Analysis, Health Benefits and Applications of Prebiotics: A Review

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Received: 30 November, 2014 ***Revised: 17 December, 2014*** ***Accepted: 21 December, 2014***

ABSTRACT

Prebiotics are polysaccharides and oligosaccharides that can withstand digestion and absorption in the small intestine, but can be selectively fermented by probiotic bacteria native to the large intestine. Over the past 15 years, a wealth of information has been gathered on prebiotics in order to understand the mechanism of actions and elucidate their beneficial health effects to the human host.

The present review sheds a light on the optimum conditions for prebiotic extraction. Moreover, determination methods of prebiotics were reviewed. Such methods include: resistance to acidic and enzymatic digestion-HPLC and NMR.

This review also focused on health benefits of prebiotics in terms of their roles as anticarcinogenic, antimicrobial, uridic, antihyperglycemic and antiosteoporrotic agents along with their stimulatent activity.

Applications of prebiotics in food industry were also reviewed. These applications include dairy, frozen desserts, fruit preservation, infant milk formulas and other food products.

***Keywords**: extraction, determination, HPLC, NMR, health benefits, applications, dairy, desserts, fruit preservation.*

INTRODUCTION

As a matter of fact, the basic concepts of nutrition are undergoing a significant change. In other words, the classical concept of "adequate nutrition that is, a diet that provides nutrients in sufficient quantities to satisfy particular organic needs, is being replaced nowadays by the concept "Optimal nutrition" which includes, besides nutrients, the potential of food to promote health, improve, general well being and reduce the risk of developing certain illness. This is where functional foods, including prebiotics, play their part (Nagi & Inoue, 2004, Grajek *et al.,* 2005, Ramadan & Al-Ghamdi, 2012, Coles, 2014).

The concept of prebiotics was introduced by Gibson & Roberfoid (1995) as an alternative approach to the modulation of the gut microbiota.

Over the past 15 years, a wealth of information has been gathered on prebiotics in order to understand the mechanism of actions and elucidate their beneficial health effects to the human host (Charalampopoulos & Rastall, 2012, Anandharai, *et al*., 2014).

Sources and chemical nature of prebiotics

Prebiotics are defined as non-digestible food ingredients that selectively stimulate the growth and/or the metabolism of health promoting bacteria in the intestinal tract, thus improving an organism's intestinal balance (Gibson & Roberfroid, 1995). The health-promoting bacteria most commonly augmented by prebiotics include those of the genus *Lactobacillus* and *Bifidobacter*, which tend to limit the presence of harmful bacteria.

The main candidates for prebiotics status are shown in Table (1).

Some prebiotics occur naturally in several foods. Prebiotic carbohydrates are found naturally in some fruits and vegetables as raw Jerusalem artichoke, raw dandelion greens, raw garlic, raw leek, raw onion, tomatoes, asparagus, bananas, and berries. It is also found in many grains include wheat, oatmeal, barley, whole wheat flour, whole grain foods such breads and cereals and legumes (lentils, kidney beans, chickpeas, white beans and black beans) (Jackson, 2010, Moongngarm, *et al*., 2011, Sharma, *et al*., 2011). Soy beans and products made from soybeans such as tofu are also an excellent source of prebiotics (Marie, 2010).

While many nutritional compounds have some degree of prebiotic activity, Roberfroid (2000) identified two groupings of nutritional compounds, inulin-type prebiotics and galactooligsaccharides (GOS).

Table 1: Properties of common non-digestible oligosaccharides

Name	Composition	Method of manufacture	Dp
Inulin	β(2-1) fructans	Extraction from chicory root	11-65
Fructo-oligosaccharides	β(2-1) fructans	Transfructosylation from sucrose,	2-10
		or hydrolysis of chicory inulin	3-5
Galacto-oligosaccharides	Oligo-galactose (85%), with some glucose and lactose	Produced from lactose by β-galactosidase	2-5
Soya-oligosaccharides	Mixture of raffinose (F-Gal-G) and stachyose (F-Gal-Gal-G)	Extracted from soya bean whey	3-4
Xylo-oligosaccharides	β(1-4) linked xylose	Enzymic hydrolysis of xylan	2-4
Pyrodextrins	Mixture of glucose-containing oligosaccharides	Pyrolysis of potato or maize starch	Vario
Isomalto-oligosaccharides	α(1-4) glucose and branched		
	α(1-6) glucose	Transgalactosylation of maltose	2-8

Dp: degree polymerization; F: fructose; Gal: galactose; G: glucose
Ref. Gibson & Fuller (2000).

Other nutritional compounds suggested as prebiotics, but not included as prebiotics, include gentiooligosaccharides, glucooligosaccharides, isomaltooligosaccharides, mannanooligosaccharides, N-acetylchitoolgosaccharides, oligosaccharides from melibiose, pectic oligosaccharides, xylooligosaccharides, gums (like gum Arabic), hemicellulose-rich substrates, resistant starches (such as resistant maltodextrin), lactosucrose, oligodextrans, polydextrose, germinated barley, gluconic acid, glutamine, lactose, and the simple sugar tagatose (a mirror image of fructose). Because research on several of these compounds for prebiotic activity is promising, it is possible that in the future one or more of these compounds might also meet the criteria specified in Roberfroid's definition of prebiotics, (Gibson & Fuller, 2000).

Analysis of prebiotics

Numerous research papers have been conducted on prebiotics in order to optimize and thereby maximize the extraction recovery. Meanwhile, different analytical methods were applied to determine prebiotics. In this section, the most abundant methods for extracting and determining prebiotics in foods will be explained:

a- Extraction of prebiotics:

The extractability of prebiotics is influenced by many factors, such as type of solvent, particle size of the extractant materials, sample to solvent ratio, agitation velocity, temperature and time of extraction (Wichienchot *et al.,* 2011).

The response surface methodology (RSM) was applied by Bhornsmithikum *et al.* (2010) for experimental design to study the extraction conditions of prebiotics from Jackfruit seeds. Such conditions included temperature (40-60°C), extraction time (15-45 min) and liquid to solid (L/S) ratios (6:1-10:1) at laboratory scale continuous extraction. The extraction efficiency was based on the extraction yield and the amount of non-reducing sugar, which is expected to be prebiotics. The optimum conditions for the extraction of the prebiotics were as follows: Extraction time of 15 min at 60°C using 50% ethanol as a solvent, L/S ratio 10:1 (w/v) which gave the maximum non-reducing sugar content of 491.70 mg/g extract from RSM modeling. The aforementioned optimum conditions were applied for pilot scale continuous extraction.

Notwithstanding, the optimal conditions of prebiotics extraction from Jackfruit seeds were: 50% ethanol solvent, particle size of a seed 1.0-2.0 mm and a solid to solvent ratio of 1:8. It was obvious that extraction time longer than 180 minutes did not increase the yield of prebiotics significantly, and that Jackfruit seeds extracted at 60°C only had slightly higher percent yield of prebiotics compared to the 30°C. Furthermore, the lower extraction temperature produced a higher yield of non-reducing sugars (Yamasaengsung *et al.,* 2009). Those authors designed a batch-scale unit for extracting prebiotics from different plant foods. To concentrate the extract, solution was filtered by vacuum filter (SIBATA: Circulating Aspirator WJ-20) and then evaporated by rotary vacuum evaporator (Buchi: Vacuum pump V-700). The concen-

trated solution was heated at 80°C for 10 min and temperature was lowered to 58°C at 1°C/ min rate and mixing speeds of 100 rpm for crystallization (Rugwong *et al.,* 2011).

Working on thirteen plants and their parts acquired from Southern Thailand, Wichienchot *et al.* (2011) explored the following extraction conditions to improve the yield of the extractant prebiotics: Ethanol (50%) as a solvent, particle size of 5 mm diameter, sample to solvent ratio 1: 2, agitation at 200 rpm, room temperature and 60-150 minute extraction time.

Table (2) shows the optimum conditions of extraction process of prebiotics from different plant foods. It is worth to note the diversity of extraction time (15-180 min), L/S ratio (2:1 – 10:1) and particle size (1-5 mm). Consequently, it is not easy to figure out optimum conditions for extracting prebiotics that are applicable to all food-stuffs.

b - Determination of prebiotics

Prebiotic extracts can be analyzed for their prebiotic properties by different analytical methods. The main methods in this respect can be reviewed under the following four main headings.

(1) Resistance to acidic and enzymatic digestion

Dried extracts of prebiotics are made into 10% solutions (w/v) with distilled water. For acidic digestion, each solution is incubated at 37°C with HCl buffer at pH 1 for 4 hrs. (Korakli *et al.,* 2002). The reaction is terminated with 1N NaOH. For enzymatic digestion, the acid-digested solutions are further incubated at 37°C with 2 unit/ mL human pancreatic α-amylase in phosphate buffer solution (20 mM) at pH 6.9 for 6 hrs (Doyle *et al.,* 1999, Wichienchot, 2010). The enzymatic digestion is terminated by heating at 80°C for 10 min. Resultant sugars after acid and enzymatic digestion of prebiotics can be determined by colorimetric methods.

To determine the amounts of indigestible polysaccharides in the extracts, they are first analyzed for their reducing sugar content (mg/g). The digesta are then analyzed for total sugar content (mg/g). The indigestible polysaccharide content (mg/ g dry extract) is calculated as follows:

Indigestible polysaccharides (mg/g) = Total sugar after acid – enzyme digestion (mg/g)-Reducing sugar (mg/g) before the digestion (Wichienchot *et al.,* 2011).

(2) Determination of sugars by HPLC:

Methods of Tieking *et al.* (2005) and Schwab &Ganzle (2006) were modified by Wichienchot *et. al.* (2011) to determine sugars in prebiotic extracts after acidic and enzymatic digestion by HPLC. The operational conditions are as follows:

Column: Agilent Zorbax LC-NH_2 4.6 mm × 250 mm, 5 μm.
Mobile phase: Acetionitrile: Water at 75: 25.
Flow rate: 1 mL/ min.
Temperature: Ambient
Dedicator: Refractometer (RI).
Standard sugars: D-glucose, D-fructose and Sucrose.

The amounts of indigestible polysaccharides in extracts are calculated as mentioned previously.

Notwithstanding, the HPLC method was used to determine inulin preparations in fermented cabbage juice (Simonova *et al.,* 2010). The HPLC method with enzymatic pre-treatment was applied. At the first, inulin in the samples was decomposed to fructose by enzyme system fructozyme L. It was found that the enzyme in the fermented cabbage

Table 2: Extraction conditions of prebiotics from different plant foods

Food	Extraction Conditions						Reference
	Solvent	Particle size (mm)	L/S* ratio	Temperature (°C)	Time (min)	Agitation (rpm)	
Jackfruit seeds	50% EtOH	1-2	8:1	60	180	--	Yamsaengsung *et al.* (2009)
Jackfruit seeds	50% EtOH	--	10:1	60	15	-	Bohrnsmithikum *et al.* (2010)
Different thirteen plant foods	50% EtOH	5	2:1	Room	60-150	200	Wichienchot *et al.* (2011)

* L/S ratio: Liquid / Solid ratio.

juices completely degraded inulin preparation at pH 4.5, room temperature (24°C) for 25 min. In the next step, the fructose released during inulin hydrolysis was determined by HPLC under the aforementioned operational conditions.

(3) Determination of molecular weight distribution in the extracts by gel permeation chromatography (GPC).

In this method, monosaccharides are first removed from the prebiotics extract by precipitating with 80% ethanol (EtOH) twice. Gel permeation chromatography (GPC) is applied for MW analysis. According to Wichienchot *et al.* (2011), the freeze dried extracts are dissolved in 0.1 M $NaNo_3$ to the concentration of 0.1% (w/v). The solutions are filtered throught nylon syringe filter 0.2 mm before 20μL samples are injected into the GPC (Polymer Laboratories, England). The proper column is Ultrahydrogel Linear (Water, USA) at a column temperature of 30°C, flow rate of 0.6 mL/min, with RI detector. Pullulons are used as standards for MW comparison and PL logical GPC software (England) can be used to analyze the results.

(4) Determination of specific prebiotics by NMR:

For the analysis of α-glucooligosaccharides (in particular isomaltooligosaccharides IMOs), the full and unambiguous as signment of ^{1}H and ^{13}C chemical shifts of standard molecules has been completed through the use of various ^{1}D and ^{2}D NMR - experiment.

Indeed, structural characteristics such as anomeric configuration, substitution pattern, as well as the diastereotopic effect on ^{13}C chemical shifts giving specific deviations useful for unambiguous structure determination. These specific deviations will represent key tools for further structural determination of unknown α-D-glucooliqosaccharides such as IMOs (Goffin *et al.,* 2009).

Figure (1) shows the procedures of prebiotic determination.

Molecular weight distribution can be applied directly to prebiotics extract by means of gel permeation chromatography (GPC). It is true also for determining α-D glucooligosaccharides by means of NMR which can be conducted directly on prebiotics extract.

In contrast, applying different analytical methods to determine sugars requires acidic and enzymatic digestion of the first prebiotics extract.

Health benefits of prebiotics

For a food ingredient to be classified as a prebiotic, it must fulfill the following:

1- Neither to be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.
2- Be selectively fermented by one or a limited number of potentially beneficial bacteria consumed to the colon.
3- Prebiotics must be able to alter the colonic microflora to a healthier bacterial flora.
4- It should be capable of inducing a physiological effect that is beneficial to health (Kolida *et al*., 2002, Caselato de Sousa *et al*., 2011).

Prebiotics may exhibit the following properties:

- Maintenance of intestinal flora and stimulation of intestinal transit.
- Change in colonic mircoflora, contributing to normal stool consistency, preventing diarrhea and constipation.
- Elimination of excess substances such as glucose and cholesterol.
- Stimulation of the absorption and production of B vitamins.
- Contribution to the control of obesity and decrease the risk of osteoporosis (Kaur & Gupta, 2002, Manning & Gibson, 2004, DeVrese &Schrezenmeir, 2008, Anandharai *et al*., 2014).

In the past decade, a large number of studies investigated the health promoting effects of prebiotics. Although some of the postulated effects have not been fully demonstrated, the data suggest clinically significant effects that warrant further study and explanation (Cummings & Macfarlane, 2002). The postulated beneficial effects of prebiotics are summarized below (Jackson, 2010, Sharma *et al.* 2011, 2012).

1- Anticarcinogenic activity.
2- Antimicrobial activity.
3- Hypotriglyceridic activity.
4- Antihyperglycemic activity.
5- Immunostimulant activity.
6- Antiosteoporotic activity.
7- Improving mineral absorption and balance.
8- Ridding the gut of harmful microorganisms.
9- Help prevent constipation and diarrhea.

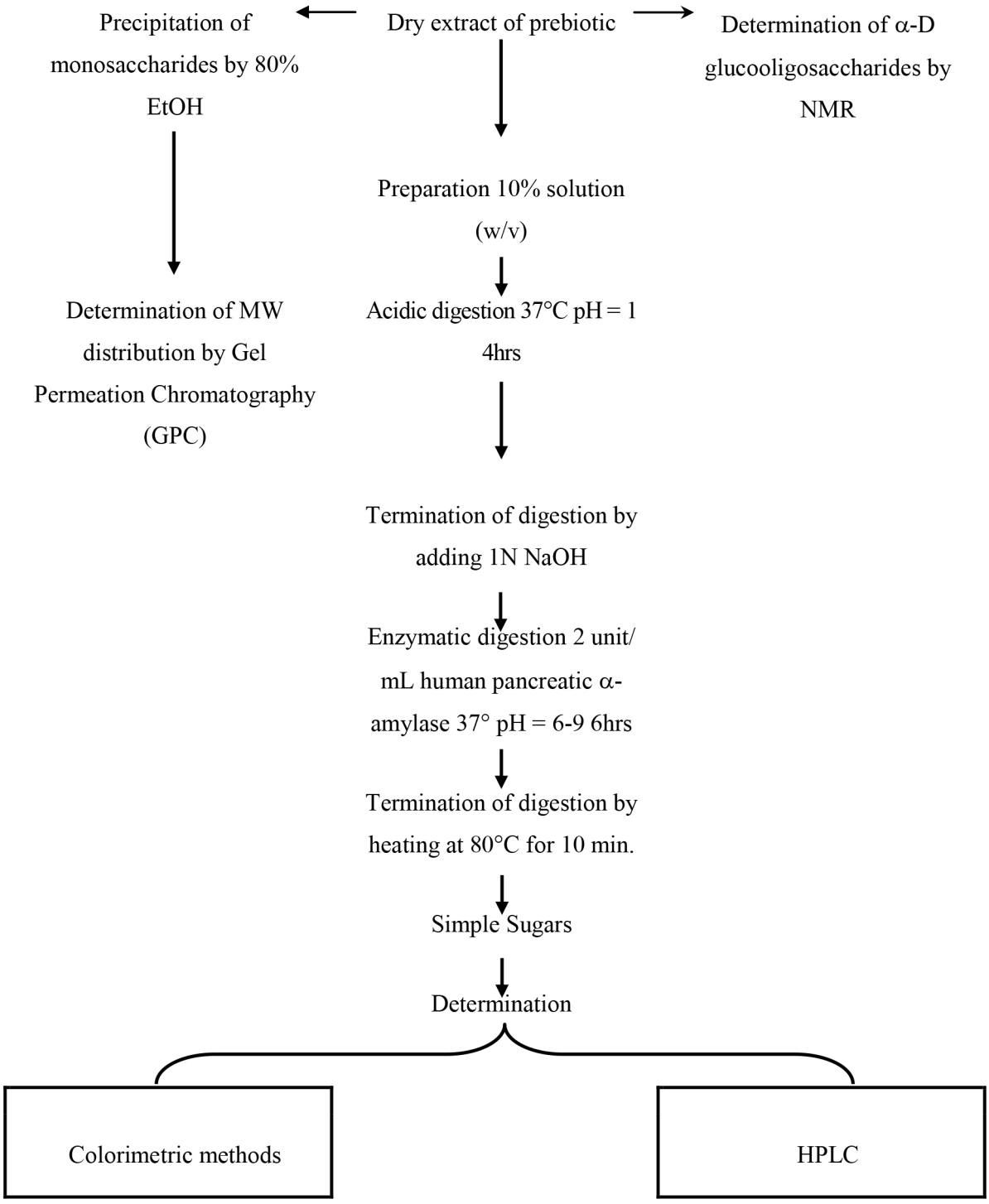

Fig. 1: Flow diagram showing the determination procedures of prebiotics

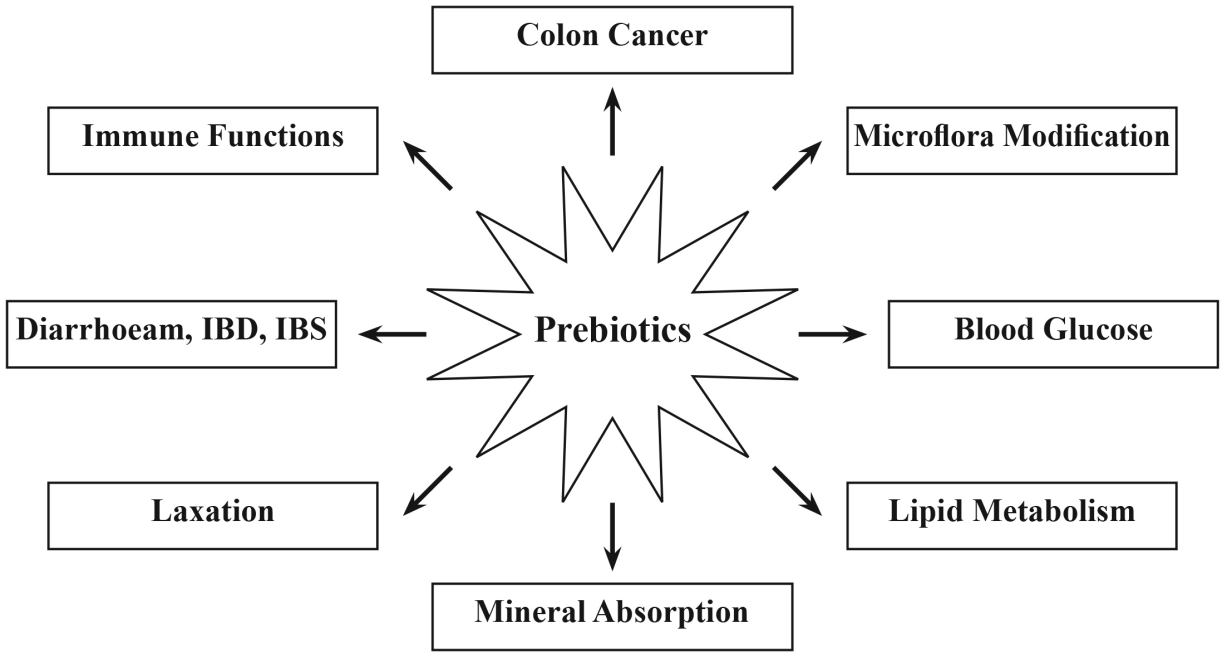

Fig. 2: The possible health benefit of prebiotics

Source: Cummings & Macfarlane (2002)

Prepiotic and cancer: At least two mechanisms have been proposed to explain the effect of prebiotics on the development of cancer:

1- Production of protective metabolites. Butyrate is a common fermentation end product and is known to stimulate apoptosis in colonic cancer cell lines, and it is also the preferred fuel for healthy coloncytes (Prasad, 1980 , Jackson, 2010).

2- Shift of colonic metabolism away from protein and lipid metabolism towards more benign and products (saccharolysis) (Manning & Gibson, 2004, Lomax & Calder, 2009).

Prebiotic and immunomodulation: The proposed mechanisms underlying the immunomodulating effects may include the following (Schley & Field, 2002, Lomax & Calder, 2009).

1- Direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine;

2- Production of short chain fatty acids (SCFA) from fermentation; and modulation of mucin production.

Hypolipidaemic effects of prebiotics: Three mechanisms have been put forward to explain a hypolipidaemic effect of prebiotics:

The first is the modification of glucose or insulin concentrations. Nondigestible carbohydrates reduce peak levels of blood glucose after a meal and consequently the induction of lipogenic enzymes *via* an increased gene transcription (Roberfroid, 2000). The second is the production of SCFA in the colon. As mentioned earlier, the ratio of acetate to propionate reaching the liver is a putative intermediate marker predicting the potential lipid lowering properties of prebiotics (Delzenne &Williams, 2002, Letexier *et al.*, 2003, Macfarlane *et al* 2006 ,Wong *et al.*, 2006). The third mechanism proposes that serum cholesterol is reduced because of precipitation and excretion of bile acids to the intestine, which requires the liver to utilize cholesterol for further bile acid synthesis (Pedersen *et al.*, 1997). Because animal studies have identified inhibition of hepatic fatty acid synthesis as the major site of action for the triglyceride- lowering effects of inulin and fructooligosaccharides, and because this pathway is relatively inactive in humans unless a high-carbohydrate diet is followed, variability in response of animals and humans may be a reflection of differences in background diet or experimental foods used (Williams &Jackson, 2002, Roberfroid *et al.*, 2010).

Antimicrobial activity:

The possible antimicrobial activity of the prebiotics may be accounted by their growth – promot-

ing effects on *Bifidobacteria* and *lactobacilli*. These bacteria can reinforce the barrier function of the intestinal mucosa, helping in the prevention of the attachment of pathogenic bacteria, essentially by crowding them out. These bacteria may also produce antimicrobial substances and stimulate antigen specific and nonspecific immune responses. (Brink *et al.*, 2005, Bosscher *et al.*, 2006). Brownawell *et al.*, (2012) reported that prebiotics has a role in reducing the risk and severity of gastrointestinal infection and inflammation, including diarrhea, inflammatory bowel disease and irritable bowel syndrome.

Improving mineral absorption

The nondigestible carbohydrates have been reported to impair the small-intestinal absorption of minerals because of their binding or sequestering action. However, the minerals that are bound or sequestered and not absorbed in the small intestine, they may be released from the carbohydrate matrix and absorbed (Roberfroid, 2000).

Moreover, a high concentration of short-chain carboxylic acids resulting from the colonic fermentation of nondigestible carbohydrates facilitates the colonic absorption of minerals, particularly Ca^{2+} and Mg^{2+}(Jackson, 2010).Gibson *et al*.(2010) and Roberfroid *et al.* (2010) stated that oligofructose and/or inulin increase calcium bioavability. It could be due to the osmotic effect of inulin and oligofructose, which would transfer water into the large intestine, allowing calcium to become more soluble.

Alleviation of constipation

All carbohydrates that reach the large intestine have a laxative effect on bowel habit. The mechanism works *via* stimulation of microbial growth, increase in bacterial cell mass and thus stimulation of peristalsis by the increased bowel content (Cummings *et al.,* 1997). It can be predicted, therefore, that prebiotics will be laxative. In carefully controlled studies it has indeed been shown that prebiotics that are fermented completely increased bowl frequency (den Hond *et al.*, 2000), bringing relief from constipation in chronically constipated subjects, and induce a fecal bulking effect of 1.5 to 2 g of feces per gram of prebiotic consumed (Gibson & Roberfroid, 1995). However, this is less than seen with non starch polysaccharide sources such as wheat bran (5.4g) or fruit and vegetables (4.7g), but similar to that produced by more rapidly fermented polysaccharides such as pectin (1.2g) (Cummings *et al*., 1997).

Three prebiotics, oligofructose, galacto-oligosaccharides and lactullose, clearly alter the balance of the large bowel microbiota by increasing *Bifidobacteria* and *Lactobacillus* numbers. These carbohydrates are fermented and give rise to short-chain fatty acid and intestinal gas. However, effects on bowel habit are relatively small. Randomized-controlled trials of their effect in a clinical context are few, although animal studies show anti-inflammatory effects in inflammatory bowel disease, while calcium absorption is increased (Macfarlane *et al.*, 2006, Slavin, 2013).

Applications of prebiotics in food:

In order for prebiotics to be incorporated into food products they should not affect negatively the organoleptic properties of the product. Also, be stable during food processing which includes, high temperatures, low pH or combination of the two, and conditions favoring Maillard reactions (Charalampopoulos & Rastall, 2012). These take place between reducing sugars and amino acids at high temperatures and result in the production of both high and low molecular weight compounds, besides affecting the organoleptic properties of the foods. So, this can potentially reduce the prebiotic activity of' the carbohydrate, if the prebiotic compound is a reducing sugar (Huebner, *et al*., 2008, Marie, 2010).

Prebiotics can be formulated either as a powder, syrup or as capsules and marketed as supplements which are available from health food stores or incorporated into food products. Prebiotic powder may be sprinkled on food or added to beverages, or capsules may be consumed with meals (Douglas & Sanders, 2008, Marie, 2010, Charalampopoulos & Rastall, 2012).

Prebiotics are being used in food industry as functional ingredients in beverages and health drinks (fruit Juices, coffee, cocoa, tea, soft drinks in general, isotonic drinks, liquid sugar and alcoholic beverages) spreads, dairy products (fermented milk, cheeses, milk powder and ice cream) infant formulate and weaning foods.

Other applications include desserts (Jellies, Puddings), confectionery, chocolates, cakes, chewing gum, bakery products (biscuits, breakfast cereals, breads, pastas), soups, sauces and dressings, meat products, dried instant foods, and canned foods

nutrition bars and meal replacement shakes (Manning &Gibson, 2004, Leach *et al.*, 2006, Caselato de Sousa, *et al.*, 2011, Sangwan, *et al.*, 2011).

The functional properties of prebiotics are illustrated in Table (3):

Fructooligosaccharides (FOS) have been the best documented oligosaccharides for their effect on intestinal Bifiodobacteria and are considered important prebiotic substrates.

FOS are available in some foods such as bananas, onion, tomato, rye, garlic, wheat, artichoke, leek, chicory and asparagus (Caselato de Sousa, et al., 2011). It is produced in large quantities in several countries and are added to various products such as biscuits, yoghurts, breakfast cereals, table spreads and sweeteners (Touhy, *et al*., 2001, Leach et al., 2006).

FOS have similar technological properties to sucrose and glucose syrups, as a result, they are frequently used as sugar replacements. They 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. It can be used in baked goods and breads to replace sugar and to retain moisture in the products (Ronda, *et al*., 2005, Charalampopoulos & Rastall, 2012).

It was found that the addition of FOS and inulin to the yogurts were regarded as smoother and thicker and yogurt with 4%.FOS (on a wet/ weight basis) had a good overall acceptability (Sharma, *et al.*, 2011).

Inulin naturally occurs in hundreds of different plant foods, such as garlic, onion chicory, asparagus, artichokes, banana, wheat and leeks (Gibson, *et al.*, 2004).

Inulin is used extensively in the food industry as a fat replacer or texture modifier. Because after mixing inulin with water, a white creamy gel is formed and this provides a short and spreadable texture and smooth fatty mouth feel, has a neutral taste and can be used to completely replace fat into foods.

So, it is used in low fat dairy products, including fermented milks, yoghurts, dairy desserts, cheeses and ice cream as a fat replacer to improve mouth feel. (Buriti, *et al.*, 2010, Meyer, *et al.*, 2011, Charalampopoulos & Rastall, 2012). It stabilizes emulsions and dispersions and improves the stability of mousses and foams (Sharma, *et al.*, 2011).

Also, Inulin is used to enrich food products with fiber, maintaining the appearance and taste of standard formulations. It is used in baked products as a texture modifier, often in combination with dietary fibers (Angioloni &Collar, 2008, Hager, *et al.*, 2011).

Galactooligosaccharides (GOS) are as other oligosaccharides, very soluble in water medley sweet (30-35% compared to sucrose). It has a pleasant taste and can increase the texture and mouth feel

Table 3: Food applications of prebiotics

Application	Functional properties
Dairy products	Fat or sugar replacement, texture and mouth feel, fiber and prebiotic
Frozen desserts	Fat or sugar replacement, texture and mouth feel, melting behaviour
Fruit preparations	Sugar replacement synergy with intense sweeteners, body and mouth feel, fiber
Beverages and drinks	Fat or sugar replacement, mouth feel, foam stabilization and prebiotics
Baked goods and breads	Sugar replacement, moisture retention, fiber, and prebiotic.
Breakfast cereals and extruded snakes	Sugar replacement, crispiness and expansion, fiber and prebiotics
Filling	Fat or sugar replacement, texture and mouth-feel.
Dietetic products	Fat or sugar replacement, fiber and prebiotic.
Sugar confectionary	Sugar replacement, fiber and prebiotics
Chocolate	Sugar replacement, heat resistance and fiber
Soups and sauces	Sugar replacement and prebiotics
Meat products	Fat replacement, texture stability and fiber.

Source: Wang, (2009).

of foods. In general it is very stable to acidic conditions and high temperatures, so, they can be potentially added to variety of acid foods (fruit juices and .other acid drinks, fermented milks, and flavoured milks (Sangwan, *et al.*, 2011). Also, it can be used in baked goods and breads to replace sugar and to retain moisture in the products (Ronda, 2005, Torres, *et al.*, 2010).

Infant milk formulas:

Breastfed infants are often healthier than formula-fed infants and can fight infections better. Breast milk naturally contains prebiotics (oligosaccharides) at a level of 10-12 g/L compared to cow's milk (<1g/L). These oligosaccarides favor the growth of *Bifidobacteria* in the colon. They can be short- or longer-chain, linear or branched chain, neutral or acidic, and apart from simple sugars like galactose, glucose, and fructose, they also contain sugar derivatives like amino sugars or uronic acids. They play a major role in the bifidoge, protective, and immunomodulating properties of human milk (Mountouris, *et al.*, 2002, Vandeplas, 2002, Boehm *et al.*, 2004, Veerman, 2005, Arslanoglu, *et al.*, 2007).

Supplementation of infant milk formula with a mixture of fructo-oligosaccharides (FOS)/ galacto-oligosaccharides (GOS) can potentially reduce the stool pH of children younger than six months and also leads to increased stool frequency and softer stools, similar to that of breastfed infants (De Vrese & Schrezenmeir, 2008, Sangwan, *et al.*, 2011, Ackerberg, *et al.*, 2012). Supplemented infant formulas usually contain 6.0 to 7.0g/L GOS together with 0.6 to 0.8 g/L FOS (Gibson & Rastall, 2006, Torres, *et al.*, 2010).

Bettler & Euler (2006) reported that Bovine milk-based formula supplemented with FOS is safe and supports normal growth for infants. Study by Kim, *et al.*, (2007) showed that inulin may be a useful ingredient in the formulation of baby formula to enhance the nutritional properties. A mixture of GOS/ inulin is comprised of short-chain GOS combined with long-chain inulin in a 9: 1 ratio is used for infant nutrition applications and is added to standard formulas for infant. Studies have shown that a formula supplemented with this mixture results in an intestinal microbiota similar to that found in breast-fed infants (Kelly, 2009).

The addition of oligofructose to cereals, leading to an average daily consumption of 1: 1 g in healthy children aged between 4 and 24 months, significantly decreased the number of infectious episodes as indicated by fever and medical visits. Control subjects had more sick days and a higher intake of antibiotics (Boehm, *et al*., 2004).

The Food and Agricultural Organization (FAO) of the United Nations supports the supplementation of formula with prebiotics in infants aged five months and older, as these infants will have a mature immune system and intestinal colonization (Ackerberg, *et al*., 2012).

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تحليل والفوائد الصحية وتطبيقات البريبيوتك: استعراض مرجعي

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البريبيوتك هي السكريات العديدة ومحدودة التسكر التي لا تهضم ولا تمتص في الأمعاء الدقيقة لكنها تتخمر اختيارياً بواسطة الداعمات الحيوية (البروبيوتك) الموجودة طبيعياً في الأمعاء الغليظة للإنسان. على مدى السنوات الخمس عشرة الماضية تجمع زخم كبير من المعلومات عن البريبيوتك لتفهم آلية عملها والتعرف على فوائدها الصحية للإنسان.

يلقي هذا الاستعراض المرجعي الضوء على الظروف المثلى لاستخلاص البريبيوتك فضلاً عن طرق تقديرها، والتي تشتمل على مقاومة نمطي التحليل الحامضي والإنزيمي، كروماتوجرافياً السائل عليه الإظهار HPLC وطريقة الرنين المغناطيسي النووي NMR.

ويركز هذا الاستعراض المرجعي على الفوائد الصحية للبريبيوتك كمضادات للسرطان والميكروبات وهشاشة العظام وارتفاع مستوى سكر الدم بالإضافة إلى نشاطها الحثي.

كذلك فقد عنى هذا الاستعراض المرجعي بتطبيقات البريبيوتك في مجال التصنيع الغذائي كمنتجات الألبان والحلوى والفواكه المحفوظة وتركيبات ألبان الأطفال الرضع وغيرها من المنتجات الغذائية.