolates, sugar confectionary, and soups and sauces and the global demand has grown considerably over the previous 10 years to amount to approximately 500,000 t per annum [75]. Currently, more and more prebiotics are used in different foods as an ingredient which helps in the growth of health promoting colon microorganisms particularly probiotics and offer additional health benefits. Some of the recent works done in the development of prebiotic foods are as follows:

### Prebiotics in infant formula

There are number of prebiotic substances present in human milk, the most abundant of which are oligosaccharides [76,77]. Many commercially available dietary food supplements are also added with oligosaccharide prebiotics. Concerning to their addition to infant food, the European Commission's Scientific Committee on Food concluded in 2003 that the addition of oligosaccharides to infant formulas had no major worry and including the studied infant formulas (formulas adapted



especially for 6- to 12-month-old infants), up to a total concentration of 0.8 g/dL in ready-to-feed formula products [78]. Few randomized controlled trials have been examined to see the effects of fortifying prebiotic oligosaccharides to infant foods [79,80]. A study showed the effect of the addition of oligosaccharides having a concentration of 1 g/dL to newly born infant formula for 1 month having 90% of GOSs and 10% of FOSs. In the oligosaccharide supplemented group the faecal bifidobacteria counts increased significantly when compared with the nonsupplemented group and the counts of bifidobacteria reached the range of a breastfed reference group. In another study, it was shown that the infants fed with the same oligosaccharide fortified formula. These infants had higher bifidobacteria counts as well as *lactobacilli* in their faecal matter. A multicenter trial was conducted that examined the efficiency of the fortification of prebiotics to infant foods. They stated the good overall tolerance and no undesirable effects during the study period of 12 weeks [81]. Also a large multicenter trials have been evaluated by United States in 2004 on the safety of FOS supplemented infant formula. The study demonstrated the effect of FOS supplemented infant formula for 12 week study period and was seen that the infant growth was maintained without having any adverse effects [82]. In weaning infant food formulas the fortification of prebiotics to solid foods shows to have a bifidogenic effect, as revealed by the results of recently published randomized controlled trials [83]. Infant food formulas containing either GOS or FOS are now marketed in the United States. On the other hand more information, including data from randomized controlled trials, is required before the efficacy of adding prebiotics to infant food formulas can be determined.

### Prebiotics in cheese

Dairy products are considered a potential vehicle for prebiotics and probiotics and are increasingly being used to produce synbiotic foods. A research conducted to evaluate the potential of prebiotic in novel petit-suisse cheeses using an *in vitro* fermentation model. Prebiotics candidate (inulin, oligofructose, honey) and probiotics (*Lactobacillus acidophilus*, *Bifidobacterium lactis*) were combined in five petit-suisse cheese formulations and were tested *in vitro* with human faecal slurry. It was seen that with addition of prebiotics to a probiotic cheese (made using starter + probiotics) fastest fermentation and high lactic acid production, promoting increased growth rates of bifidobacteria and *lactobacilli*, were achieved. Addition of probiotics as starter culture to control cheese also resulted in high lactic acid production. *In vitro* conditions, cheese made from the combination of different prebiotics and probiotics shows potential functional petit-suisse cheese [84]. An investigation conducted to see the influence of prebiotic compounds (FOS and inulin), probiotic bacteria (*Lactobacillus casei*-01, *Bifidobacterium lactis* B94) and ripening time (0-60 days) on the free fatty acid (FFA) profile of cheese, with special prominence on the conjugated linoleic acid (CLA) content. 10<sup>9</sup>-10<sup>10</sup> cfu g<sup>-1</sup> cheese were recorded in both probiotic and synbiotic cheeses after 60 days of ripening, in spite of harsh conditions of low pH values (4.1-5.1) and low moisture content (<30%, w/w). It was observed that the increases in total FFA and CLA during the ripening period, particularly in synbiotic cheeses having FOS and inulin (50:50) inoculated with *B. lactis* B94. Addition of prebiotic compounds in probiotic cheese is advantageous so far as functional CLA compounds are concerned plus an enhanced nutritional quality [85].

### Prebiotics in fermented milk

The physicochemical composition (day 1 of storage) and rheological (days 1, 7, 14 and 21) were evaluated for the microfiltered cow's milk samples fermented with and without inulin, at temperatures

of 4.0 ± 0.1°C and 6.0 ± 0.1°C. The total solid content of the prebiotic fermented milk increased with the addition of inulin, also increased its acidity. The Power Law and Mizhari and Berk models described the flow of the fermented milks as shear thinning and non-Newtonian fluid behaviour. The apparent viscosity of the product added with inulin increased with storage time. The prebiotic fermented milk showed greater thixotropy and the hysteresis got lowered with an increase in the temperature [86]. Debon et al. used permeate obtained from the best microfiltration process for the preparation of fermented milks, with and without of inulin (5 g 100 g<sup>-1</sup>), stored at 5 ± 1°C for 28 days. It has been seen that the storage period and addition of inulin increased the total solids and carbohydrate contents, the caloric value and the acidity but decreased the pH. Lower syneresis index resulted with the addition of inulin in a fermented product also with greater firmness and cohesiveness. The inulin addition in a product resulted with a greater tendency toward a greenish coloration [87]. Cruz et al. evaluated the effect of addition on physicochemical, rheological and microbiological characteristics of non-flavoured yogurt. There was no influence on the pH, proteolysis or the viability of *Streptococcus thermophilus* or *Lactobacillus bulgaricus* during 28 days of refrigerated storage with the addition of oligofructose. Yogurt added with oligofructose was characterized as a weak gel, showing thixotropic and pseudoplastic behaviour as per the rheological measurements. As per the survival analysis 25% of the consumers rejected the yogurt added with different levels of oligofructose, and the level of oligofructose that can be added to the yogurt was shown to be 2.58% wt. V-1 [88].

### Prebiotics in dairy fruit beverages

Guergoletto et al. evaluated the survival of *Lactobacillus casei* (LC-1) when adhered to dehydrated prebiotic fibers. After vacuum drying in oat bran with 9% β-glucan and green banana flour, viability of LC-1 was 79% and 76%, respectively. Images revealed by scanning electron microscopy showed no morphological changes in the cells adhered to these fibers and the cell protectant trehalose addition had a positive and significant effect on the survival of LC-1. Greater viability of LC-1 has been seen when adhered to the oat bran (7.1 log CFU g<sup>-1</sup>) than in the free form (2.4 log CFU g<sup>-1</sup>). Sensory evaluation showed that the probiotic oat bran added to a dairy fruit beverage was well accepted by consumers [89].

### Prebiotics in cereals

Several studies have publicized the prebiotic activity of cereal derived arabinoxylan oligosaccharides (AXOS). A study investigated the *in situ* AXOS production during bread making process. Firstly AXOS producing ability of different xylanases was compared in whole meal bread making. Different dosages of xylanase were used originating from *Bacillus subtilis*, *Aspergillus niger* and *Hypocrea jecorina*, and thermophilic xylanase from *H. jecorina* (HjXynA). Dosages which do not impair dough manageability, arabinoxylan fraction are solubilised and cleaved to the largest extent by HjXynA, resulting in an AXOS content of 2.1% on dry basis and an average degree of polymerisation of 9. Secondly the HjXynA effect on the AXOS levels in dietary fibre enriched breads was studied. Breads fortified with rye or wheat bran treated with HjXynA yielded high-quality breads with AXOS levels above 2.0% with an average degree of polymerization of 26 and 19, respectively [90]. Due to wide occurrence of celiac disease and wheat allergy has led to an increasing demand for gluten-free foods that present an appropriate sensory acceptance. A consumer test was performed with 65 celiac people. Additionally, 15 trained assessors were used in the sensory profiling by using quantitative descriptive analysis (QDA). By using QDA, the samples differed significantly with respect

to all parameters, and partial least squares (PLS) regression was used to recognize the factors of liking of gluten-free breads. The results show that the softness, traditional bread aroma, sweetness and crumb color are the most desired sensory properties of such products which can be considered as drivers of liking of prebiotic gluten free breads [91]. In a clinical study partially hydrolysed guar gum (PHGG) and fructooligosaccharides (FOS) were incorporated in biscuit and was assessed in human volunteers. The study showed consumption of biscuits added with FOS and PHGG gave a significant increase in faecal bifidobacterial numbers ( $P=2.15 \times 10^{-3}$ ). Bifidobacteria increased from 9.18 log<sub>10</sub> cells/g faeces to 9.59 log<sub>10</sub> cells/g faeces after the experimental treatment. There was small change in the total numbers of bacteria, *Bacteroides spp.*, *Clostridium spp.* or *Lactobacillus-Enterococcus spp.* present in faecal samples collected over the course of the trial. No changes were observed in bacterial populations on eating of the placebo biscuits [92].

### Prebiotics in salad dressing

A study conducted by Mantzouridou et al. to develop a low-oil salad dressing emulsion, based on oat flour, that has both health promoting effect as well as provides growth to probiotic *Lactobacillus paracasei* subsp. *paracasei* DC412 with the functional properties also e.g. emulsion stabilization and thickening ability. Probiotic viability, following emulsion storage and enzymatic treatment, was persistent in oat flour presence at the levels near to those found for inulin. The dressing emulsion was further investigated by applying response surface methodology and the optimum formulation (g/100 g) was as: oil (20), xanthan (0.475) and oat flour (3.0); viable cell counts of emulsions reached levels as high as 108 cfu/g after the refrigerated storage period of 7.5 weeks and treatment with simulated gastric and intestinal juices. In addition to enhancing cell viability, appreciable improvement of the emulsion rheological properties and physical stability of salad dressing occur by oat flour incorporation [93].

### Prebiotic as thermoprotector

*Bifidobacterium bifidum* viability to spray-drying and with storage time can be considerably improved by adding aguamiel as a thermoprotector prebiotic offering resistance to oxygen diffusion. *B. bifidum* harvested in the late log phase were mixed with three different protective colloids blends exhibiting relatively high, medium and low activation energies with or without aguamiel were spray dried at 130, 140 and 155°C, and stored at 4°C at a water activity of 0.32. The blend exhibiting highest activation energy (40.7 kJ mol<sup>-1</sup>), with aguamiel, and dried at 140°C showed best viability when microencapsulating the microorganism. After 5 weeks storage time at 4°C the viability ranged from 1.26×10<sup>8</sup> cfu g<sup>-1</sup> immediately after drying to 6.0×10<sup>6</sup> cfu g<sup>-1</sup> [94].

### Prebiotics in edible coating

The idea for encapsulating probiotic living cells by prebiotic edible films was investigated. In combination of gelatine based matrices and plasticizer glycerol, four soluble fibres (inulin, polydextrose, glucose oligosaccharides and wheat dextrin) were selected as prebiotic co-components for the immobilisation of *L. Rhamnosus* GG. Glucose-oligosaccharides and polydextrose considerably improved the viability of *L. rhamnosus* GG during air drying (by 300% and 75% respectively), whereas a 33 and 80% decrease in viable counts was seen for inulin and wheat dextrin. On the other hand it was seen that inulin was the most effective at controlling the sub-lethal effects on *L. rhamnosus* GG during storage. Yet in all cases the prebiotics supplementation of edible films increased the storage stability of *L. Rhamnosus* GG [95].

### Prebiotics in custard

Rheological and sensory properties were studied for low-fat

custards added with (7.5 g/100 g) of long-chain inulin (TEX) and short-chain inulin (CLR) combined in different proportions (25:75, 50:50, and 75:25). Low-fat samples added with inulin blends did not cope to fully imitate the rheological behaviour of full-fat custard. However, inulin blends in the ratio of 50:50 of TEX: CLR added to the low-fat samples perceived to have the same creaminess and thickness as the full-fat sample. When this blend was used in combined with carrageenan gave thicker, creamier custard, which was preferred over the full-fat custard [96]. A seven-day period of refrigerated storage was studied for the changes in rheological properties and microstructure of inulin-enriched custard desserts. The comparison in desserts prepared with 7.5% short-chain inulin (CLR), long-chain inulin (TEX), native inulin (IQ), and a mixture of short and long-chain inulin (MIX) were carried out. During the seven-day storage period of CLR samples the rheological changes and inulin aggregates formation were not observed. Conversely TEX, MIX and IQ samples formed inulin aggregates and as a result became more thixotropic, consistent and elastic. The order of the rate of the inulin aggregates formation and the percentage of volume occupied by them was TEX > MIX > IQ. Although for both skimmed-milk and whole-milk samples the same trend was observed and the effects of inulin type and storage time on rheology and microstructure were less prominent in the whole-milk samples [96].

### Conclusion

Prebiotics have a noteworthy effect on human health and have greater potential for incorporation into a broader range of common foodstuffs as they have both technical as well as nutritional properties. They act as substrate for the growth of probiotic bacteria (bifidobacteria and lactic acid bacteria), thus enhance the gastrointestinal functions and immune system. Prebiotics also increase the absorption of calcium and magnesium, influence blood glucose levels and improve plasma lipids. To confirm the health benefits of prebiotics by incorporating in formulated foods a long term clinical trials are needed, hence a lot of work is yet to be done.

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