Near East University

Yakın Doğu Üniversitesi, Lefkoşa KKTC

FACULTY OF ENGINEERING

DEPARTMENT OF FOOD ENGINEERING

FDE 402

FOOD ENGINEERING RESEARCH AND

DESING PROCESS II

‘PREBIOTICS AND FOOD APPLICATIONS’

Submitted to : Prof.Dr.Feryal Karadeniz

Submitted by : Selen Kamun (20092331)

Nicosia -2013

ABSTRACT

In recent years, much attention has been paid to physiological functions of foods due to increasing concerns for health. People have turned to natural food sources such as plants and herbs for these enhancers, rather than artificial substances. Increases in consumer demand have resulted in emerging of various health promoting products in the market. They are called dietary supplements, designers foods, super food, as well as functional foods. These terms are actually referred to foods that have special beneficial effects on the human. Functional food is now defined as food that is, or appears similar to a conventional food. It must be a part of standard diet, which is consumed on regular basis and in normal quantities. Other than that, it should also been proven to reduce the risk of specific chronic diseases or beneficially affect target functions beyond its basic nutritional functions. This showed that consumers are more aware on what they eat and drink as they have become more proactive in improving their health. There are a lot of products containing functional ingredients in the market—infant milk formula, bakery products, chocolate, dairy products and health drinks. Prebiotics are among those functional food ingredients which raise much attention recently.

CONTENTS

ABBREVIATIONS

GI	Gastrointestinal
GIT	Gastrointestinal Tract
SCFA	Short Chain Fatty Acid
NDO	Non-digestible Oligosaccharide
GOS	Galacto-oligosaccharide
FOS	Fructo-oligosaccharide
XOS	Xylo-oligosaccharide
IMO	Isomalto-oligosaccharide
SOS	Soy bean oligosaccharide
IBS	Irritable Bowel Syndrome
IBD	Inflammatory Bowel Disease
IUB	International Union of Biochemistry
IUPAC	International Union of Pure and Applied Chemistry
FISH	Fluorescence in situ Hybridization
DP	Degree of Polymerization

TABLES INDEX

PAGE NO

FIGURE INDEX

PAGE NO

1. INTRODUCTION

Biological functions of the human large intestine include waste storage (and its excretion) and the absorption of water as well as essential minerals. However, because of a slow transit time, near-neutral pH and high substrate availability, the colon harbours a very complex and diverse bacterial microflora. The microflora in the human large intestine is thought to compromise about 95% of total cells in the body, representing 10^{12} cells/g dry weight contents, making the organ a highly specialized and active area of the body. Through the activities of the resident microflora, the colon plays a major role in host nutrition and welfare. Dietary modulation of the human gut flora can be of some benefit to health. In recent years, the functional food concept has moved towards the situation where by improved gut (microbial) functionality is the main current driving force. The colon is by far the most intensely populated region of the gastrointestinal tract and is therefore a major target for dietary intervention (Gibson, 2004).

Nowadays, consumers are demanding for foods with increasingly properties, such as pleasant flavor, low-calorie value or low fat content, and benefic health effects. Within this context, food industry has been trying to offer products with improved flavor and appearance. In addition, functional dairy products offer requirements, benefits to health that are strengthened by the addition of probiotics as well as by certain types of soluble fibers known as prebiotics. Established amounts for food fibers are at least 3–6% (w/w) in solid foods and 1.5–3% (w/w) in liquid foods (Oliveira et al., 2009).

Many prebiotics are already used in a broad range of food applications. However, it is still possible to identify desirable targets for enhancement of their efficacy as prebiotics. According to the claims of the producers, these products are effective in supporting the health of human and are also safe. On the other hand, there are doubts with regard to the general concept of prebiotics and to these claims. Thus, there is clearly a need to increase our knowledge of gut microflora and interactions with prebiotics (Wang, 2009).

2. DEFINITION OF PREBIOTIC

In 1995, Gibson and Roberfroid defined a prebiotic 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' (Gibson & Roberfroid, 2008). The stimulated bacteria should be of a beneficial nature, namely bifidobacteria and lactobacilli (Rad et al., 2012). This definition only considers microbial changes in the human colonic ecosystem. Later, it was considered timely to extrapolate this into other areas that may benefit from a selective targeting of particular microorganisms and to propose a refined definition of a prebiotic as: a selectively fermented ingredient that allows specific changes, both in the composition and/ or activity in the gastrointestinal (GI) microflora that confers benefits upon host wellbeing and health (Gibson & Roberfroid, 2008). According to this last definition, to be classified as prebiotic, a food ingredient has to resist gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, be fermented by the intestinal microbiota and selectively stimulate the growth and/or activity of intestinal bacteria associated with health and wellbeing (Licht et al., 2012).

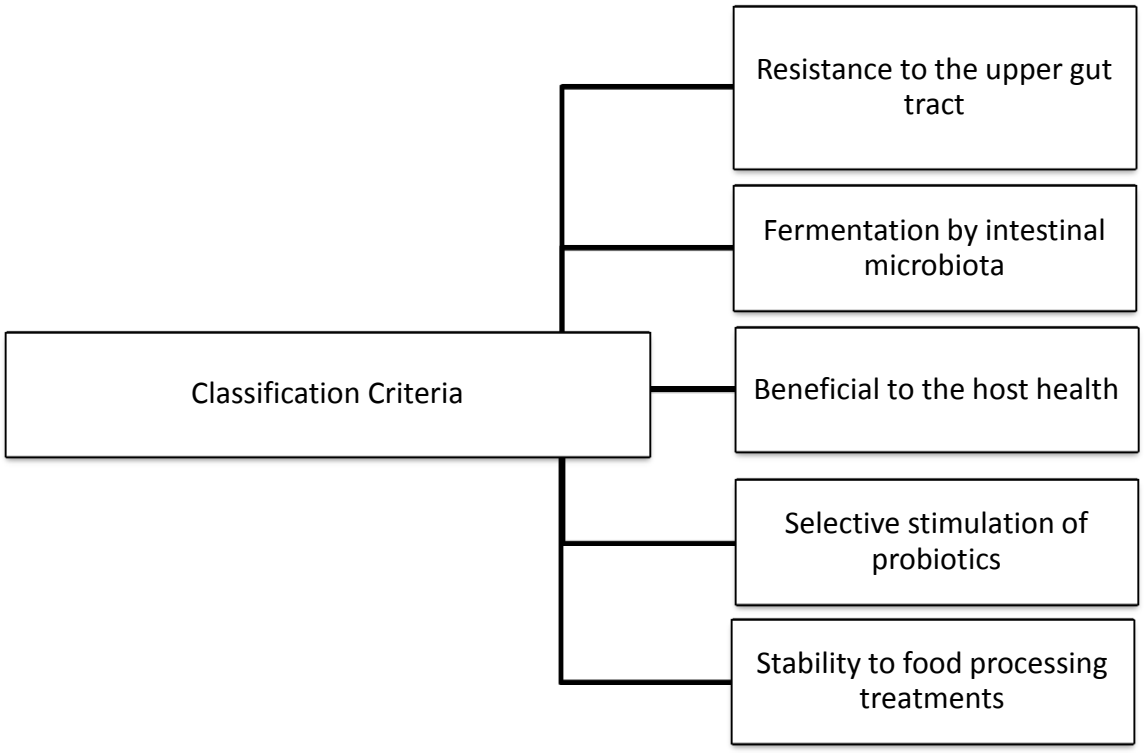

Figure 1: Criteria for classification of a food ingredient as prebiotic (Wang, 2009).

Prebiotics exploit selective enzyme production by those gut micro-organisms that may impart health benefits to the host. While some peptides, proteins and certain lipids are potential prebiotics, non-digestible carbohydrates have received the most attention. Certain carbohydrates, oligo- and polysaccharides, occur naturally and meet the criteria of prebiotics. Some non-digestible carbohydrates have a number of functional effects on the GIT which have been used to validate functional and health claims. These include delayed gastric emptying, modulation of GIT transit times, improved glucose tolerance, reduced fat and cholesterol absorption via binding of bile acids, increased volume and water carrying capacity of intestinal contents, and modulation of microbial fermentation with increased short chain fatty acid (SCFA) production, decreased pH and ammonia production (Ziemer & Gibson, 1998).

Prebiotics have been associated with a variety of health benefits including an increase in the bioavailability of minerals, particularly calcium, modulation of the immune system, prevention of the incidence or improvement in the severity and duration of gastrointestinal infections, such as traveller's diarrhoea, acute diarrhoea and antibiotic-associated diarrhoea, modification of inflammatory conditions, such as irritable bowel syndrome (IBS) ulcerative colitis and inflammatory bowel disease (IBD), regulation of metabolic disorders related to obesity and reduction of risk cancer (Charalampopoulos & Rastall, 2012).

3. THE EFFECT OF PREBIOTICS ON HEALTH

There is extensive evidence in experimental animals that prebiotics, such as inulin-type fructans, can increase the absorption of a variety of minerals, including calcium, magnesium, iron and zinc (Gibson & Roberfroid, 2008).

3.1 THE EFFECT OF PREBIOTICS ON MINERAL ABSORPTION

3.1.1 Calcium

Many animal and human studies have shown that prebiotics increase calcium absorption. The most compelling data is from humans where studies have

demonstrated that regular consumption of prebiotic inulin-type fructans lead to increased calcium absorption in some, but not all, subjects and lead to improvements in clinically relevant outcomes including bone mineral density (Gibson & Roberfroid,2008).

Calcium Absorption

Calcium absorption can be either active or passive. Active absorption is vitamin D-dependent, saturable and occurs mostly in the small intestine. Passive transport occurs along the length of the gastrointestinal tract by paracellular concentration gradient-dependent diffusion. Overall, passive diffusion has been estimated to account for between 8% and 23% of calcium absorption in humans. In humans, calcium absorption is more than 95% complete in the small intestine, with less than 5% of calcium absorption occurring in the small bowel (Gibson & Roberfroid, 2008).

A variety of mechanisms have been proposed to explain in the effect of prebiotics on calcium absorption , although the most widely favoured explanation concerns their effect on passive calcium absorption in the large intestine. This theory states that non-absorbed prebiotics enter the large intestine undigested where they are fermented in to short chain(volatile) fatty acids such as acetate, butyrate and propionate. These fatty acids lower the pH of the large intestine contents, increase solubility of calcium (and other minerals) in the luminal contents and so increase passive concentration-dependent calcium absorption in the colon (Gibson & Roberfroid, 2008).

Effect of Specific Fatty Acids on Calcium Absorption

One study has examined the effect of different short chain fatty acids on colonic calcium absorption in humans. In a novel design, subjects received rectal infusions of calcium and polyethylene glycol(PEG)- containing solutions. Multiple samples of the rectal fluid were taken for 30 min, and calcium absorption estimated from the disappearance of calcium from the rectal fluid using the calcium: PEG ratio. The effects of acetate and propionate on calcium absorption were assessed by adding various amounts of these short chain fatty acids to the infused solution. Both acetate and propionate increased calcium uptake from the rectal solution. The effect

was not pH mediated as addition of sodium chloride to the infused solution lowered the pH more than addition of acetate or propionate, but did not increased calcium uptake. At relatively low concentrations (18.7 mmol/L) acetate and propionate had similar effects on calcium absorption. However, at higher concentrations (56.3mmol/L) calcium absorption was twice as high when propionate was added to the infusate than when butyrate was added. These concentrations are similar to those seen in experimental animals fed prebiotic rich diets where cecal acetate concentrations between 14 and 58 mM. These data suggest that short chain fatty acids increase calcium absorption directly, rather than through pH-dependent mechanisms, and that propionate was more effective at increasing calcium absorption than is acetate (Gibson & Roberfroid, 2008).

3.2 EFFECTS OF PREBIOTICS IN THE GASTROINTESTINAL TRACT

3.2.1 Proven Effects Treatment of Constipation

Several RCTs (Randomised Placebo-controlled clinical trials) have demonstrated that lactulose is an effective treatment of constipation. The mechanism involved in the laxative effect is not fully understood, and seems to be multifactorial. At high doses, lactulose (and probably all non-digestible oligosaccharide (NDO)) can induce an osmotic diarrhoea, however at low doses (at which lactulose has a significant effect in patients), the osmotic effect is limited by fermentation. An increase in faecal hydration, faecal bacterial mass and a stimulation of colonic motility by end products may contribute to clinical efficacy. Lactitol and other NDOs such as galacto-oligosaccharides, fructo-oligosaccharides and lactose are also probably effective in alleviating constipation as well although this needs to be confirmed (Marteau, 2001).

Treatment of hepatic encephalopathy

Substances derived from the metabolism of the gut flora are involved in the pathogenesis of hepatic encephalopathy. The therapeutic efficacy of lactulose has been demonstrated in RTCs. The side effects calculated from 18 studies concerning 298 patients were the following: flatulence 18%, diarrhoea 14.5%, abdominal pain 13%. The possible mechanisms of action include stimulation of bacterial growth, incorporation of ammonia into bacterial proteins, colon acidification, laxative effect

and possibly a shift in production of medium chain fatty acids to short chain fatty acids (Marteau, 2001).

3.2.2 Other Potential Applications of Prebiotics

Prevention of colon carcinoma

Fermentation reduces colonic pH and may reduce the 7-dehydroxylation of primary bile salts. A role of carbohydrate fermentation in colon cancer prevention has thus been hypothesized and studies in animal models have been encouraging. Several studies have shown that lactulose administration to healthy volunteers lowered faecal concentrations of secondary bile salts. However, in one study, administration of 60 mL of lactulose/d for 12 weeks did not influence crypt cell proliferation assessed in rectal biopsis. Roncuci et al. (1993) reported that lactulose decreased the recurrence rate of colon adenomas. Two hundred and fifty five patients with colon adenomas were randomized after removal of the adenomas to receive vitamins, lactulose (20g/d) or no treatment. Colonoscopy was performed thereafter every 6 months. After a mean follow-up of 18 months, the percentages of recurrence of adenomas were 5.7% in the vitamin group, 14.7% in the lactulose group and 35.9% in the untreated patients. This study which may have important consequences needs to be confirmed (Marteau, 2001).

3.3 EFFECT OF PREBIOTICS ON INTESTINAL MICROFLORA

It is possible to modify the composition of the intestinal microflora by the oral administration of live, selected microbes, mostly lactic acid bacteria and bifidobacteria (probiotics) or by the administration of non-digestible food, selectively stimulating the growth and/or the activity of one or of a limited kind of bacteria in the colon (prebiotics). Taking breast –feeding as the natural example of infant nutrition, the prebiotic approach should be considered as the most physiologic approach to influence intestinal microflora early in life (Bruzzese et al., 2006).

Several evidences demonstrate that the administration of different prebiotics (inulin, fructo-oligosaccharides) induces a significant modification in intestinal microflora, increasing the number of Bifidobacteria and lactic acid bacteria. Recently it has been showed that the supplementation of infant formula with a mixture of

galacto-oligosaccharide (GOS)/ fructo-oligosaccharide (FOS) is effective in modifying the composition of intestinal flora in both term preterm infants. After a 28- day feeding period, the number of bifidobacteria and lactobacilli significantly increased in infants supplemented with GOS/FOS. The increase in bifidobacteria was dose dependent as the number of bifidobacteria was higher in group of children fed with 0.8 g/dl GOS/FOS as compared to the group fed with 0.4 g/dl GOS/FOS. These changes associated with a reduction of tool pH and with a modification of SCFA pattern, ultimately resulted in an intestinal microflora composition more similar to that of breast-fed individuals than that of those fed with standard infant formula. The effects on intestinal microecology were closely dependent on the GOS/FOS mixture and were not confirmed by using other oligosaccharides (Bruzzese et al., 2006).

Infant formula supplemented with two different concentrations of fructo-oligosaccharides (1.5 g/l and 3.0 g/l) had minimal and not significant effect on fecal flora of 2-6 week healthy infants. During the FOS supplementation an increase incidence of flatulence and loose stools was registrated. The incidence of adverse effect was higher in children supplemented with 3.0 g/l of FOS (Bruzzese et al., 2006).

More recently a new mixture of oligosaccharides (80% GOS/FOS and 20% acidic oligosaccharides) has been evaluated. The adjunct of acidic oligosaccharides, derived from pectin hydrolysis, had the objective to increase the similarity with human milk, which contains 75-85% neutral and 15-25% acidic oligosaccharides. However, the adjunct of acidic oligosaccharides did not modify the effects on intestinal microflora induced by GOS/FOS alone (Bruzzese et al., 2006).

These data show that it is possible to manipulate the intestinal microflora by adding prebiotic components to infant formula. They also indicate that the resulting microflora is similar to that found in infants fed with human milk as compared to those using formula milk. The key question is whether this approach is effective in obtaining relevant effects beneficial for health. These effects should be measurable in randomised controlled trials (Bruzzese et al., 2006).

4. PREBIOTICS

4.1 OLIGOSACCHARIDES

Properties

The carbohydrates can be classified according to their molecular size or degree of polymerization (number of monosaccharide units combined), into monosaccharides, oligosaccharides and polysaccharides. According to IUB-IUPAC nomenclature, oligosaccharides defined as saccharides containing between 3 and 10 sugar moieties. Other authorities classify saccharides including anyone from 3 to 19 monosaccharide units in this group (Mussatto & Mancilha, 2007).

Oligosaccharides are water soluble and typically 0.3- 0.6 times as sweet as sucrose. The sweetness of the oligosaccharide product is dependent on the chemical structure and molecular mass of the oligosaccharides present, and the levels of mono- and disaccharides in the mixture. This low sweetness intensity is quite useful in the various kinds of foods where the use of sucrose restricted by its high sweetness property. The relatively low sweetness makes the oligosaccharides useful in food production when a bulking agent with reduced sweetness is desirable to enhance other food flavours. Compared with mono-and disaccharides, the higher molecular weight of oligosaccharides provides increased viscosity, leading to improved body and mouth feel. The oligosaccharides can also be used to alter the freezing temperature of frozen foods, and to control the intensity of browning due to Maillard reactions in heat- processed foods. They also provide a high moisture-retaining capacity, preventing excessive drying, and also a low water activity, which is convenient in controlling microbial contamination (Mussatto & Mancilha, 2007).

Although oligosaccharides possess important physicochemical properties, most of the interest in their use as food ingredients is due to their many physiological properties beneficial for health. One of these is that unlike starch and simple sugars, the NDOs are not utilized by mouth microflora. Consequently, the production of acids or polyglucans does not occur. Therefore, the NDOs can be used as low cariogenic sugar substitutes in products like confectionery, chewing gums, yoghurts and drinks (Munsatto & Mancilha,2007).

Many NDOs are not digested by humans because the human body lacks the enzymes required to hydrolyze the β- links formed among the units of some monosaccharides. Such compounds include carbohydrates where fructose, galactose, glucose and/or xylose are the monosaccharides units presents. This property makes the NDOs suitable for use in sweet, low- calorie diet foods and for consumption by individuals with diabetes. In the case of very sweet foods, they may be used as bulking agents in conjunction with intense artificial sweeteners such as aspartame, phenylalanine or sucralose. Oligosaccharides can be used to mask the after tastes produced by some of these intense sweeteners (Mussatto & Mancilha, 2007).

4.1.1 Galacto-oligosaccharides

GOS are manufactured from lactose by β- galactosidases (Barile & Rastall, 2013). GOS molecules (for example, Gal(β 1-4) Gal(β1-4)Glc) are typically synthesized by the enzymatic activity of β-galactosidase on lactose in a reaction known as transgalactosylation. Other carbohydrate- modifying enzymes, such as β-glucosidases and β-galactosidase belongs to a class of hydrolytic enzymes and has long been used in the dairy industry to hydrolyse lactose, producing glucose and galactose. This hydrolytic activity increases the sweetness of a dairy product and also lowers lactose concentration, which can be beneficial to lactose intolerant consumers. The competing transgalactosylation reaction and formation of GOS during β-galactosidase catalysed conversion of lactose was first observed in the early 1950's. Since this time, GOS have become a commercially important product manufactured in Asia and Europe, with a manufacturing facility also opening recently in Australia. Six thousands of tonnes of GOS were manufactured in 2005 in Japan alone (Gosling et al., 2010).

GOS are in general very stable to acidic conditions and high temperatures and for this reason they can be potentially added to a variety of acid or heated foods, such as yogurts, fermented milks, buttermilk, pasteurised fruit juices and bakery products (Charalampopoulos & Rastall, 2012).

With increasing public awareness of nutrition there has been increased demand for foods with demonstrable health benefits. An example of this can be found with yoghurts, where consumers put a high preference on products perceived to be low in fat. Dairy products processed exploiting the GOS-forming activity of β-galactosidase could contain lower sugar content and higher soluble fibre and therefore have a competitive edge. This makes further increases in GOS manufacture likely (Gosling et al., 2010).

GOS were shown to have a positive impact on immune function. In a 24-week crossover study of 70 healthy older subjects (average age 64-80 years) fed Bimuno GOS or placebo, improvements in phagocytosis and natural killer cell activity were seen together with a shift to a more anti-inflammatory cytokine balance, with an increase in IL-10 (interleukin 10, human cytokine synthesis inhibitory factor which is anti- inflammatory cytokine) and decreases in IL-1β (interleukin 1β known as catabolin, is a cytokine protein), IL-6 (interleukin -6 which is an interleukin that acts as pro-inflammatory and anti- inflammatory cytokine) and tumor necrosis factor (TNF-α). Whereas these results can be considered as a positive effect of immunity, the impact of disease susceptibility or resistance was not determined. The same GOS product, however, has shown positive effects in traveller's diarrhoea. One hundred and fifty-nine travellers to high diarrhoea risk destinations were fed 5.5 g GOS or maltodextrin placebo daily over a two-week trip. The GOS resulted in a statistically significant decrease in diarrhoea incidence from 38.5 to 23.5%. Decreases were also seen in duration of diarrhoea from 4.6 to 2.4 days and in duration of abdominal pain from 3.5 to 2 days (Barile & Rastall, 2013).

GOS has also shown potential to improve the symptoms of IBS. In one study on 44 IBS patients fed 3.5 or 7 g/day GOS or 7 g/day maltodextrin placebo for twelve weeks, there were changes in fecal microbiota as determined by 16S-FISH, and there was a selective increase in bifidobacteria. Flatulence, bloating, stool consistency, anxiety and a subjective global assessment of severity were all significantly improved in the GOS fed subjects (Barile & Rastall, 2013).

4.1.2 Lactulose

Lactulose (4-0-β-D-galactopyranosyl-D-fructofuronose), a synthetic disaccharide composed of two sugar molecules fructose and galactose bonded together with β-1,4 glycosidic bond.

Lactulose is 1.5 times sweeter than lactose and can be crystallized from alcohol solution. The β-glycosidic linkage of the disaccharide lactulose is not hydrolysed by mammalian digestive enzymes and ingested lactulose passes the stomach and small intestine without degradation. It is characteristically utilized by all the species of *Bifidobacterium,* which resides in the human intestine tract. It does not help in the growth of oral bacteria responsible for tooth decay. In the colon, large number of bacteria metabolizes lactulose and consumes it as their own food. In doing so, these bacteria produce lactic, acetic and formic acid as well as carbon dioxide gas. These acids biochemically draw fluid into the bowel which softens the stool, hence the lactulose can be used as a laxative. It has prebiotic property, because it stimulates the growth of health- promoting bacteria in the gastrointestinal tract, such as bifidobacteria (*Bifidobacteria bifidum*, *Bifidobacteria longum, Bifidobacteria infantilus, Bifidobacteria adolescentis*)and lactobacilli (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus* etc.) and at the same time inhibits pathogenic bacteria such as *Salmonella* (Panesar & Kumari, 2011).

Lactulose can be produced by the isomerization of lactose by regrouping the glucose residue to the fructose molecule. The large number of complex reagents, alkalies or enzyme can be used as catalyst for the isomerization of lactose to lactulose. The catalyst must have properties of being low cost, easy to remove from the medium, eco-friendly, safe and non-toxic. Systematic methods by using a catalyst for lactulose production can be divided into two principal groups i.e. chemical and enzymatic methods.

In chemical methods, industrial lactulose production is exclusively carried out by chemical isomerization of lactose via the Lobry de Bruyn- Alberda van Ekenstein (LA) rearrangement. The formation of lactulose by means of LA rearrangement has been realized in different matrices either using additional catalysts or catalysing

processes. The technology of lactulose production is mainly based on the isomerization reaction of lactose in alkaline media. The chemical catalysts used for the isomerization of lactose to lactulose have both positive and negative aspects. The process includes expensive separation and purification steps to remove the by product. Lactulose was produced first time by heating a mixture of lactose and lime at a temperature of 35 ^{o} C. After that, a method was developed in which calcium hydroxide was used as catalyst for the isomerization reaction of lactose to lactulose in which a solution containing 60% lactose was combined with 0.1% calcium hydroxide at 100- 102 ^{o} C for 15 min and the final solution was demineralized by combination of electrolysis and ion exchange resins. It can also be obtained from lactose by using alkaline reagents such as sodium hydroxide, magnesium oxide, tertiary amines (Panesar & Kumari, 2011).

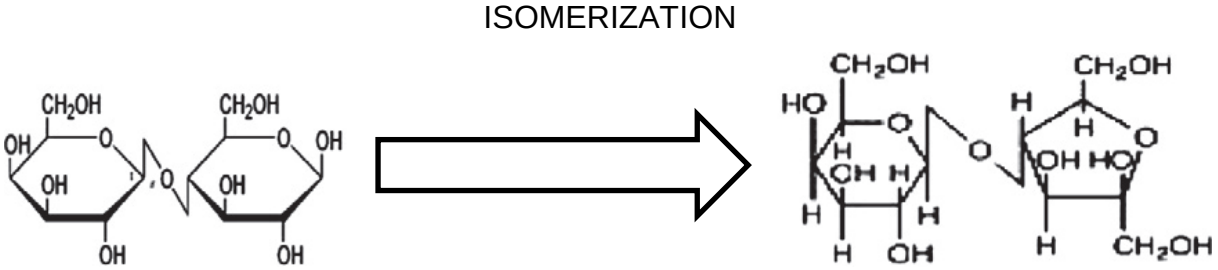

Figure 2: Schematic isomerization process of lactulose formation (Panesar & Kumari, 2011).

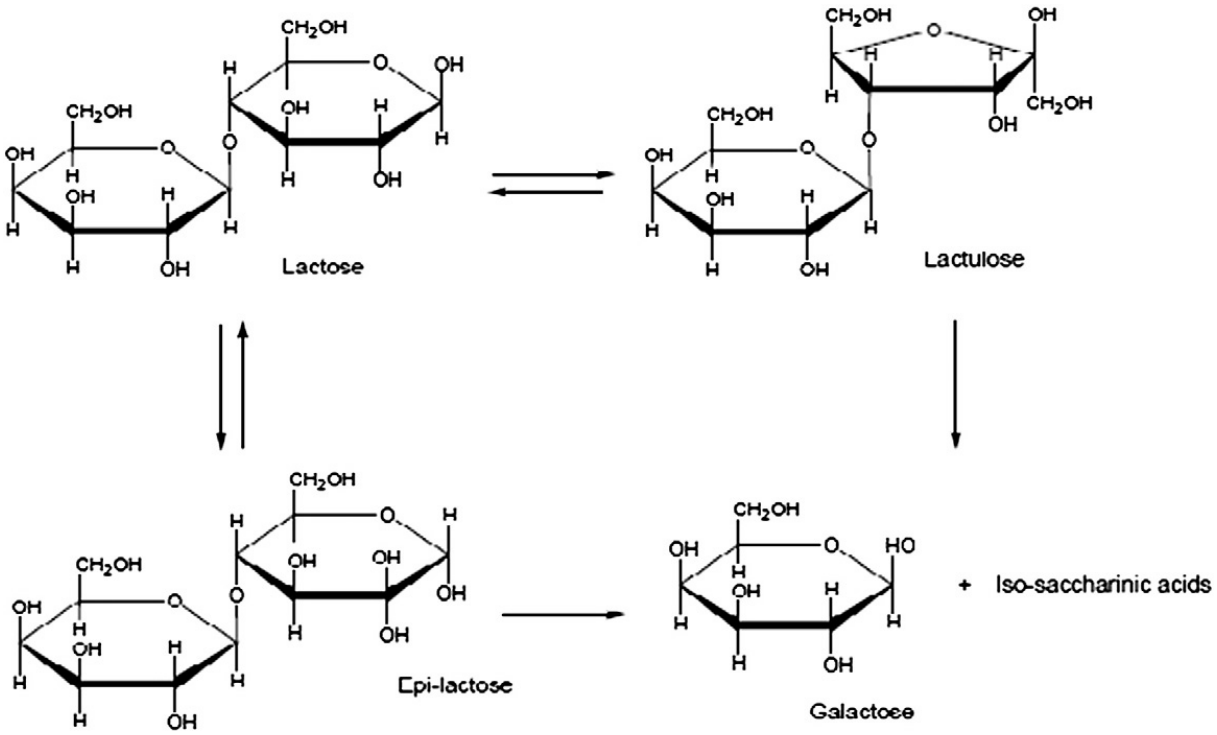

Figure 3: Alkaline isomerization of lactose (Panesar & Kumari, 2011).

In enzymatic synthesis of lactulose is commonly carried out with classes of enzyme β-galactosidase and glycosidase. β- galactosidase is a known well biocatalyst for transgalactosylation reaction and for the synthesis of lactose based derivatives including galactooligosaccharides. This enzyme can be obtained from a wide variety of sources such as microorganisms, plants and animals, however, according to their source, their properties differ markedly. Enzymes of plants and animal origin are of little commercial value but several microbial β- galactosidases are of technological interest. Further, microorganisms offer a number of advantages such as easy handling, higher multiplication rate and high production yield over other available sources of this enzyme. Free β- galactosidases as well as in whole cell and immobilized form can be used for lactulose production.

Lactulose is applied in a wide variety of foods as a bifidus factor or as a functional ingredient for intestinal regulation. In addition to providing useful modifications to food flavour and physicochemical characteristics, many of these sugars possess properties that are beneficial to the health of consumers. Additionally, lactulose can be used as a sweetener for diabetics, as a sugar substitute in confectionery products, beverages, infant milk powders, bakery

products, yoghurts, dairy desserts and in various liquid or dried food preparations which are routinely manufactured for old people.

Lactulose also has some properties with desirable effects in food product such as flavour enhancing properties, favourable browning behaviour, excellent solubility in water etc. Many tests have been performed on yoghurt, cookies, cake, chocolate, etc. to find the change in behaviour of lactulose during processing of products. Lactulose has also been reported to improve the survival of available probiotic strains in yoghurt. The survival of the probiotic strains was monitored for 5 weeks at 4 ^{o} C. It has been observed that *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* were extremely stable and survives slightly better time period in the presence of lactulose (Panesar & Kumari, 2011).

4.1.3 Isomaltulose (palatinose) oligosaccharides

Isomaltulose also known as palatinose, is a potential disaccharide sugar substitute produced from sucrose by isomaltulose synthase from *Protaminobacter rubrum, Serratia plymuthica, Erwinia rhapontici, Klesbsiella planticola* among others. Isomaltulose is a natural occurring disaccharide composed of α-1,6 linked glucose and fructose. Isomaltulose occurs in honey and sugarcane juice. Commercial isomaltulose is produced from sucrose by enzymatic rearrangement and has been used as a sugar in Japan since 1985 (Lina et al, 2002 ; Neto & Menao, 2009).

Isomaltulose is a white crystalline powder that is thermally stable (melting point 122 ^{o} C) even in acid conditions, exhibits low hygroscopicity and has a sweetness approximately 42% of that of sucrose Caramels, gums and chocolates formulated with this sugar, exhibited a long shelf life. It is particularly suitable as a non-cariogenic sucrose replacement and is favourable in products for diabetics and prediabetic dispositions.

Studies with rats and pigs indicated that isomaltulose is completely hydrolysed and absorbed in the small intestine; however, the rate of hydrolysis is very slow compared to that of sucrose. Isomaltulose is particularly suitable for inclusion in dietetic products (Neto & Menao, 2009).

4.1.4 Xylo-oligosaccharides

XOSs are produced by hydrolysing xylan. XOSs are oligosaccharides commercialized as a white powder containing two to ten xylose molecules linked by β 1-4 bonds, but molecules with degree of polymerization (DP) ≤ 20 have been considered XOS. XOSs are considered non-digestible oligosaccharides, non-cariogenic in humans and have important biological properties. They are used as dietary sweeteners in low- calorie diet foods and for consumption by individuals with diabetes.

XOS stabilities can differ greatly depending on the types of oligosaccharide and sugar residues, linkages are stronger, ring forms and anomeric configurations. Generally, β- linkages are stronger than α- linkages, and hexoses are more strongly linked than pentoses. Most oligosaccharides can be hydrolysed, resulting in the loss of nutritional and physicochemical properties at pH < 4.0, when treated at high temperatures for short time periods, or when subjected to prolonged storage under room conditions.

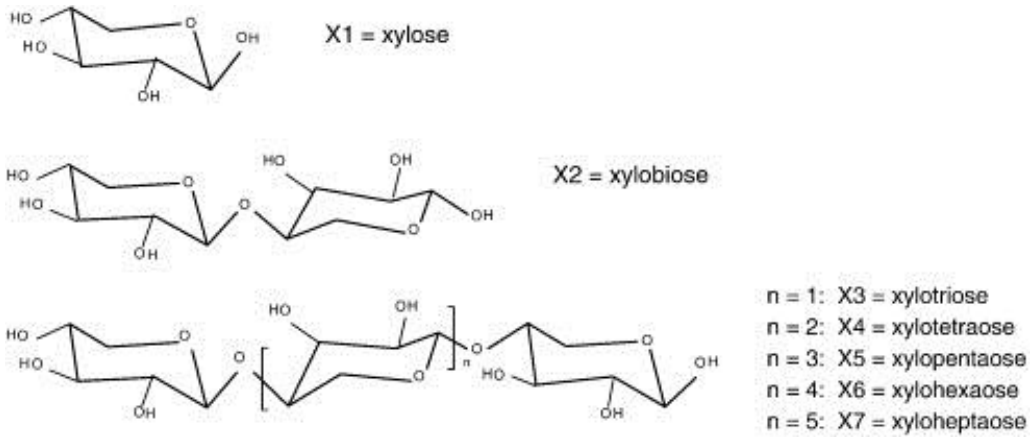

Figure 4: Schematic structure of xylose and xylo-oligosaccharides (Carvalho et al., 2012).

XOS are stable over a wide range of pHs (2.5-8.0), even the relatively low pH value of gastric juice and temperatures up to 100 ^{o}C. This is an advantage compared with other NDOs such as FOS and inulin.

In health benefits of xylo-oligosaccharides; XOS are not digested by humans because the human body lacks the enzymes required to hydrolyse the β- links, so they are considered prebiotics and soluble fiber because they are not degraded in

the stomach and reach the large intestine tract. Therefore, they are classified as NDOs and they show several health benefits. This properly allows XOSs to be used as dietary sweeteners for low-calorie diet foods and for consumption by individuals with diabetes.

The biological activity of a XOS depends on its molecular weight distribution. XOSs with fewer than four monomer units are important for prebiotics applications because they promote the proliferation of bifidobacteria, which are considered as beneficial microorganisms in the human intestine. XOSs provide health benefits as the active ingredients in functional foods. These components may be present in food or as added industrial products (Carvalho et al., 2012).

The addition of XOS to food adds physiological properties beneficial for the body, including improvement in bowel function, calcium absorption and lipid metabolism, prevention of dental caries, protection against cardiovascular disease and reduction in the risk of colon cancer due to the formation of short-chain fatty acids. In addition, XOS provides beneficial effects on: skin, blood, immunological system, anti-oxidant, antiinflammatory effects and antiallergic activities. XOS can improve the microbiota increasing the presence of beneficial bacteria, particularly *Bifidobacterium*, that inhibit the growth of pathogenic and putrefactive bacteria. Some studies indicate that 4 g of XOS per day for 3 weeks improved the intestinal microbiota among people who are above 65 years old. XOS also have favorable technological features, including stability at acidic pH, heat resistance, the ability to achieve significant biological effects at low daily doses, low calorie content, non-toxicity and other properties that have yet to be studied.(Carvalho et al., 2012).

Xylo-oligosaccharides benefits for the healthy human as summurized:

- Modulation of the colon microbiota increasing the number of bifidobacteria and Lactobacillus.
- Decrease in the number of pathogenic and putrefactive bacteria.
- Growth of health promoting bacteria in the intestinal tract leading to the production of SCFAs, which cause beneficial effects for metabolism (e.g., reduction of osteoporosis). Protection against cardiovascular disease and reduction of the risk of colon cancer due to the formation of these acids.

- Improvements in bowel function and calcium absorption; the prevention of dental caries.
- Biological effects at low doses. Effects related to skin and blood, immunological action, anti-oxidant activity, anti-inflammatory and antiallergic action.
- Low calorie (Carvalho et al., 2012).

4.1.5 Lactosucrose

Lactosucrose (4^G -β-D-lactosylfructoside, galactosylsucrose) is a trisaccharide consisting of glucose, galactose, and fructose. Lactosucrose can be obtained via a transfructosylation reaction catalyzed by either a levansucrase or a β-fructofuranosidase, with lactose and sucrose serving as substrates. In this reaction the fructosyl moiety of sucrose is transferred to lactose thus forming lactosucrose. However, the enzyme not only catalyzes the transfer reaction, but also catalyzes the hydrolysis of sucrose and lactosucrose. For this reason, the maximum attainable lactosucrose yield in a batch process assuming equimolar initial reactant concentration, and the absence of any parallel and consecutive reaction, is around 52%, at 50 °C. Lactosucrose is indigestible in the human digestive tract and is therefore low in calories and suitable for use in low-calorie foods. It is a putative growth stimulator of intestinal bifidobacteria and therefore could be classified as a prebiotic (Cheul et al., 2009, Mussatto & Mancilha; 2007).

4.1.6 Malto-oligosaccharide

Malto-oligosaccharides contain α-D-glucose residues linked by α-1,4 glycosidic linkages. They are produced commercially from starch by the action of debranching enzymes such as pullulanase and isoamylase, combined with hydrolysis by various α-amylases. These α-amylases have differing reaction specificities and can be used to produce syrups that are rich in malto-oligosaccharides of different chain lengths.

Malto- oligosaccharides are not generally claimed to increase the numbers of bifidobacteria in the human colon. They are hydrolysed and absorbed in the small intestine and do not reach the colon intact. However, an author, in his review paper

on malto-oligosaccharides, reported that the consumption of maltotetraose-rich corn syrup has been demonstrated in human trials to reduce the levels of intestinal putrefactive bacteria such as *Clostridium perfringens* and members of the family Enterobacteriaceae. Therefore, malto-oligosaccharides may be effective in improving colonic conditions (Crittenden & Playne, 1996).

4.1.7 Isomalto-oligosaccharides

Isomalto-oligosaccharide (IMO) are derived from starch by a two- step enzymatic process and are mixtures of α-1,6 glucosides such as isomaltose, isomaltotriose, panose and isomaltotetrose. Starch is first liquefied through the hydrolytic activity of α-amylase. The liquefied starch is then treated with both β-amylase and α-glucosidase to produce IMO. B-amylase converts the starch to maltose. The hydrolytic and glucotransferase activity of α-glucosidase then converts the maltose to a mixture of IMO. The IMO mixtures also contain oligosaccharides with both α-1,6 and α-1,4 linked glucose. IMO do not conform with the non-digestibility criterion of potential prebiotics as they are partially digested by isomaltase in the human jejunum and the residual oligosaccharides are fermented by bacteria in the colon. However, it has been suggested that higher DP molecules can reach the colon ,intact and be selectively fermented by the beneficial flora therein. Many researchers investigated breath H_2 excretion in 38 healthy volunteers during FOS and IMO supplementation of gradually increasing daily doses from 10 to 20 g/day. FOS ingestion mediated high H_2 excretion while H_2 during IMO ingestion was slight. They suggested that IMO was readily hydrolysed by small intestine enzymes.

A number of studies have suggested that IMO are bifidogenic. According to Kohmoto et al. in an in vivo study of 6 healthy adult men and 18 senile persons observed an increase in bifidobacterial numbers following the administration of IMO at a dose of 13.5 g/day for 2 weeks. The same researchers reported that, according to in vitro work, IMO could only be utilized by bifidobacteria and the *Bacteroides fragilis* group but not by E. coli or other gut bacteria. In a further in vivo study of healthy men, the minimum dose of IMO to induce a significant increase in numbers of bifidobacteria was established at 8-10 g/day.

Koneko et al. studied the fermentation of different saccharide fractions of IMO and established that growth activity of bifidobacteria in the human large intestine proportionally increased with the DP of IMO components. This observation was attributed to differing digestibility of IMO in the small intestine, with increased DP being associated with resistance to intestinal digestion (Gibson & Roberfroid, 2008).

The other researchers investigated the effect of IMO on bowel function of 7 elderly males suffering from constipation. Volunteers went on a 30-day low fiber control diet following which they crossed over to a 30-day period where diet was supplemented with 10 g/day IMO. During IMO ingestion, defecation frequency significantly increased and no complaints of bloating or diarrhoea were noted. Mean wet fecal weight increased by 70% and mean dry fecal weight by 55%. However, no bacteriological analysis was performed to monitor the effect of IMO supplementation on fecal flora (Gibson & Roberfroid, 2008).

4.1.8 Soybean-oligosaccharides

Soybean whey is a by-product of the production of soy protein. It contains the oligosaccharides raffinose, stachyose together with glucose, sucrose and fructose. These sugars are directly extracted from soy bean whey and concentrated to produce syrup. Because there is no α-galactosidase activity in the human small intestine to digest the α-1,6 linkages present in raffinose and stachyose, soybean oligosaccharides (SOS) may be able to reach the colon intact.

A number of studies that SOS exert a bifidogenic effect on colonic flora. According to many researchers, studied the effect of SOS on bifidobacteria in vitro, in a two-stage continuous culture system inoculated with fecal slurry from a healthy volunteer. It was observed that bifidobacteria increased in numbers relative to other bacterial groups. The other researchers administered 15 g/day raffinose to 7 healthy adults observed a significant increase in bifidobacteria while total bacterial counts remained stable. Furthermore, during raffinose intake *Bacteroides* spp. and *Clostiridium* spp. counts were significantly lower than those prior to and after raffinose intake (Gibson & Roberfroid, 2008).

4.1.9 Gentio-oligosaccharides

Gentio-oligosaccharides consist of several glucose residues linked by β-1,6 glycosidic bonds. They are produced from glucose syrup by enzymatic transglucosylation. These oligosaccharides are not hydrolysed in the stomach or small intestine anda are claimed by the manufacturer to promote the growth of bifidobacteria and lactobacilli (Crittenden & Playne, 1996).

4.1.10 Cyclodextrins

Cyclodextrins are cyclic α-1,4-linked maltooligosaccharides consisting of 6-12 glucose units. They are formed from starch digests by the action of cyclomaltodextrin glucanotransferase. These oligosaccharides are capable of forming inclusion complexes with various organic compounds by incorporating them into the cavity of their cyclical structure. This can lead to desirable changes in the physical and chemical properties of the incorporated compound. Uses of cyclodextrins include stabilization of deliquescent or volatile compounds in foods and chemicals; emulsification of oils and fats; protection of substances that are susceptible to oxidation and photodegradation; and masking bitterness in foods and drugs (Crittenden & Playne, 1996).

4.1.11 Inulin-Type Prebiotics

Inulin-type prebiotics include inulin, oligofructose, and FOS, oligo- or polysaccharide chains comprised primarily of linked fructose molecules that are bifidogenic. Inulin-type prebiotics are used as functional food ingredients in beverages, yogurts, biscuits, and spreads; they are also used as dietary supplements. Inulin-type prebiotic compounds are naturally occurring constituents in many plants. Root vegetables including Jerusalem artichokes, burdock, chicory, leeks, and onions are especially rich sources.

Inulin-type prebiotics are members of a larger group called “fructans.” Fructans represent a category of compounds that encompasses all naturally occurring plant oligo- and polysaccharides in which one or more fructosyl-fructose linkages comprise

the majority of glycosidic bonds; hence, they are primarily polymers of fructose units. Fructans can have at least one fructosyl-glucose linkage – identical to that found with sucrose and, when present, is typically a starting link in the polymer chain. When the starting molecule is sucrose in the fructan chain, the bond between the starting glucose and the second carbon (of fructose) can be hydrolyzed to some degree by sucrase enzymes, secreted by the tips of the small intestinal epithelial villi, and produce free glucose. The presence of this sucrose sugar moiety is not a necessary precondition for the compound to be considered a fructan; therefore, many fructans begin with fructose. Structurally, fructans can be linear or branched fructose polymers.

An individual fructan having a glucose molecule preceding fructose is designated as GFn – G referring to the terminal glucose unit, F referring to fructose units, and n designating the number of fructose units found in the fructan chain. Hence, GF_2 is a fructan oligosaccharide with a terminal glucose followed by two fructose units (Figure 1). This fructan has one fructosyl-glucose linkage (a sucrose molecule) followed by one fructosyl-fructose linkage. A fructan with no glucose would be designated as either F_n or F_m. Both are used in the scientific literature with n (or m) referring to the number of fructose units occurring in the fructan. In this review, n will be used. A fructan designated as F_3 (Figure 2) has three fructose units and two fructosyl-fructose linkages (Kelly, 2008).

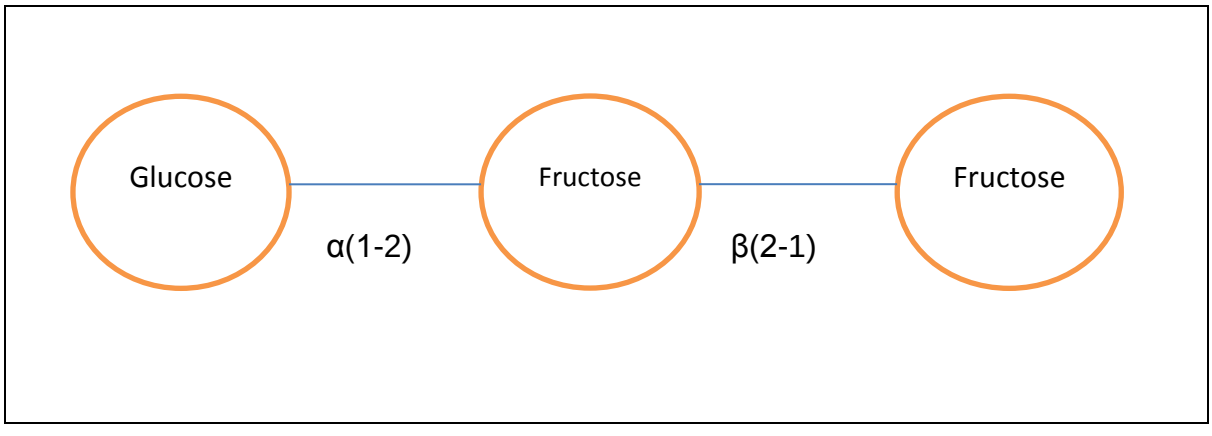

Figure 5: GF_2 Fructan (Kelly, 2008).

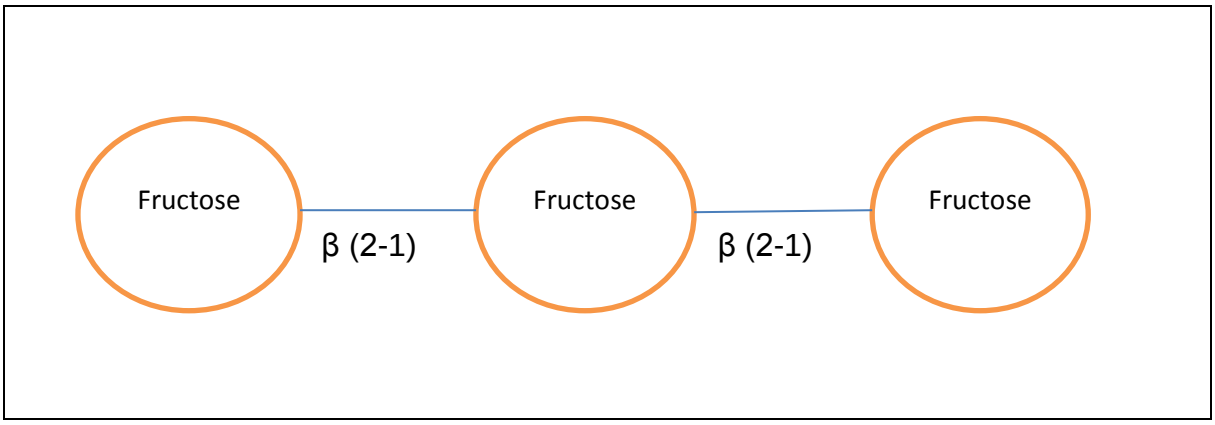

Figure 6: F_3 Fructan (Kelly, 2008).

Fructans can also be described by degree of polymerization (DP). DP refers to the number of repeat units in an oligomer or polymer chain, so DP of an individual fructan would be its number of repeating fructose units and identical to n.

Inulin is a generic term that covers all linear fructans with beta (2−1) fructosyl-fructose glycosidic bonds. This specific type of glycosidic bond gives inulin its unique structural and physiological properties. Because of the beta configuration of the bonds between fructose monomers, inulin-type fructans resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes – specific for alpha-glycosidic bonds. As a result, inulin-type fructans are indigestible and are fermented in the colon.

Production of the inulin-type prebiotics oligofructose, inulin, and inulin HP begins with a plant source rich in fructans. Production of FOS begins with a more fundamental molecule (typically sucrose). Commercially available inulin-type prebiotics differ in purity, fructan and free-sugar content, and fructan portfolios. By applying specific production technologies to either a food source of fructans (such as chicory root) or sucrose, the food industry can produce a diversity of inulin-type prebiotic products with different DP and sugar portfolios with slightly different strengths and weaknesses.

The starting plant material for production of oligofructose, inulin, and inulin HP is in most cases chicory root , preferred because it contains high amounts of inulin-type fructans. The inulin is extracted by hot water. The result of this extraction is ~92-percent inulin-type fructans of both GFn and Fn types with DP ranging from 2-60 and

a DP_{av} of ~10-12. About 10 percent of the fructans in this minimally processed inulin might have a DP ranging from 2-4 and 20 percent might range from 5-9. This extract will also contain a small amount (6-10%) of free sugars (the monosaccharides fructose and glucose and the disaccharide sucrose), present in the starting root material and not a result of hot water extraction.

Inulin can be further processed into more purified inulin-type prebiotic products (oligofructose or inulin HP). Total enzymatic hydrolysis results in monosaccharide molecules of fructose and glucose. Partial enzymatic hydrolysis can produce oligofructose mixtures with a DP ranging from a hot-water extracted inulin to a pure mix of completely enzymatically hydrolyzed monosaccharides. An endoinulase is used for partial enzymatic hydrolysis of inulin.

Depending on the DP_{max}, DP_{av}, DP range, and the amount of free sugars desired, the degree of partial hydrolysis can produce oligofructose products with different portfolios of fructans and free sugars. To appropriately compare products, the DP_{max}, DP_{av}, DP range, and free-sugar content must be known.

Whether or not inulin undergoes partial enzymatic hydrolysis to produce oligofructose, physical separation techniques can be applied to produce products of higher purity and more uniformity. Using partial hydrolysis and/or physical separation techniques, products with 99-percent purity can be produced.

Because of additional processing, commercially available products with the generic names inulin or oligofructose are not identical in purity. Standard inulin (92% fructans and 8-10% free sugars) and low-sugar versions of inulin (99.5% fructans and ~0.5% sugars) are both referred to as inulin. Oligofructose syrups with ~60-percent fructans and ~40-percent sugars are commercially available as are syrups with ~95-percent fructans and ~5-percent sugars. Oligofructose syrups and powders with other specifications are also commercially available.

FOS is produced by an entirely different method. Using the fungal enzyme beta-fructosidase, derived from *Aspergillus niger*, FOS is enzymatically synthesized using a process called transfructosylation. The starting molecule used is sucrose, and the enzyme activity sequentially adds fructose units with new β(2−1) linkages

placed in the chain. Unlike inulin and oligofructose, which have all glycosidic bonds between fructose units in the β(2−1) configuration, transfructosylation does not result exclusively in β(2−1) fructosyl-fructose glycosidic bonds; other linkages occur in limited numbers (Kelly, 2008).

Similar to inulin and oligofructose, FOS products vary in their free-sugar content. During enzymatic synthesis, some glucose and fructose molecules are formed as by-products. FOS can also contain unreacted sucrose. These free sugars can be removed or left in the finished product depending on the sweetness characteristics desired. (Kelly, 2008).

Table 1: Prebiotics and Their Sources (Broek & Voragen, 2008).

Fructo-oligosaccharides	Oligofructose is produced from (i) partial enzymic hydrolysis from chicory inulin or (ii) synthesis from sucrose using the transglycosylation activity of a β-fructofuranosidase
Inulin	A polydisperse β-(2-1) fructan from e.g. chicory, artichoke, garlic.
Isomalto-oligosaccharides	Manufactured from starch by enzymatic hydrolysis and using the transglycosylation activity of an α-glucosidase
Lactosucrose	Non-reducing oligosaccharide produced from a mixture of lactose and sucrose using the transglycosylation activity of a β-fructofuranosidase
Lactulose	A semi-synthetic disaccharide prepared from lactose by alkaline isomerisation.
Soybean–oligosaccharides	α-Galactosyl sucrose derivatives like raffinose and stachyose e.g. extracted from soy.
Xylo-oligosaccharides	Derived from xylan or arabinoxylan from e.g. corncobs, oats, and wheat by enzymatic hydrolysis

5. FOOD APPLICATIONS OF PREBIOTICS

A number of NDOs have been introduced as functional food ingredients during the last few decades, and their industrial applications are continuously increasing. Major uses focus in beverages (fruit drinks, coffee, cocoa, tea, soda, health drinks and alcoholic beverages), milk products (fermented milk, instant powders, powdered milk and ice cream), probiotic yogurts (based on live microorganisms that exert beneficial effects for the host via improvement of the microbiological balance in the intestine) and synbiotic products (containing a mixture of probiotics and prebiotics that beneficially effects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare (Munsatto & Mancilha, 2007).

Other current applications of NDOs in the food industry include desserts such as jellies, puddings and sherbets; confectionary products such as candy, cookies, biscuits, breakfast cereals; chocolate and sweets; breads and pastries; table spreads and spreads such as jams and marmalades; and meat products such as fish paste and tofu. Nevertheless, since the specific physicochemical and physiological properties of NDOs products vary depending on the type of mixture prepared, the most appropriate oligosaccharide for a particular food application also vary. Bread, for example, is a suitable food for galacto-oligosacharides inclusion because during the fermentation with yeast and the baking of bread, they are not broken down, and render bread excellent in taste and texture. Infant-food and food special for old-aged or hospitalized people are promising examples of products for galacto-oligosaccharides inclusion, since these people are more susceptible to modifications in the intestinal microflora (Munsatto & Mancilha, 2007).

Inulin-type prebiotics are increasingly being used for food applications. This has potential clinical relevance since consumers might be consuming sufficient quantities of inulin-type prebiotics in foods and beverages to generate physiological responses, including gastrointestinal side effects. Because inulin, oligofructose, and FOS are classified as soluble fibers they can be used as a means of increasing dietary fiber or to replace sugars or fats. Depending on the taste, texture, and other

attributes desired, different mixtures are considered for inclusion in food products. In these applications they are considered to be a functional food ingredient, added to make health claims and/or persuade the consumer the product is a healthier choice than one that does not contain inulin-type prebiotics (Kelly, 2008).

Inulin-type prebiotics have a wide range of food applications, although they are not suitable for use in soft drinks and fruit jams because the acids in these foods hydrolyze the inulin-type fructans into monosaccharides (Kelly, 2008).

Certain mixes of inulin-type prebiotics can act as potential fat replacers in foods. Using a specific processing technique inulin is combined with water to produce the same texture and mouth feel as fat. As a fat replacement, this patented inulin-type prebiotic can be used in water-based foods such as dairy products and spreads, but not dry foods. Long-chain, high-molecular weight inulin HP is most desirable as a fat replacer. Longer chain lengths reduce the solubility of inulin-type fructans and result in the formation of what Niness describes as “inulin microcrystals” when mixed with water or milk, which are not discretely perceptible and have a smooth, creamy mouth feel. According to Niness, inulin HP has “almost twice the fat mimetic characteristics of standard inulin, with no sweetness contribution.” When inulin-type prebiotics are used to produce low-fat spreads, for example, inulin HP is the preferred choice because of its superior fat mimetic properties and lack of sweetness (Kelly, 2008).

Certain inulin-type prebiotics have properties that make them suitable as sugar replacers. In contrast to longer-chain, higher-molecular weight inulin-type fructans that are less soluble and fat mimetic, shorter-chain, lower-molecular weight oligofructose and FOS are preferred as sugar replacers. These shorter-chain oligomers are more soluble than sucrose, possess functional qualities similar to sugar or glucose syrup, and can have ~30-50 percent the sweetness of table sugar (Kelly, 2008).

Fructo-oligosaccharides have been applied in a variety of dairy products, as they are the ideal ingredients to give bulk with fewer calories and increase the functional value without compromising on the taste and mouth feel of the products.

They are also used in baked goods and breads to replace sugar and to retain moisture in the product (Charalampopoulos & Rastall, 2012).

Table 2: Food Applications of Prebiotics (Wang, 2009).

Applications	**Functionality**
Yoghurts and desserts	Fat or sugar replacement, texture and mouthfeel, fiber and prebiotics
Frozen desserts	Fat or sugar replacement, texture, and mouthfeel, melting behavior
Beverages and drinks	Sugar replacement, mouthfeel, foam stabilization and prebiotics
Fruit preparations	Sugar replacement, synergy with intense sweeteners, body and mouthfeel, fiber and prebiotic
Baked goods and breads	Sugar replacement, moisture retention, fiber and prebiotic
Breakfast cereals and extruded snacks	Sugar replacement, crispiness and expansion, fiber and prebiotic
Fillings	Fat or sugar replacement, texture and prebiotic
Tablets and sugar confectionery	Sugar replacement, fiber and prebiotic
Chocolate	Sugar replacement, heat resistance and fiber
Dietetic products and meal replacers	Fat or sugar replacement, synergy with intense sweeteners, body and mouthfeel, fiber and prebiotic
Meat products	Fat replacement, texture, stability and fiber
Soups and sauces	Sugar replacement and prebiotic
Salad dressing	Fat replacement, mouthfeel and body
Table spreads and butter products	Fat replacement, texture and spreadability, stability, fiber and prebiotic
Baby food	Texture, body and mouthfeel, fiber, stability and prebiotic

6. CONCLUSIONS

Presently, the use of foods that promote a state of wellbeing, better health and reduction of the risk of diseases have become popular as the consumer is becoming more and more health conscious. In this sense, there has been a lot of attention paid to specific types of dietary carbohydrates, namely the non-digestible oligosaccharides.

Oligosaccharides are relatively new functional food ingredients that have great potential to improve the quality of many foods. In addition to providing useful modifications to food flavor and physicochemical characteristics, many of these sugars possess properties that are beneficial to the health of consumers. These include non-cariogenicity, a low calorific value and the ability to stimulate the growth of beneficial bacteria in the colon. Both the production and the applications of food-grade oligosaccharides are increasing rapidly. Major uses are in beverages, infant milk powders, confectionery, bakery products, yoghurts and dairy desserts. Research continues into the development of new oligosaccharides with a range of physiological properties and applications in the food industry.

7. REFERENCES

Barile, Daniela., Rastall, R. A. (2013). Human milk and related oligosaccharides as prebiotics. *Current Opinion in Biotechnology, 24,* 214–219.

Broek, V. D., Voragen, A. G. J. (2008). Bifidobacterium glycoside hydrolases and (potential) prebiotics. *Innovative Food Science and Emerging Technologies, 9*, 401–407.

Bruzzese, E., Volpicelli, M., Squaglia, M., Tartaglione, A., Guarino, A. (2006). Impact of prebiotics on human health. *Digestive and Liver Disease, 38:2,* S283–S287.

Carvalho, A. F. A., Neto, P. O., Silva, D. F., Pastore, G. M. (2012). Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. *Food Research International, 51,* 75–85.

Charalampopoulos, D., Rastall, R. A. (2012). Prebiotics in foods. *Current Opinion in Biotechnology, 23,* 187–191.

Cheul, H. W., Byun, S. H., Sohn, E. H. (2009). Production of Lactosucrose from Sucrose and Lactose by a Levansucrase from Zymomonas mobilis. *J. Microbiol. Biotechnol, 10,* 901-908.

Crittenden, R. G., Playne, M. J. (1996). Production, properties and applications of food- grade oligosaccharides. *Trends in Food Science & Technology, 7,* 353-360.

Gibson, G. R., (2004). Prebiotics. Best Practice & Research Clinical Gastroenterology, *18:2*, 287-298.

Gibson, G. R., Roberfroid, M. B. (2008). Handbook of Prebiotics.

Gosling, A., Stevens, G. W., Barber, A. R., Kentish, S. A., Gras, S. L. (2010). Recent advances refining galactooligosaccharide production from lactose. *Food Chemistry, 121,* 307–318.

Kelly, G. (2008). Inulin-Type Prebiotics – A Review: Part 1. *Alternative Medicine Review, 13,* 315-327.

Licht, T. R., Ebersbarch, T., Frokiaer, H. (2012). Prebiotics for prevention of gut infections. *Trends in Food Science & Technology, 23,* 70-82.

Lina, B. A. R., Jonker, D., Kozianowski, G. (2002). Isomaltulose (Palatinose): a review of biological and toxicological studies. *Food and Chemical Toxicology 40, 10,* 1375–1381.

Marteau, P. (2001). Prebiotics and probiotics for gastrointestinal health. *Clinical Nutrition, 20:1,* 41-45.

Mussatto, S. I., & Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers, 68,* 587–597.

Neto, P. O., Menao, P. T. P. (2009). Isomaltulose production from sucrose by *Protaminobacter rubrum*immobilized in calcium alginate. *Bioresource Technology 100, 18*, 4252–4256.

Oliveira, R. P. S., Florence, A. C. R., Silva, R. C., Perego, P., Converti, A., Gioielli, L. A., Oliveira, M. N. (2009). Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acids profiles in nonfat symbiotic fermented milk. *International Journal of Food Microbiology, 128,* 467–472.

Panesar, P. S., & Kumari, S. (2011). Lactulose: Production, purification and potential applications. *Biotechnology Advances, 29,* 940–948.

Rad, A. H., Akbarzadeh, F., Mehrabany, E. V. (2012). Which are more important: Prebiotics or probiotics? *Nutrition, 28,* 1196–1197.

Wang, Y., (2009). Prebiotics: Present and future in food science and technology. *Food Research International, 42,* 8–12.

Ziemer, C. J., & Gibson, G. R. (1998). An Overview of Probiotics, Prebiotics and Synbiotics in the Functional Food Concept: Perspectives and Future Strategies. Int. Dairy Journal, 8, 473-479.