

Inulin: therapeutic potential, prebiotic properties and immunological aspects

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The protective influence of dietary components on diseases development is a topic of major interest. Identification of such dietary components, understanding of their mechanisms of action as well as their development and use in human diet are some of the objectives of functional food science. Inulin oligosaccharides are among the substrates considered as prebiotic for their non-digestible carbohydrate properties often found in many vegetables, fruits and cereals. There is convincing data to suggest that consumption of prebiotics such as inulin can modulate immunological parameters in gut-associated lymphoid tissues, microflora and may present potential health implications in protection against colon diseases. This review shows the prebiotic properties, therapeutic potential and immunological aspects of inulin.

Keywords: inulin; prebiotics; colon disease; immunity

Introduction

Prebiotics are a category of nutritional compounds that selectively promote selectively the growth of bacteria known to confer health benefit to the host. The potential health benefits of prebiotics have been investigated, with special interest in the effects on the immunological system, the host's ability to fight infection and inflammatory processes and conditions (Lomax & Calder, 2009). Substrates considered prebiotics include the oligosaccharides inulin, fructooligosaccharides (FOS), galacto-oligosaccharides (GOS) and lactulose (Sherman et al., 2010).

Inulin-type fructans containing prebiotics form a category of nutritional compounds naturally occurring in plants, in which one or more fructosyl–fructose linkages comprise the majority of glycosidic bonds. Inulin and its derivative compounds (oligofructose, FOS) are usually called fructans because they are essentially based on linear fructose chains (Marteau et al., 2011) and are present in significant amounts in several fruits and vegetables (Van Loo, Coussemant, de Leenheer, Hoebregs, & Smits, 1995).

Chemically, inulin-type fructans are a linear polydisperse carbohydrate material consisting mainly, if not exclusively, of β -(2-1)-fructosyl-fructose glycosidic bond

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linkages (Liu, Waterhouse, & Chatterton, 1993), which gives inulin their unique structural and physiological properties, allowing them to resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes (Kelly, 2008). Fructans are proposed to be classified as “functional fiber” according to recent concepts drawn from physiological effects on human individuals (Madriral & Sangronis, 2007).

The average daily consumption has been estimated to be between 3 and 11 g in Europe (Van Loo et al., 1995) and between 1 and 4 g in the USA (Moshfegh, Friday, Goldman, & Ahuja, 1999). The most common sources are wheat, onion, banana, garlic and leek.

Inulin is produced industrially from chicory root (*Cichorium intybus*) and it is widely used as ingredient in functional foods. Clinical research has led to discussion about inulin-type prebiotics, including effects on infant nutrition, gastrointestinal health, bone mineralisation, fatty liver disease, obesity, blood sugar and lipid metabolism, colon cancer prevention and immunity (Kelly, 2009).

The impact of 15 g chicory native inulin daily consumption on fecal levels of bifidobacteria, stool parameters and quality of life of elderly constipated volunteers was investigated in a randomised, double-blind, controlled versus placebo clinical trial. Inulin supplementation led to a significant increase in total fecal bacteria and bifidobacteria concentrations after 28 days of consumption. Daily supplementation of 15 g of inulin improved constipation and quality of life in an elderly sample with constipation (Marteau et al., 2011).

Nutritional status of populations living in the developed countries is affected by poor habits, such as excessive consumption of fats, especially of the saturated, high intake of sugars and considerable reduction in the consumption of starch, fibre, vitamins and minerals that may be causing the high incidence of chronic diseases in such countries (Silva, Engstrom, & Zaborowski, 2002).

Parallel to this phenomenon is an accelerated development of foods having, in addition to adequate technological and nutritional characteristics, components that exert biological functions to prevent disease and promote health functional foods, especially prebiotics and probiotics (Roberfroid, 1999). Given this context, several studies have called attention to the health benefits of prebiotics, including inulin (Fuchs, Borsato, Bona, & Hauly, 2005).

Food is one of the determining factors in population health and its levels express “the social and economic organization of the country” (Brasil, 2011).

This review discusses the prebiotic properties, therapeutic potential and immunological aspects of inulin.

Inulin as prebiotics

Probiotics are defined as microorganisms in sufficient amounts that reach the intestine in an active, viable state and exert positive health effects. *Prebiotics* are “selectively fermented ingredients that allow specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health” (Macfarlane, Steed, & Macfarlane, 2008).

Only bifidogenic and non-digestible oligosaccharides (particularly inulin and its hydrolysis product oligofructose and trans-galactooligosaccharides) selectively promote the growth of certain types of bacteria, for example, Bifidobacteria (Gibson, Beatty, Wang, & Cummings, 1995) and fulfil all the criteria for prebiotic

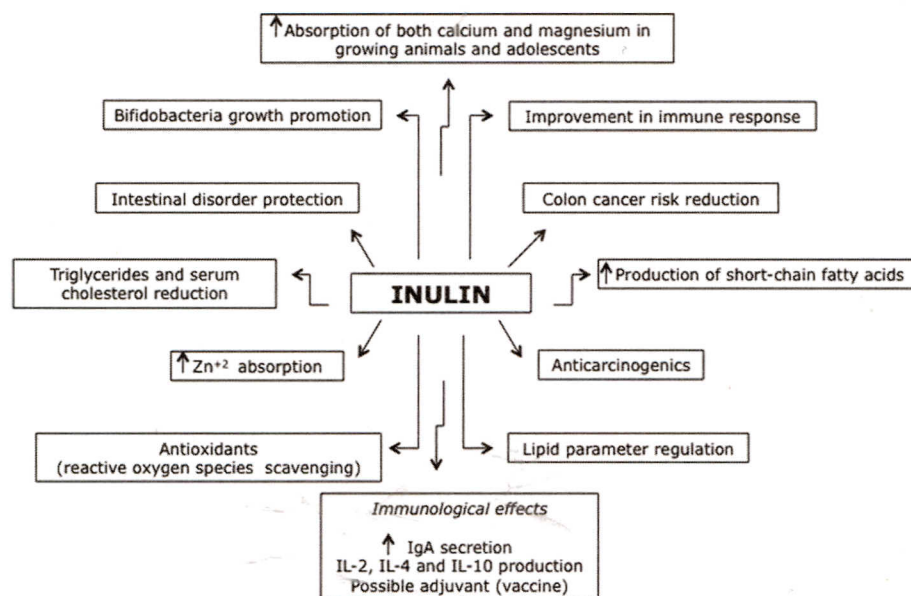


Figure 1. Schematic overview of the biological effect of inulin in humans.

classification. It is also known that the prebiotic factor is able to stimulate the growth of bifidobacteria.

Prebiotics include dietary fibres with a well-established positive impact on the intestinal microflora (de Vrese & Schrezenmeir, 2008). Inulin is a natural prebiotic which produces many desirable effects in humans, including increased production of short-chain fatty acids, growth and activity of bifidobacteria, reduced triglycerides and serum cholesterol and reduced risks of colon cancer and intestinal infections (Meyer & Stasse-Wolthuis, 2009; Ramirez-Farias et al., 2009).

Prebiotic fibres likely have unique modes of action with respect to cholesterol metabolism, specifically on fermentability and microflora modulation (Parnell & Reimer, 2010).

Some studies have shown both probiotics and prebiotics (non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of resident colonic bacteria) to suppress preneoplastic lesions and tumours in the colons of animals treated with chemical carcinogens (Rafter et al., 2007).

The predominance of beneficial bacteria in gut microflora of breast-fed infants is thought to be, at least in part, supported by the metabolism of the complex mixture of oligosaccharides present in human breast milk, as a more adult-type intestinal microbiota can be achieved in formula-fed infants. Inadequate gut colonisation (*dysbiosis*) may lead to an increased risk of infectious, allergic and autoimmune disorders in later life (Sherman et al., 2010).

Prebiotics such as inulin-type fructans and alternative natural sweeteners have also become more popular as food ingredients. Accumulating evidence suggests that carbohydrates and carbohydrate-containing biomolecules can be considered true antioxidants, capable of scavenging reactive oxygen species (ROS). It has been found

that inulin and stevioside are superior scavengers of both hydroxyl and superoxide radicals, being more effective than mannitol and sucrose (Stoyanova, Geuns, Hideg, & Van Den Ende, 2010).

Waligora-Dupriet et al. (2007) verified that bifidobacteria, tended to increase slightly with oligofructose supplementation when compared with placebo. Simultaneously, a decrease in potential pathogens, significant for *Clostridia* but not for *Staphylococcus*, was observed in the oligofructose group. Oligofructose supplementation was accompanied by less flatulence, diarrhoea, vomiting and fever events. Related to this, it has been reported by Kapiki et al. (2007) that infant formula containing a small quantity of prebiotic oligosaccharides is well accepted and leads to rapid growth of bifidobacteria in the gut of bottle-fed preterm infants while decreasing the number of pathogenic microorganisms. Likewise, results from Martin et al. (2008) provide evidence for the potential use of prebiotics for modifying the gut microbial balance, as well as host energy and lipid homeostasis beneficially.

Influence of prebiotics during childhood

During and following birth, the sterile gastrointestinal tract of newborn infants becomes inoculated and colonised with bacteria derived from the mother and the environment (Fanaro, Chierici, Guerrini, & Vigi, 2003). As prebiotics, inulin-type fructans act essentially via a modification of the endogenous intestinal microflora, modified microflora directly or indirectly cause most, if not all, of their effects (Roberfroid, 2007).

Euler, Mitchell, Kline, and Pickering (2005) proposed a study to determine the prebiotic effect of infant formula supplemented with fructo-oligosaccharides. They verified that infant formula supplemented with 1.5 or 3.0 g/L fructo-oligosaccharides was safe, but had a minimal effect on fecal flora and *Clostridium difficile* toxin.

Kapiki et al. (2007) investigated the effect of a fructo-oligosaccharide supplemented formula on gut flora of pre-term infants. They verified that infant formula containing a small quantity of prebiotic oligosaccharides is well accepted and leads to rapid growth of bifidobacteria and decreased numbers of pathogenic microorganisms in the gut of bottle-fed pre-term infants.

Infant formulas supplemented with inulin and FOS diets were studied clinically and *in vitro* and were compared with mature breast milk. There were no significant differences in the fecal numbers of lactobacilli, total aerobes, anaerobes, yeasts and fungi. In contrast, bifidobacteria numbers in the stools of infants receiving the supplemented formula increased significantly during the study. The comparative *in vitro* test showed that a bifidogenic effect was similarly found in experiments using infant formula and breast milk in terms of bifidobacteria number. Consumption of infant formula with added inulin and FOS stimulated the bifidogenic effect both clinically and *in vitro* (Lugonja et al., 2010).

According to Gregory (2008), fructans are a category of nutritional compounds that encompasses naturally occurring plant oligo- and polysaccharides in which one or more fructosyl-fructose linkages comprise the majority of glycosidic bonds. "Inulin-type" fructans must have beta (2-1) fructosyl-fructose glycosidic bonds, which give inulin its unique structural and physiological properties.

Daily consumption of a combination of prebiotic inulin-type fructans significantly increases calcium absorption and enhances bone mineralisation during

pubertal growth. Effects of dietary factors on calcium absorption may also be modulated by genetic factors including specific vitamin D receptor gene polymorphisms (Abrams et al., 2005). In humans, the most convincing data to date have been obtained in adolescents (Griffin, Davila, & Abrams, 2002; Griffin, Hicks, Heaney, & Abrams, 2003; van den Heuvel, Muys, van Dokkum, & Schaafsma, 1999).

Calcium supplementation has been shown to increase bone mineralisation in children and adolescents. Genetic and lifestyle factors can be used to guide optimal calcium intake during childhood (Abrams, 2005).

Inulin in colon disease

Most studies involving prebiotic oligosaccharides have been carried out using inulin and its fructooligosaccharide derivatives together with various forms of GOS. Although many intestinal bacteria are able to grow on these carbohydrates, most investigations have demonstrated that growth of bifidobacteria, and to a lesser degree lactobacilli, is particularly favoured. Even though literature dealing with the health significance of prebiotics is not as extensive as that concerning probiotics, considerable amount of research supports the notion that consumption of GOS and fructooligosaccharide can have significant health benefits, related to mineral absorption, lipid metabolism and anti-inflammatory and immunological effects and anti-cancer properties (Macfarlane et al., 2008).

Colon cancer is, by definition, a genetic disease of colonial epithelial cells in which accumulated epigenetic and/or genetic mutations promote hyperplasia and dysplasia that result in cancer (Markowitz & Bertagnoli, 2009). Certain dietary fibres were found to be factors preventing the start and, possibly, the promotion of carcinogenesis. These products were classified as *anticarcinogens* (Wattenberg, 1992).

The impact of specific dietary components on colon tissue probably depends on a host of genomic processes which influence growth, development and differentiation of the epithelial cells in the colon crypt surface. The expanded use of high-throughput technologies will contribute to the understanding of gene expression patterns and protein fingerprints and ultimately elucidate the physiological significance of bioactive food components in cancer protection (Kim & Milner, 2007).

Among the earliest detectable neoplastic lesions in the colon are the aberrant crypt foci (ACF). ACF lesions may be identified by highmagnification-chromoscopic-colonoscopy which otherwise normally appear as colonic mucosa. They consisted of crypts that are microscopically elevated above the normal mucosa, are composed of excessively thickened epithelia and have altered luminal openings clearly circumscribed from the normal adjacent crypts. Although the progression of the ACF to polyp, adenoma and adenocarcinoma parallels the accumulation of several genetic and biochemical alterations, only a small fraction of ACF evolve to colon cancer. Currently, it is not clear which crypts develop into tumours and which do not. However, many studies support the concept that the formation of ACF precedes the development of colon cancer (Alrawi et al., 2006).

In studies reported by Pool-Zobel (2005), some fibres which promoted a stable butyrate-producing colonic ecosystem decreased the rate of ACF, thus adding to the line of evidence that a stable butyrate producing colonic ecosystem, reduces risks of developing colon cancer.

The study done by Hijova, Chmelarova, Bomba, and Zitnan (2009) evaluated the effects of prebiotics in chemically induced carcinogenesis using Wistar albino rats fed a high-fat diet. The study showed that prebiotics may provide potential protection against colon cancer. Munjal, Glei, Pool-Zobel, and Scharlau (2009) prepared a fermentation supernatant fraction of inulin and studied biological properties in human colon cell lines, LT97 and HT29 (representing early and late stages of colon cancer, respectively). The results indicated growth-inhibiting and apoptosis-inducing effects in fermentation supernatant fractions containing inulin. There are potential implications for chemoprevention, as early adenoma cells (LT97) were found to be more sensitive to the growth-inhibitory than late stages of colon cancer (HT29) cells. Inulin-induced protection against colorectal cancer was observed. Probiotic therapy can be potentially improved through combination with a Prebiotic, known as a synbiotic, that promotes growth of the probiotic in the large bowel. Several colorectal cancer biomarkers can be altered favourably by synbiotic intervention (Rafter et al., 2007). Furrer et al. (2005) have verified that consumption of synbiotic twice daily over 4 weeks significantly reduced mucosal inflammatory markers in active ulcerative colitis. This was concurrent with a reduction in colitis at the macroscopic and microscopic levels. A synbiotic was developed for use in ulcerative colitis patients combining a probiotic, *Bifidobacterium longum*, isolated from healthy rectal epithelium, and a prebiotic, a preferential inulinoligofructose growth substrate for the probiotic strain. Tumour Necrosis Factor (TNF alpha) and interleukin 1 (IL-1), which are inflammatory cytokines that drive inflammation and induce defensin expression, were also significantly reduced after treatment.

"Synergyl" is a prebiotic composed of inulin and oligofructose in equal proportion. Gourineni, Verghese, Boateng, Shackelford, and Bhat (2011) showed the chemopreventive potential of Synergyl and soybean in reducing azoxymethane-induced ACF in Fisher 344 male rats.

Although statistical power was limited by the relatively small sample size in a study, Limburg et al. (2011) do not provide convincing evidence of colorectal cancer risk reduction from 6-month interventions with atorvastatin, sulindac or oligofructose-enriched inulin.

Another study suggests that in addition to the number, size and growth rate of adenomatous polyps, the signalling pattern of adenomas should be considered when evaluating preventive dietary strategies (Misikangas et al., 2008). There is also considerable evidence that inulin modulates parameters within the risk group of colon cancer cells in humans and animals. These include suppression of tumour cell survival and reduced exposure to other risk factors (Pool-Zobel & Sauer, 2007).

Because Crohn's disease and ulcerative colitis, also called chronic inflammatory bowel diseases, result from a combination of genetic, environmental and immunological factors, inulin may promote beneficial effects. In light of the efficacy of providing probiotic bacteria to patients with inflammatory bowel diseases, there has been interest in the prophylactic and therapeutic potential of prebiotics that are easily administered and do not require large numbers of live bacteria. Studies using beta-fructan oligosaccharides for the treatment of chronic intestinal inflammation have shown benefit in animal models of colitis (Leenen & Dieleman, 2007).

Further studies are required to elucidate which mechanisms are essential in the tumour inhibiting and/or anticarcinogenic effects of non-digestible carbohydrates.

This information will enable their introduction as food compounds lead to reducing the risk of cancer.

Immunological aspects of inulin

Diet is known to modulate immunological functions in multiple ways and to affect host resistance to infections. Besides the essential nutrients, non-essential food compounds such as non-digestible carbohydrates may also have an impact on the immunological system, especially the gut-associated lymphoid tissue (GALT). Boder (2008) reviewed the research done on the immunity-enhancing effects of prebiotics and noted that there is enough evidence showing prebiotics such as inulin modulating immunological functions to suggest that the consumption of prebiotics can modulate immunological parameters in GALTs, secondary lymphoid tissues and peripheral circulation.

The innate immune system acts as a first line defence by preventing entry of infectious agents or eliminating invading pathogens. It comprises physical barriers such as skin or mucous membranes, cells in blood and tissue, such as phagocytes, natural killer (NK) cells and soluble mediators, such as complement proteins and cytokines. In animal studies, inulin primarily activates immunological cells in Peyer's patches including IL-10 production and NK cell cytotoxicity. Other immunological functions modulated by inulin include IgA signalling in the ileum and caecum, splenic NK cell cytotoxicity and splenocyte cytokine levels. Results from Watzl, Gierbach, and Roller (2005) propose that inulin primarily modulates immunological parameters in the GALT (but splenocytes are also activated).

Human studies are needed to find out whether inulin has the potential to modulate systemic immunity in well-nourished individuals and to lower the risk of diseases such as colon cancer. It is known that prebiotics may increase zinc (Zn^{2+}) absorption, an ion known to play a central role in the immunological system. Zinc-deficient states are characterised by suppressed immunological function, while prebiotics may improve both gut and cell-mediated immunity. Ryz, Meddings, and Taylor (2009) proposed that the higher proportion and number of dendritic cells in Peyer's patches of inulin-fed rats indicate a need for further research on how prebiotics and their metabolites affect immunological function of intestinal dendritic cells action.

Further studies with pro- and prebiotics in small children in the developing and developed countries are necessary to elucidate their influence on health in general and the cellular and humoral immune status, in particular. In this context, Haschke, Firmansyah, Meng, Steenhout, and Carrié (2001) showed that after vaccination with measles vaccine IgG antibody titres were higher in the group receiving the prebiotic mixture.

Benyacoub et al. (2008) investigated the effects of inulin mixtures in the murine response to *Salmonella* vaccine and its relevance towards protection. Peritoneal macrophage phagocytic activity was significantly increased in FOS-inulin-fed mice compared with control. No detectable effects were observed on the percentage of lymphoid cell subsets in the spleen. However, production of cytokines, interferon-gamma, interleukin-12 and tumour necrosis factor-alpha, was numerically increased in spleen cell cultures stimulated with mitogens from FOS-inulin-fed mice at post-immunisation.

The increase in interleukin-2 and interleukin-4 measured in blood was also noted in rats fed with inulin and oligofructose. The increase in activity of peritoneal macrophages was confirmed by enhanced superoxide anion (O_2^-) evaluation and phagocytosis, and immunological-enhancing effects of prebiotics were confirmed by morphological investigation of peripheral lymphoid organs (Trushina, Martynova, Nikitiuk, Mustafina, & Baĭgarin, 2005).

A challenge to the innate immune system often leads to the activation of the adaptive immunological system. This system consists of two major cell types, the T- and B-lymphocytes, which enable the specific recognition of and response to invaders. T-lymphocytes develop in functionally different cell types with specific cytokine patterns. The cellular immunological response (Th1 pattern) consists mainly in production of IFN- γ and IL-2, while the humoral immunological response (Th2 pattern) shows high production of IL-4 and IL-6 (Watzl et al., 2005). The use of a mixture containing galacto- and FOS in dietary compounds might provide an opportunity to stimulate the adaptive immunological response towards the Th1 pattern, inhibiting infections subsequently and to modulate Th2-related immunological disorders in humans, such as allergies (Vos et al., 2006).

Another important immunological point is that despite better tolerability, pure soluble, recombinant and synthetic antigens are often much less immunogenic than live or killed whole organism vaccines. Thus, the development of safer and more potent vaccines has created a need for better adjuvants, in particular, adjuvants capable of boosting the cellular (Th1) immunological response, with acceptable toxicity.

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 (Figure 1). The inulin could be directly influencing immunological cells with access to the epithelial surface by different cytokine pathways and stimulating a more immunomodulatory and tolerant immune response. Although further studies are needed to elucidate its potential to modulate systemic immunity and prevent chronic diseases such as colon cancer, inulin promises to be an effective and functional food.

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