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Mini Review

Applications of inulin and probiotics in health and nutrition

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Abstract

Interest in consumption of prebiotics and probiotics to improve human gastrointestinal health is increasing. Consumption of beneficial probiotic bacteria combined with oligosaccharides may enhance colonic bacterial composition and improve internal health. The objectives of this article are to review existing literature concerning the effect of synbiotic foods on the composition and activity of the colonic microbiota, and efficiency of functional attributes of synbiotic foods in formulation and development of new dairy products.

Introduction

It is well documented that the large intestine is one of the most densely populated ecosystem in nature consisting of over 500-1,000 different species of bacteria (Xu and Gordon, 2003; Meyer and Stasse-Wolthuis, 2009) of which bifidobacteria are generally considered to be health promoting and beneficial (Kimura *et al*., 1997). The equilibrium of the ecosystem is dynamic and may be negatively affected by aging, daily diet and other environmental factors (Collins and Gibson, 1999). It is believed that the maintenance of the gastro-intestinal bacterial population, which mainly contains beneficial species, is important for overall gastro-intestinal health and wellbeing (Crittenden, 1999). Recently, research has focused on the ability of probiotic bacteria to ferment prebiotics and produce short-chain fatty acids (SCFA) which is thought to be beneficial to gut health (Collins and Gibson, 1999; Kaur and Gupta, 2002). Prebiotics are non-digestible food ingredients that pass through the upper gut unchanged and are selectively fermented by colonic bacteria (Ramirez-Farias *et al*., 2009). This leads to specific changes in the composition and activity of the gut microbiota that confers benefits upon host health (Cummings *et al*., 2001). The dietary fructans are particularly well studied prebiotics as they have the potential to increase bifidobacterial population in the colon without being utilized by other intestinal bacteria (Roberfroid *et al*., 1998; Gibson *et al*., 2004; Macfarlane *et al*., 2006). Major classes of dietary fructans found in higher plants include inulin, levan, and graminan (Chalmers *et al*., 2005), with inulin being significant by its unique molecular structure, which confirms the bifidogenic effect of inulin as a functional food ingredient. Inulin naturally occurs in several plant foods including onion, garlic, chicory, artichoke and leek (Gibson *et al*., 1994). Inulin is composed of a series of oligo- and polysaccharides of fructose with (2→1) linkages, where the terminal sugar in most chains is glucose. The configuration of the anomeric C2 in fructose monomers prevents fructans from digestion and this is responsible for its reduced caloric value and dietary fibre effects (Coussement, 1996; Kaur and Gupta, 2002). Some of the functional effects of inulin in the gastro-intestinal tract include modulation of microbial fermentation, reducing fat and cholesterol absorption, and pH reduction; these therefore have a direct effect on reducing intestinal disturbances, constipation, hyperlipidaemia, hyperglycemia and intestinal cancer (Ziemer and Gibson, 1998; Kaur and Gupta, 2002). This review presents an overview of the effects of prebiotic and probiotic combination on the composition and activity of the colonic bacterial flora, and discusses about functional attributes of synbiotic foods in development of new dairy products.

Biomarkers for gut health

The gut flora are the micro-organisms that

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normally live in the digestive tract and can perform a number of useful functions for their hosts. The average human body consists of about 10^{13} (ten trillion) cells, but has about ten times more than that number of microbial cells in the gut (Kaur and Gupta, 2002; Meyer and Stasse-Wolthuis, 2009). Different studies have confirmed the significant role of gut microflora in human health through their ability to ferment unused energy substrate, improve the immune system, digest food ingredients, prevent growth of harmful species and produce vitamins(such as Biotin and vitamin K) for the host (Kolida and Gibson, 2007).

The colon is the most heavily populated part of the gastro-intestinal tract, consisting of over 500-1,000 different species of bacteria (Xu and Gordon, 2003; Lopez-Molina *et al*., 2005; Meyer and Stasse-Wolthuis, 2009). The intestinal bacteria can be divided into three categories including a) *Lactobacillus* and *Bifidobacterium*; b) potentially pathogenic bacteria, such as certain species of *clostridia*, and c) other bacteria such as bacteroides, which may have both positive and negative effects (Gibson, 2004; Lopez-Molina *et al*., 2005). It is assumed that *Bifidobacterium* and *Lactobacillus* are primarily carbohydrate-fermenting bacteria; whereas *Bacteroides* and *Clostridium* are mainly proteolytic and amino acid fermenting (Meyes and Stasse-Wolthuis, 2009).

The first part of the colon is mostly responsible for fermenting substances that cannot be digested by the host in the upper gut (such as resistant starch, non-digestible carbohydrates like inulin and oligosaccharides), while in the lower part of the colon proteins and amino acids are broken down (Kolida and Gibson, 2007). The two main types of fermentation that are carried out in the colon are saccharolytic and proteolytic (De Preter *et al*., 2011). Saccharolytic fermentation is more preferred to the host than proteolytic because of the types of metabolic end products (Kilda and Gibson, 2007; De Preter *et al*., 2011). The main end product of carbohydrate fermentation in the colon is the generation of short-chain fatty acids (SCFA), predominantly acetate, propionate, and butyrate (Topping and Clifton, 2001; De Preter *et al*., 2011). Most of the SCFA formed by the intestinal bacteria are absorbed and metabolized, thereby contributing towards the host energy gain. Acetate is metabolized in a systemic area such as muscle and used to produce adenosine-5'-triphosphate (ATP), whereas propionate can be transported to the liver for gluconeogenesis. Butyrate is an important source of energy for the colonocytes and has anti-tumour properties. It inhibits cell proliferation and stimulates cell differentiation in epithelial cell lines of neoplastic origin *in vitro* (De Preter *et al*., 2011).

Increased SCFA synthesis in the colon creates a more acidic environment in the gut (Scheppach *et al*., 2001) which enhances the colonization resistance against pathogenic bacteria (Topping *et al*., 2003), reduces the formation of secondary bile acids which has been implicated in colonic carcinogenesis (Nagengasti *et al*., 1988; Zampa *et al*., 2004) and impairs the activity of specific enzymes such as proteases (Macfarlane *et al*., 1998). Furthermore, SCFAs have been shown to possess anti-inflammatory capacities affect satiety hormones and play a role in insulin resistance. A role for SCFA in prevention of some human pathological conditions such as ulcerative colitis and colon carcinogenesis has been presumed although conclusive evidence is still lacking.

Therefore, saccharolytic fermentation of inulin by *Bifidobacteria* spp. in the gut will result in increase in their numbers, thus shifting in the gut microbiota balance towards a 'healthier' composition. However, it is important to note that alterations in a single biomarker cannot provide explicit proof of a health benefit or reduced risk of disease and more consistent data on various gut biomarkers and their effects on gut health is required (Meyer and Stasse-Wolthuis 2009).

Synbiotic foods

Synbiotic food is defined as "a mixture of probiotics and prebiotics that beneficially affects the host by 1) improving the survival and implantation of live microbial dietary supplements in the gastro-intestinal tract, and 2) selectively stimulating the growth and activity of one or a limited number of health-promoting bacteria, and thus improving host health and welfare" (Gibson and Roberfroid, 1995). The synbiotic concept is a promising trend in the functional food sector as the combination of probiotics and prebiotics may confer greater benefits to using individual ingredients. It is expected that adding prebiotic would benefit the survival of *bifidobacteria* during the shelf life of the dairy products (Lourens-Hattingh and Viljoen, 2001). The effect of synbiotics on faecal microflora of experimental animals is demonstrated by increasing the total anaerobes, aerobes, *lactobacilli*, and *bifidobacteria* counts and decreasing in *Clostridia*, *Enterobacteriaceae* and *Escherichia coli* counts in the colon of rats (Suskovic *et al*., 2001). In humans, Bouhnik *et al*. (1996) reported an overall increase in faecal bifidobacterial numbers in healthy volunteers after the consumption of synbiotic mix of inulin and *Bifidobacterium* spp. Kiebling *et al*. (2002) also observed the significant

decrease in LDL/HDL cholesterol ratios after long-term consumption of synbiotic yoghurt (*L. acidophilus* and *B. longum* plus inulin), similar to the previous observation of Schaafsma *et al*. (1998).

Synbiotic products often are composed of a combination of inulin-type fructans, bifidobacteria, and lactulose in conjunction with lactobacilli (Lourens-Hattingh and Viljoen, 2001). Currently, only limited variety of synbiotic products such as probiotic yoghurt and dairy drinks are available in the market. Future market of synbiotic foods not only depends on their effectiveness on human health but also the increase of consumers' awareness about the consumption of these products.

A review of probiotics and prebiotics

Over the last 20 years, there has been a significant interest, by both consumers and food manufactures in the production and consumption of prebiotics in daily diet (O'Sullivan, 2001; Bruno and Shah, 2004) as consumers are becoming more aware of maintaining their 'internal health' by modifying gastro-intestinal microflora. Probiotics are 'live micro-organisms' which when administered in adequate amounts in the diet, deliver health benefits to the host (Fuller, 1991; Bruno and Shah, 2004). Probiotic bacteria are able to suppress potentially pathogenic micro-organisms in the gastro-intestinal track and improve the balance in favour of beneficial micro-organisms (Ibrahim and Bezkorovainy, 1993). The beneficial impact of the probiotic approach on the gastro-intestinal health can be defined by provision of the protection against diet-related diseases (Fuller, 1991; Bruno and Shah, 2004).

The human gastro-intestinal tract (GIT) is composed of a complex ecosystem of anaerobic and aerobic micro-organisms (Bruno and Shah, 2004), of which the large intestine contains over a hundred distinct strains of anaerobic bacteria (>10 ^{11} CFU per gram of colonic content) (Gibson *et al*., 1994; Bruno and Shah, 2004). Among these bacteria, bifidobacteria are believed to have the most beneficial effects for improving the gastro-intestinal health (Bruno *et al*., 2002). *Bifidobacteria* can improve the host immune functions by suppression the activity of harmful bacteria such as *Escherichia coli* and *Clostridium perfrigens* (Yaeshima *et al*., 1997) and protect the GIT against colon tumourigenesis by mutagenic and carcinogenic factors (Singh *et al*., 1997). Bifidobacteria are consumed as a part of fermented foods such as yoghurt, soy yoghurt, or as dietary supplements. Some previous studies have shown that dairy products may not be an ideal medium for the maintenance of bifidobacteria due to reduced viability of these organisms (Medina and Jordano, 1994; Dave and Shah, 1998). Loss of viability of bifidobacteria has been observed in fermented milk rather than in unfermented milk due to acid injury to the organism.

Lankaputhra and Shah (1997) observed that viability of *Bifidobacterium* infantis in skim milk (12% total solids) at pH 4.3 was reduced by 30% and 82% after 12 and 24 days of storage at 4°C, respectively. Although more recent study by Bruno *et al*. (2002) has shown that with addition of prebiotics particularly hi-maize in fermented milks, the viability of bifidobacteria could be improved. However, more research needs to be carried out in order to investigate the effect of consuming capsules containing freeze-dried bifidobacteria supplemented with a prebiotic on gastro-intestinal health in the average healthy adult population.

Prebiotics are non-digestible carbohydrate substrates in the diet that are the preferred foods for bifidobacteria and lactobacilli and result in their increased number in the large intestine (Gibson and Roberfroid, 1995; Ziemer and Gibson, 1998). The definition of prebiotics overlaps significantly with the dietary fibre definition; with the exception of its selectivity for certain species of the gut bacteria. According to Salminen *et al*. (1998) and Gibson (2004), in order to being considered as an effective prebiotic, any food ingredient must demonstrate the following characteristics:

-Non-digestibility and non-absorption in the GIT
-Fermentability by the gut microflora
-Selective stimulation of the growth and activity of one or a limited number of colonic bacteria
-An ability to increase the number of saccharolytic species and decrease putrefactive microorganisms such as *Clostridia* in order to alter the colonic microflora balance towards a healthier composition.

The concept of prebiotic is to improve the gut microflora through dietary means. According to Saxelin *et al*. (2003), while the large intestine contains several hundred strains of anaerobic bacteria, the prebiotic concept assumes that there is already favorable microflora in the GIT and therefore, the prebiotics only need to stimulate the growth and metabolic activities of those bacteria. The most important functional effects of prebiotics on the gut microflora include (1) gut microflora modification, (2) maintaining the intestinal mucosa with the capacity to prevent pathogen activation, (3) modification of dietary proteins by the intestinal microflora, (4) reducing the risk of tumour induction by improving the bacterial enzyme activity, and (5) improving gut mucosal permeability (Salminen *et al*., 1996).

Using prebiotics in food formulation process

has some advantages over the probiotic strategy as they could reduce the problem of keeping the organisms alive during transit through upper gastro-intestinal tract as well as during storage (Crittenden, 1999). Thus, the prebiotic approach involves the interaction of a non-digestible food ingredient and beneficial micro-organisms such as bifidobacteria and lactobacilli in the human colon.

The most researched prebiotics are non-digestible oligosaccharide molecules, containing 3 to 10 monosaccharide residues connected by glycosidic linkages (Niness, 1999). Most of them occur naturally as native components in plants e.g. raffinose and stachyose in beans and peas, oligofructose (OF) and inulin in chicory, garlic, artichoke, onion and leek (Van Loo *et al*., 1995). Inulin is a prebiotic for which sufficient data has been generated to allow an evaluation of its classification as a functional food ingredient. Inulin includes native inulin, enzymatically hydrolyzed inulin or oligofructose, and synthetic fructooligosaccharides (De Leenheer and Hoebregs, 1994).

Inulin - chemistry and nomenclature

Inulin belongs to a category of carbohydrates known as fructans. In general, fructan is a term used for any carbohydrate in which fructosyl-fructose linkages constitute the majority of the glycosidic bonds (Kaur and Gupta, 2002). Fructans are linear or branched fructose polymers, which are either β 2 → 1 linked inulin or β 2 → 6 linked levan (Kaur and Gupta, 2002). In comparison to starch and cellulose, which are large glucose polymers, fructans are not large molecules and only include a small proportion of plant polymer chains exceeding 50 fructose units (Roberfroid *et al*., 1998). Fructans are known as storage polymers in many plants such as *Cichorium intyubus* (chicory), *Innula helenium* (elecampane), and *Helianthus tuberosus* (Jerusalem artichoke). It is believed that the synthesis and storage of these molecules usually occur in the plant vacuole (Vijn and Smeekens, 1999).

In higher plants, major classes of fructans are found and they include inulins (linear; G1-2F1-2Fn; β 2 → 1), levans (linear; G1-2F6-2Fn; β 2 → 6), graminans (branched; mixed levans; β 2 → 6 and β 2 → 6) (Chalmers et al., 2005).

Inulin (C_{6n} H_{10n+2} O_{5n+1}) has been described as a polydisperse carbohydrate, comprised of linear chains of 1, 2- linked-β-D-fructosyl units bound to a terminal glucose molecule (Roberfroid and Delzenne, 1998). It is distinguished among the fructan family by its unique molecular structure which not only confirms inulin's status as a soluble dietary fibre but also qualifies it as an effective prebiotic. It is mainly of plant origin, whereas some fungi and many bacteria are important producers of inulin (Fuchs, 1991). Inulin usually found in plant species belonging to the order Asterales, such as chicory and Jerusalem artichoke (Vijn and Smeekens, 1999). Schematically, the molecular formula of inulin is GFn and Fm, where G is glucosyl unit, F is fructosyl unit and n indicating the number of fructose units linked to the terminal glucose unit, and m is an integer number of fructose units linked to each other in the carbohydrate chain.

Inulin is a mixture of GFn molecules with $2 < n < 60$ while OF as a subgroup of inulin is composed of GFn and Fm with $2 \leq n$, and $m \leq 10$ (Niness, 1999; Franck, 2000). The term OF was initially introduced as a synonym for fructo-oligosaccharide in 1989 for labelling purposes (Coussement, 1996). OF is manufactured by two different processes which lead to slightly different end-products. Those produced via partial enzymatic hydrolysis of inulin contain both fructose chains (Fm) and fructose chains with terminal glucose units (GFn), whereas OF produced via transfructosylation of sucrose contains only GFn. The number of saccharides in the fructans molecule is commonly referred to as the degree of polymerization (DP). The DP of plant inulin is rather low and depends on plant source, growing stages, climatic conditions and the duration and conditions of post-harvest storage. Native inulin which refers to inulin extracted from fresh roots/tubers without fractionation procedure has an average DP of 10-12 while inulin from which smaller oligosaccharides have been removed has an average DP of 27-29 (De Leenheer and Hoebregs, 1994).

For synthesis of the linear inulin at least two enzymes are required (Vijn and Smeekens, 1999): sucrose: sucrose 1-fructosyltransferase (1-SST) initiates fructan synthesis by catalysing the transfer of a fructosyl residue from sucrose to another sucrose molecule, leading to the production of a trisaccharide 1-kestose (glucosyl-1, 2-fructosyl-1, 2-fructose) (Figure 1) (Roberfroid *et al*., 1998). Chain elongation is mediated by the second enzyme, fructan: fructan 1-fructosyl transferase (1-FFT), leading to forming the inulin molecules (Flamm *et al*., 2001). 1-FFT transfers fructosyl residues from a fructan molecule with a DP>3 to another fructan molecule or to starch (Vijn and Smeekens, 1999).

Although this model of inulin synthesis was proposed by Edelman and Jefford in 1968 (Van Laere, 2002), it took more than 30 years before it was shown to be correct. In 1996, results of several studies also confirmed that the interaction of this set of fructosyltransferases with starch resulted in the

formation of inulin with a polymer length of up to 20 fructosyl residues (Vijn and Smeekens, 1999; Van Laere, 2002). The cDNAs encoding 1-SST has been cloned from Jerusalem artichoke (Pan *et al*., 2009), chicory (Vijn *et al*., 1998), artichoke (Hellwege *et al*., 1998), and onion (Vijn *et al*., 1998), whereas 1-FFT has been cloned only from Jerusalem artichoke and artichoke (Hellwege *et al*., 1998). In terms of inulin chain length, it has been suggested that the size of the fructosyl polymers produced by a plant mainly depends on the enzymatic activity of their 1-FFTs (Hellwege *et al*., 1998).

In comparison, fungal or bacterial inulin generally has a much higher degree of polymerization (DP up to 150) and less structural diversity than plant inulin (Vijn and Smeekens, 1999). Fungal inulin is generally assumed to be synthesized by sequential transfer of fructosyl residues from fructosyl donor (sucrose) to the growing inulin. Chain elongation is mediated by inulosucrase (sucrose 1F- fructosyltransferase) without the presence of any trisaccharide as intermediate. Thus, inulin synthesis in bacteria is simpler than plant inulin biosynthesis because only a single biosynthetic enzyme is involved (Vijn and Smeekens, 1999).

As discussed above, inulin is a mixture of poly- and oligosaccharides of fructose units linked with β (2→1). Inulin is indigestible in the gastrointestinal tract of all higher animals (Coussement, 1996) due to lack of degradation in digestive tract. Inulin would be able to selectively enhance the proliferation of gut bifiobacteria, decrease pH and lower the oxidation-reduction potential in the gastrointestinal tract (Gibson, 1994).

Plant sources of inulin

Approximately 15% of flowering plant species (e.g. *Liliaceae*, *Amaryllidaceae*, *Gramineae*, and *Compositae*) produce fructans in significant amounts (Vijn and Smeekens, 1999) and store them as a reserve in at least one of their organs during their lifecycle (Vijn and Smeekens, 1999; Kaur and Gupta, 2002). Inulin is present in roots and rhizomes of a number of regularly consumed vegetables, fruits, and cereals, including leek, onion, garlic, wheat, chicory (Roberfroid, 1993) and in a wide range of bacterial species (Vijn and Smeekens, 1999). Despite the high inulin content of the aerial parts of many Gramineae (e.g. grains), only a limited number of these plant species are suitable for industrial food applications (Fuchs, 1999). This may be due to presence of some interfering components in these plants which inulin cannot be easily extracted and processed to purified products.

Inulin content can vary with some plants being quite low in inulin (banana, around 0.5% of root dry weight) and other plants being very high in inulin (raw-dried onion around 18.3% of root dry weight, dried garlic 28.2% of root dry weight) (Vijn and Smeekens, 1999). In chicory, inulin is stored as a reserve carbohydrate in the fleshy tap root and constitutes about 42% of root dry weight (Kaur and Gupta, 2002). Some important sources of inulin together with their inulin content (g/100g dry weight) are shown in Table 1.

Inulin as a dietary fibre

Dietary fibre are components of edible plant cells and classified as carbohydrates which are resistance to digestion by small intestine enzymes but are fermented by the bacteria in the large bowel (Roberfroid, 1993). Because of the β-(2→1) fructosyl-fructose linkages, inulin is classified as a 'storage carbohydrate (oligo-and polysaccharides) that resists digestion by mammal digestive enzymes in the upper gastro-intestinal tract but fermented by the colonic microflora. By increasing faecal biomass and water content of the stools, inulin improves bowel habits. For all these reasons, inulin can be considered as dietary fibre (Roberfroid, 1993).

Inulin presents in significant amounts in several edible fruits and vegetables like wheat, chicory, onions, banana, garlic, and leek (Flamm *et al*., 2001). The average daily intake of inulin has been estimated to be 3-11 g/person/day in Europe and 1-4 g/person/day in the United States.

However, no such study has been made in India, Asia and Middle East (Kaur and Gupta, 2002). The U.S. Department of Agriculture (USDA) study by Moshfegh *et al*. (1999) showed that the mean intake of inulin varied by gender and age group, with a range from 1.3 g/day for young children to 3.5 g/day for teenage boys and adult males. Per 1000 calories, the daily mean intake of inulin ranged from 0.9 to 1.5 g/day in the American diet. The primary sources of inulin in the American diet include wheat, onion, garlic and banana with the contribution percentage of 69%, 23%, 3% and 3% to the average daily intake of inulin, respectively (Moshfegh *et al*., 1999).

Initially, inulin and other fructan-containing foods were not consumed for the health benefits per se, due to availability, cost and personal preferences (Kaur and Gupta, 2002). A series of conferences held in Japan in 1982, 1984 and 1986 and more recent studies have linked nutritional health changes in humans resulting from eating inulin to changes in the numbers and varieties of intestinal micro-organism (Roberfroied and Delzenne, 1998; Flamm *et al*.,

2001). In the future, inulin could be classified as a functional food ingredient for disease risk-reduction claims.

Prebiotic effects of inulin in the gastro-intestinal tract

Fructo-polysaccharides such as inulin are non-digestible carbohydrate substrates in the diet that target certain components of the gut microbiota in the human large intestine such as bifidobacteria and lactobacilli (Kaur and Gupta, 2002).

Published results from *in vitro* studies and human subjects (Gibson *et al*., 1995) have demonstrated that inulin can stimulate the growth and/or activity of these types of bacteria in the colon and this stimulation can improve the intestinal flora composition, enhance the immune system and thereby contribute to the health of the host (Rao, 2001; Lopez-Molina *et al*., 2005). Results from Kaur and Gupta (2002) have shown that ingestion of inulin compared to other sources of carbohydrate like sucrose, could significantly reduce the count of pathogenic bacteria such as bacteroids, fusobacteria and clostridia and increase the count of positive microorganisms such as bifidobacteria (Kaur and Gupta, 2002) (Table 2). Similar human studies in European, Japanese and North American adult populations looking for using different daily doses of inulin have reported similar results (Gibson *et al*., 1995; Klessen *et al*., 1997; Kruse *et al*., 1999). These study results have suggested that the beneficial effect of inulin could be due to the ability of bifiodobacteria to inhibit the activity of detrimental bacteria, change the colonic composition, obtain an adhesion sites on the gut epithelium and finally stimulate the immune system (Kaur and Gupta, 2002). The prebiotic effect of inulin has been extensively confirmed by several studies. Some of these studies are listed in Table 3.

Fermentibility of inulin in the upper gastro-intestinal tract

Schematically, inulin molecule is comprised of β configuration between the two atoms of carbon (C2) in its molecule (Kaur and Gupta 2002). Several studies have shown that this configuration is resistant to hydrolysis by human digestive enzymes (α-glucosidase; maltase-isomaltase; sucrase) which is specific for its glycoside linkages (Roberfroid and Delzenne, 1998; Flamm *et al*., 2001). The non-digestibility of inulin has been confirmed by several studies conducted *in vivo* and *vitro* experiments (Roberfroid and Delzenne, 1998). They have shown that 'the stomach hydrolysis of inulin is likely to be of limited physiological significance'. The ingested inulin remains unchanged for up to 1 or 2 h in the different segments (duodenum, jejunum, and ileum) of rat or human small intestine which has been confirmed by *in vivo* studies of rats and humans. Knudsen and Hessove (1995) and Ellegard *et al*. (1997) have studied the action of the upper gastrointestinal tract on inulin in humans. To study the physiology of the digestive system in humans and to quantify the amount of inulin exiting the small intestine, they used the ileostomy model in both studies. The ileostomy model serves as the basis for the study of carbohydrate digestion and carbohydrate effects in the human small intestine and includes the surgical formation of an opening of the ileum on the abdominal wall (Knudsen and Hessove, 1995). Different clinical studies have demonstrated that this model is making the exact amount of any substrate passing to ileostomy effluent easily measurable (Cummings *et al*., 1996; Englyst *et al*., 1996). Results of both above studies demonstrate that 86-88% of the ingested dose of inulin is recovered in the ileostomy effluent. They concluded that inulin proceeds undigested through the upper part of gastro-intestinal tract into the colon, and further, there is no evidence that inulin is absorbed to any significant extent in the small intestine.

The small loss of inulin during the passage through the small intestine could be due to fermentation by the microbial population colonizing the small intestine in individuals with ileostomies. This microbial population is known to be up to 100 times greater in the people with ileostomy than in normal individuals (Flamm *et al*., 2001).

During passage through the gastro-intestinal tract, inulin as a non-digestible carbohydrate never produces fructose, glucose, lactic acid and short chain carboxylic acid (the end products of glycolysis and anaerobic fermentation) in the small intestine (Reboerfroid *et al*., 1995). Knudsen and Hessov (1995) conducted a case control trial among seven subjects (six females and one male) with a median age of 38 (range 22-73) years and measured the amount of lactic acid and short chain acids before and after inulin intake. The results have shown no difference in total acids exiting the small intestine before and after inulin intake.

They suggested that although no digestive process appears to occur during the inulin intake in small intestine however, there was a shift in the nature of fermentation substrate from starch and non-starch polysaccharide to inulin. This shifting trend could lead to a decrease in the SCFA and an increase in lactic acid amount in small intestine.

Thus it has been proposed to classify inulin as a 'colonic food', which means a 'food entering to and fermenting in the colon and serving as a

substrate for the endogenous bacteria to provide the host with energy, metabolic substrate and essential nutrients'(Flamm *et al*., 2001).

Physiological effects of inulin fermentation in the gastro-intestinal tract

Inulin is classified as a low calorie food ingredient as it contains less than half amount of calorie content of digestible carbohydrates, providing a calorie value of 1.0-2.0 kcal/g (Kaur and Gupta, 2002). Therefore, inulin can be used as a suitable food ingredient substitute to lower the total calorie content of daily diet especially for obese people.

In addition, animal *in vivo* studies have concluded that a diet supplementation with inulin would reduce the cecal pH by production of SCFA (end products of colonic fermentation of inulin), increase the size of the cecal pool for SCFA production, and increase the wall thickness in the small intestine and in the cecum which results in an increase in blood flow (Andersson *et al*., 2001).

Effect of inulin on constipation

Constipation is a multi-factorial ailment often encountered in elderly people. Different reasons may contribute to the development of constipation such as aging, medications, inadequate fluid intake, lack of fibre-containing products in a daily diet, inadequate physical activity, and decrease in intestinal motility.

Different human studied have suggested that fermentation of carbohydrate stimulate colonic motility (Roberfroid, 1993; Kleessen *et al*., 1997), and therefore, administration of oligofructose and inulin to a daily diet could improve constipation, abdominal discomfort, and increase in stool frequency. Hidaka *et al*. (1991) observed that the administration of oligofructose relieved constipation and inulin ingestion improved constipation in 9 of 10 subjects. Abdominal discomfort, mainly flatulence, was reported rarely, and by only a few patients. A significant increase in stool frequency was observed in healthy volunteers having one stool every 2–3 days by including inulin with DP more than 25 in the diet (Hond *et al*., 2000).

Effect of inulin on mineral absorption

Some studies have suggested that chicory inulin as a soluble fibre may increase the body absorption of calcium, improve bone mineral density, and reduce the risk of osteoporosis development. Delzenne *et al*. (1999) have demonstrated that rats fed with inulin absorbed more calcium and magnesium compared to control rat, despite an increase in total faecal mass.

Increased calcium absorption could be due to its increased availability by transfer of calcium from the small intestine into large intestine and the osmotic effect of inulin that transfers water into the large intestine, thus allowing it to become more soluble (Kaur and Gupta, 2002). The improved absorption was associated with decreased pH of ileal, cecal and colonic contents, resulting in an increased concentration of ionized minerals. Ohta *et al*. (1994) reported that ingestion of inulin improved calcium and magnesium absorption in normal rats, although only magnesium absorption was increased in cecectomized rats. This suggested that the effect of fermentation in the cecum was particularly important for calcium absorption. Other study by Coudray *et al*. (1997) noted that inulin improved the absorption of calcium but not of magnesium, iron and zinc in humans. Mechanism by which ingestion of non-digestible carbohydrates improves mineral absorption is not clear.

Effect of inulin on glycemia/insulinemia

The effect of inulin and oligofructose on glycemia and insulinemia are not yet fully understood, and existing data are contradictory, indicating that these effects may be due to physiological nature of the disease (Kaur and Gupta, 2002). Oku *et al*. (1984) revealed 17% and 26% reductions in postprandial glycemia and insulinemia respectively among rats after feeding by a diet containing 10% short-chain fructooligosaccharides (FOS) for 30 days. The reduction in glycemic response to saccharose or maltose is possibly due to reduction of disaccharidase activity in the gastro-intestinal tract. Luo *et al*. (1996) confirmed the previous results and showed that in diabetic rats, ingestion of a diet containing 20% oligofructose for 2 months decreased postprandial glycemia, despite a lack of modification of the glycemic or insulinemic response to a saccharose or maltose load. However, the results from some human studies are not consistent with above studies. For example, Yamashita *et al*. (1984) showed that in diabetic subjects, taking 8 g of FOS/day for 14 days in diabetic subjects led to a decrease in fasting blood glucose (Yamashita *et al*., 1984). When 10 g of artichoke inulin was added to 50 g of wheat-starch meal in healthy human subjects, the blood glycemic response was lower, despite no apparent interference by inulin on starch absorption (Rumessen *et al*., 1990). When rats are fed with 10% and 20% FOS in their diet for 6 weeks, the tests showed that mouth to anus transit time was shortened by 25 and 50%, respectively. This reduction in transit time confirms a dose-dependent effect (Oku *et al*., 1984), possibly similar to other dietary fibres; inulin and oligofructose

influence the absorption of macronutrients, especially carbohydrates, by delaying gastric emptying and shortening small-intestinal transit time.

Boillot *et al*. (1995) have demonstrated the reduced hepatic gluconeogenesis induced by inulin intake could be mediated by the short-chain carboxylic acids, especially propionate. Propionate given in the diet of rats for 4 weeks reduced fasting blood glucose and inhibited gluconeogenesis in isolated hepatocytes, probably via its metabolic conversion into methylmalonyl-coenzyme A (CoA) and succinyl-CoA, both of which are specific inhibitors of pyruvate carboxylase. In addition, propionate may also influence hepatic glucose metabolism indirectly by lowering plasma fatty acid concentration, a factor known to be closely related to gluconeogenesis (Lee *et al*., 1996).

Effect of inulin on lipid metabolism

Kaur *et al*. (1989) have shown that inclusion of inulin in the diet of saturated fat fed rats significantly reduced the high triglyceride content of blood and liver. Delzenne and Kok (1999) suggested that triacylglycerol (TG) lowering effect of oligofructose occurs via reduction in very low density lipoprotein (VLDL)-TG secretion from the liver as a result of the reduction in the activity of lipogenic enzymes, and in the case of fatty acid synthase via modification of lipogenic gene expression. Oligofructose decreased serum TG when it was included in the standard, fibre free or high fat diet of rats. Addition of oligofructose in a carbohydrate rich diet reduced the de novo liver fatty acid synthesis (Delzenne and Kok, 1999). Studies on incorporation of 14© acetate into TG in hepatocytes isolated from control and oligofructose-fed rats also supported the above results (Kok *et al*., 1996). Hepatic glycerol-3- phosphate concentrations were significantly higher in oligofructose fed rats than in controls. This relative increase in glycerol-3-phosphate content of the liver might be due to its decreased utilization for fatty acid esterification. Indeed, the administration of oligofructose slightly but significantly, reduced hepatocyte capacity to esterify 14 © palmitate into TG (Fiordaliso *et al*., 1995).

Prebiotics in food applications

Prebiotics are classified as "food products that contain prebiotic ingredients in sufficient concentration, so that after their ingestion, the postulated benefit is obtained" (Saxelin *et al*., 2003). The potential interest for using prebiotic in daily foods is mainly due to its low calorie value, hypocariogenic and bifidogenic properties, and dietary fibre effects (Roberfroid, 1993). Dairy products such as yoghurts, yoghurt drinks, spreads, fresh cheeses, and milk, and other emerging food products such as sport products, functional waters, nutrition bars, weight loss products, soymilk, and mineral supplements can be supplemented with prebiotics (Niness, 1999; Kaur and Gupta, 2002).

Bifidus promoting agents

Inulin-type fructans are amongst the well-established prebiotic ingredients. Their selective stimulation of the growth of bifidobacteria and the production of SCFA as end products of fermentation has been confirmed in many *in vitro* and *in vivo* studies (Gibson and Wang, 1994; Roberfroid, 2002). They are increasingly used in functional foods, especially dairy products and breads at typical amounts of 3-8 g per serving to allow the bifidogenic claims (Coussement, 1999; Franck, 2000).

Compared to inulin, other types of non-digestive oligosaccharides such as oligofructose are either branched or composed of several types of glycosidic bonds, which makes them less readily accessible for bacterial hydrolysis (Roberfroid, 1998). However, both inulin and oligofructose have been demonstrated to be effective prebiotics (Menne *et al*., 2000). The other commercially available prebiotic (Murphy, 2001; Cummings *et al*., 2001) and currently marketed as bifidus factor for infant formula is lactulose. Its ingestion contributes to the growth of gut microflora in bottle-fed babies in the same way as breast-fed babies (Strohmaier, 1998; Salminen *et al*., 1998).

Fibre enhancer

Another remarkable functionality of prebiotics in food formulations is their roles as a dietary fibre. The dietary fibre is defined as "remnants of plant cells resistant to hydrolysis by the human digestive enzymes" (Trowel and Burkitt, 1986). As discussed previously, several prebiotic substances such as inulin fall under this definition (Flamm *et al*., 2001). Moreover, from a physiologic point of view the effects of these prebiotics on intestinal function, blood lipid parameters, and caloric value meet the properties of dietary fibres (Roberfroid, 1993; Gibson *et al*., 1995). These effects are related to reduce the risk of coronary heart disease, colon cancer and other colonic disorders.

Compared to insoluble fibre such as bran, soluble prebiotic ingredients are more palatable and have greater functional properties (Dreher, 1999). Resistant starch is related to increased fibre content in baked goods and pasta products without any grainy appearance (Murphy, 2001). Supplementation

with inulin in baked goods allows not only fibre enrichment, but also better moisture retention properties and improved texture (Franck, 2000). Their solubility also allows fibre incorporation in drinks, dairy products, soup and table spread. Such additions are in the range of 3-6 g per serving and increase up to 10 g in extreme cases (Coussement, 1999).

Sugar replacer

Several types of non-digestive oligosaccharides and polyols can be used as sugar replacers due to their physiological characteristics such as having minimal contribution to energy intake and performing bulking properties (Frank, 2000).

Oligofructose delivers some functional properties similar to glucose syrup and is often used to replace sugar in various foods, mainly dairy and bakery products such as chocolate filling biscuits, chewing gums, confectionary, dairy desserts, and ice-cream (Franck, 2000). In addition, oligofructose depresses the freezing point of frozen desserts and acts as a binder in nutrition bars, in much the same way as sugar. The solubility of oligofructose is higher than sucrose but its sweetness is about 30% of sucrose (Angus *et al*., 2005). In combination with other sweeteners such as aspartame, oligofructose can provide a desired sweetness and better flavour profile (Weidmann and Jager, 1997; Kaur and Gupta, 2002).

Lactulose carries out some flavour enhancing properties similar to sucrose and therefore, it can be used to replace sucrose in some foods such as yoghurt, cookies and cakes (Schumann, 2002). However, because of its laxative characteristics, lactulose is often utilised in limited quantities in foods.

Sugar alcohols such as sorbitol, mannitol, xylitol, lactitol, and non-digestive oligosaccharides such as oligofructose and lactulose contribute fewer calories (1-2 kcal /g) than sugar (4 kcal /g) (Salminen *et al*., 1998; Murphy, 2001) allowing the development of sugar-reduced low-energy products. This is particularly true in sugar-free confections such as hard candies, chewing gums and marshmallows, sugar-free added baked goods and ice-creams. More importantly, these low-calorie ingredients offer advantages over traditional digestible carbohydrates such as sucrose, glucose and fructose in terms of having low glycemic index (especially helpful for those patients with diabetes, heart problems and obesity (Hidaka *et al*., 1986; Schumann, 2002).

Fat replacer

Specific kinds of prebiotics such oligosaccharides have been developed as fat replacers and texture modifiers as they would be able to 1) reduce total fat or partial fat content, 2) modify smoothness and creaminess, 3) increase perception of body and richness, 4) improve an overall eating quality and an acceptable appearance.

Inulin is well-recognized prebiotic for its ability to replace fat in the manufacturing of low-calorie foods (Silva, 1996; Franck, 2000). When inulin is mixed with water, it forms gels composed of a tri-dimensional gel network of insoluble sub-micron crystalline inulin particles with large amounts of immobilized water. This inulin gel provides the same texture and mouth feel as fat (Silva 1996; Franck 2000). The chain length of inulin plays a key role in gel quality. A high DP inulin facilitates gel formation at lower concentrations and can be formulated to replace fat up to 100%. Fat replacement by inulin is successfully applied in most water-based foods such as dairy products, frozen desserts, dressings, table spreads, sauces, soups and even meat products, but not in dry foods such as snacks, bakery and confectionery products (Murphy, 2001). Typically, 1 g of fat can be replaced by a 0.35 g of inulin in most foods (Coussement 1999). Formulating foods with inulin also helps to maximize freeze-thaw stability and minimize emulsion separation phased due to its ability to immobilize water and to work with most gelling agents such gelatin, gellan gum, and maltodextrin (Bishay, 1998). Inulin also gives a richer texture to liquid products and spreads and provides crispness and expansion to extruded snacks and cereals. In addition to inulin, resistant starch is also used as a fat mimetic and a texture enhancer in low-moisture foods e.g. crackers and cookie. In extruded cereals, the use of resistant starch improves crispness and expansion (Murphy, 2001).

Health effects of probiotics

Probiotic cultures are described as live microbial feed supplements that improve intestinal microbial balance and are intended for maintenance of health or prevention of disease (Fuller, 1991; Bruno and Shah, 2004). Probiotic bacteria are able to suppress potentially pathogenic microorganisms in the gastro-intestinal track and improve the balance in favour of beneficial microorganisms (Ibrahim and Bezkorovainy, 1993). The scientific evidence obtained through various studies on *Lactobacilli* and *Bifidobacterium* spp. has strengthened the positive effects of these micro-organisms on human health. Such examples are presented in Table 4.

It is noted that no strain provides all the proposed health benefits and strains of the same species often exhibit distinct effects, therefore, the health properties

of each strain need to be investigated independently (Doleyres and Lacroix, 2005). Strain of *L. rhamnosus* GG (Valio) is the most extensively studied probiotic in human clinical trials (Fonden *et al*., 2003), particularly involving in the management of rotavirus diarrhoea, and antibiotic-associated diarrhoea (Clostridium difficile). Strains of *L. acidophilus* NCFB 1748, B. lactis Bb 12, *L. plantarum* DSM9843 (299V), *L. reuteri* (BioGaia Biologics), *L. johnsonii* La-1 and *L. casei* Shirota (Yakault) are also well established for the clinical effects (Fonden *et al*., 2003; Shah, 2006).

Application of probiotics bacteria in functional foods

The demand for probiotic foods is increasing in Europe, Japan, Germany and the U.S, reflecting the heightened awareness among the public of the relationship between diet and health. Probiotics are widely used in dairy products, particularly yoghurts where the fermentation is often carried out with strains of *Lactobacillus* spp. and *Bifidobacterium* spp. (Fonden *et al*., 2003). In Germany, the mixture of *L. acidophilus* and *B. Bifidum* were introduced during the late 1960s for producing mildly acidified yoghurts, later known as "AB yoghurt" due to their expected adaptation to the intestine and the sensory benefits. Later the trend has been to incorporate *L. casei* in addition to *L. acidophilus* and *Bifidobacterium* as these strains are believed to act synergistically on each other.

Typical examples of other probiotic products available in the market are probiotic drinks including drinking yoghurts, fruit juices, fermented soy products, sour cream, buttermilk, ice-cream and frozen desserts, spread, cheeses, and milk powders (Lourens- Hattingh and Viljoen 2001). Currently, probiotic milk drinks are manufactured in various ways. The bacteria may be added without fermentation, so-called sweet milk or the milk is cultured with probiotic bacteria such as Yakault (Tamime *et al*., 2005).

Fermented milks containing *B. longum* or *B. breve* have obtained "foods for specific health uses" (FOSHU) approval in Japan (Champagne *et al*., 2005). In recent times, probiotics have also been marketed as dietary supplements consisting of freeze-dried bacteria, mainly *L. acidophilus* in tablet, capsule or powder form (Hamilton-Miller, 2005). Some of the commercial companies producing such dietary supplements include Blackmores Ltd. (Balgowlah, NSW, Australia), Probiotics International Ltd. (Stokesub- Hamdon, Somerset, UK) and Natren Inc. (Westlake Village, CA, USA).

Industrial interest in developing probiotic foods is driven by the market potential for foods that target health and well-beings. To date, over 100 bifidus- and acidophilus-containing products are available worldwide (Tamime *et al*., 2005). In Japan, probiotic-containing foods have been launched since the 1920s and more than 53 different types of milk products are estimated to be on the market (Shah, 2006). Using probiotic is largely restricted to the manufacturing of yoghurt in Europe and acidophilus milk in the USA. It was estimated that in 2000, Europeans spent $899 million on probiotic yoghurts and milks and on average the market share of probiotic yoghurts was ca. 10% of the total yoghurts (Stanton *et al*., 2001). In 2007, the Australian market for yoghurt and dairy desserts accounted for 17% of dairy category sales value with probiotic yoghurt a leader, growing up by 12 % (Dairy Australia, 2007).

Conclusion

As different studies have suggested, combining probiotics and prebiotics in what has been called a synbiotic could beneficially affect the host by 1) improving survival and implantation of live microbial dietary supplements in the gastro-intestinal flora, 2) selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the GIT, and 3) improving the gastro-intestinal tract's microbial balance. However, the creation of a synbiotic food has not been investigated. Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of the gastro-intestinal tract, thus enhancing their effects in the large bowel. Moreover, probiotic and prebiotic effects might be additive or even synergistic. This has been the case when combining the anti-carcinogenic effects of inulin and bifidobacteria in experimental animals.

A large number of researches have focused on the production and bifidogenic effects of inulin and less on synbiotic foods. Hence, further research is required to focus on the combination of prebiotics and probiotics in development of new dairy products on and the task for assessment of the viability of commercial probiotic cultures in the presence of these prebiotic compounds. The emergence of new analytical techniques such as metabolite profiling has revealed new pathways affected by dietary intervention. However, an important challenge for current and future research is to relate changes in bacterial metabolism to concrete health benefits. Potential targets and expected benefits have been identified: reduced risk for the metabolic syndrome and prevention of colorectal cancer.

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