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Review

Non-digestible oligosaccharides: A review

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Abstract

Non-digestible oligosaccharides (NDOs) are low molecular weight carbohydrates of intermediate in nature between simple sugars and polysaccharides. They can be obtained by direct extraction from natural sources, or produced by chemical processes hydrolyzing polysaccharides, or by enzymatic and chemical synthesis from disaccharides. The NDOs possess important physicochemical and physiological properties, and are claimed to behave as dietary fibers and prebiotics. Enrichment of diet with NDOs gives opportunity for improving of gut microecology including bacterial populations, biochemical profiles and physiological effects. Therefore, their industrial applications have rapidly increased in the last few years, both in prebiotic formulations and in symbiotic products (containing probiotic organism and prebiotic oligosaccharide).

Keywords: Non-digestible oligosaccharides; Carbohydrates; Intestinal microflora; Human health; Prebiotics

1. Introduction

Presently, the use of foods that promote a state of well-being, better health and reduction of the risk of diseases have become popular as the consumer is becoming more and more health conscious. In this sense, there has been a lot of attention paid to specific types of dietary carbohydrates, namely the non-digestible oligosaccharides (NDOs). These compounds present important physicochemical and physiological properties beneficial to the health of consumers, and for this reason, their use as food ingredients has increased rapidly. Such properties include non-cariogenicity, a low calorific value and the ability to stimulate the growth of beneficial bacteria in the colon. They are also associated with a lower risk of infections and diarrhea, and an improvement of the immune system response. Moreover, due to the decrease of the intestinal pH caused by their fermentation, NDOs provoke a reduction of the pathogens flora, an increase of bifidobacteria population, and an increase of the availability of minerals. In the food industry, these compounds have great potential to improve the quality of many foods, providing modifications to food flavor and improving its physicochemical characteristics (Crittenden & Playne, 1996; Rivero-Urgell & Santamaria-Orleans, 2001). So that, the NDOs popularity as food ingredients has strongly increased, mainly in the last few years. As a consequence, several researches have been performed aiming the discovery of new NDOs, as well as the development of new products containing these compounds.

The present review summarizes the main types of non-digestible oligosaccharides that can be commercially found, their physicochemical and physiological properties, the natural sources where they are present as well as the industrial production processes, and potential industrial applications.

2. Oligosaccharides properties

The carbohydrates can be classified according to their molecular size or degree of polymerization (number of monosaccharide units combined), into monosaccharides, oligosaccharides or polysaccharides. According to IUB-IUPAC nomenclature, oligosaccharides are defined as

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saccharides containing between 3 and 10 sugar moieties. Other authorities classify saccharides including anyone from 3 to 19 monosaccharide units in this group. However, there is not a rational physiological or chemical reason for setting these limits (Voragen, 1998). Consequently, oligosaccharides are low molecular weight carbohydrates. At the same time, based on the physiological properties, the carbohydrates can be classified as digestible or non-digestible. The concept of non-digestible oligosaccharide originates from the observation that the anomeric C atom (C1 or C2) of the monosaccharide units of some dietary oligosaccharides has a configuration that makes their osidic bounds non-digestible to the hydrolytic activity of the human digestive enzymes (Roberfroid & Slavin, 2000). The main categories of NDOs presently available or in development as food ingredients include carbohydrates in which the monosaccharide unit is fructose, galactose, glucose and/or xylose (Fig. 1).

The NDOs are known to promote the growth of beneficial bacteria in the colon, mainly the Bifidobacteria species, and are thus recognized as prebiotics. Sako, Matsumoto, and Tanaka (1999) described 13 classes of NDOs that present bifidogenic functions, and are commercially produced (Table 1). The chemical differences among these NDOs include chain length, monosaccharide composition, degree of branching, and purity. As can be noted in Table 1, NDOs are made from one, two or even three different types of monosaccharides. Although these oligosaccharides are composed at least by three monosaccharides units, lactulose is a disaccharide that possesses similar properties to the oligosaccharides and for this reason it is also included in the oligosaccharides class (Crittenden & Playne, 1996). Similarly, xylobiose is a compound of polymerization degree = 2 that is considered to be a xylooligosaccharide because it presents technological properties and cause effects on health similar to those caused by the xylooligosaccharides of higher polymerization degree (Vázquez, Alonso, Dominguez, & Parajó, 2000).

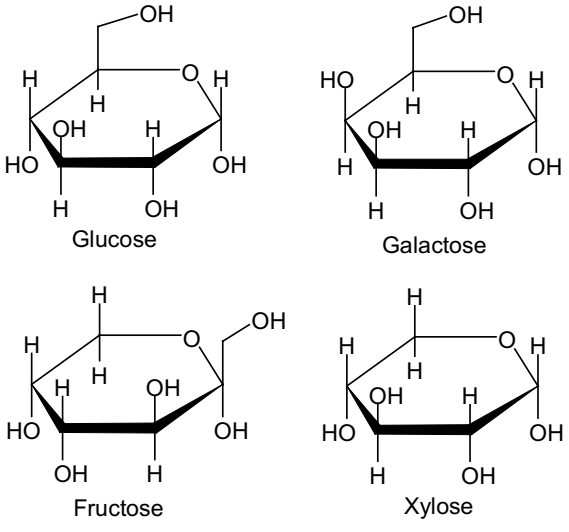

Fig. 1. Monosaccharides components of non-digestible oligosaccharides.

Table 1
Non-digestible oligosaccharides with bifidogenic functions commercially available (Sako et al., 1999)

Compound	Molecular structure[a]
Cyclodextrins	$(Gu)_n$
Fructooligosaccharides	$(Fr)_n$–Gu
Galactooligosaccharides	$(Ga)_n$–Gu
Gentiooligosaccharides	$(Gu)_n$
Glycosylsucrose	$(Gu)_n$–Fr
Isomaltooligosaccharides	$(Gu)_n$
Isomaltulose (or palatinose)	$(Gu–Fr)_n$
Lactosucrose	Ga–Gu–Fr
Lactulose	Ga–Fr
Maltooligosaccharides	$(Gu)_n$
Raffinose	Ga–Gu–Fr
Soybean oligosaccharides	$(Ga)_n$–Gu–Fr
Xylooligosaccharides	$(Xy)_n$

[a] Ga, galactose; Gu, glucose; Fr, fructose; Xy, xylose.

2.1. Physicochemical properties

Oligosaccharides are water soluble and typically 0.3–0.6 times as sweet as sucrose. In fact, the sweetness depends on chemical structure, the degree of polymerization of the oligosaccharides present and the levels of mono- and disaccharides in the mixture (Crittenden & Playne, 1996; Voragen, 1998). According to Roberfroid and Slavin (2000) the sweetness decreases with longer the oligosaccharide chain length. This low sweetness intensity is quite useful in the various kinds of foods where the use of sucrose is restricted by its high sweetness property.

The relatively low sweetness makes the oligosaccharides useful in food production when a bulking agent with reduced sweetness is desirable to enhance other food flavors. In the case of very sweet foods, they may be used as bulking agents in conjunction with artificial sweeteners such as aspartame or sucralose, for example, with the advantage to mask the aftertastes produced by some of these intense sweeteners. In addition, when compared with mono- and disaccharides, the higher molecular weight of oligosaccharides provides increased viscosity, leading to improved body and mouthfell (Crittenden & Playne, 1996).

The stability can greatly differ for the various classes of oligosaccharides depending on the sugar residues present, their ring form and anomeric configuration and linkage types. Generally β-linkages are stronger than α-linkages, and hexoses are more strongly linked than pentoses. Nevertheless, as a whole, at pH < 4.0 and treatments at elevated temperatures or prolonged storage at room conditions, oligosaccharides present in food can be hydrolyzed resulting in loss of nutritional and physicochemical properties (Voragen, 1998). The oligosaccharides can also be used to alter the freezing temperature of frozen foods, and to control the intensity of browning due to Maillard reactions in heat-processed foods. They also provide a high moisture-retaining capacity, preventing excessive drying, and a low water activity, which is

convenient in controlling microbial contamination (Crittenden & Playne, 1996). The caloric value of NDOs has been estimated to be 1.5–2.0 kcal/g. This is proximately 40–50% of those of digestible carbohydrates such as sucrose (Sako et al., 1999).

2.2. Physiological properties

Although oligosaccharides possess important physicochemical properties, most of the interest in their use as food ingredients is due to their many physiological properties beneficial for health. One of these is that unlike starch and simple sugars, the NDOs are not utilized by mouth microflora. Consequently, the production of acids or polyglucans (cariogenic compounds) does not occur. Therefore, the NDOs can be used as low cariogenic sugar substitutes in products like confectionery, chewing gums, yoghurts and drinks (Crittenden & Playne, 1996).

Many NDOs are not digested by humans because the human body lacks the enzymes required to hydrolyze the β-links formed among the units of some monosaccharides. Such compounds include carbohydrates where fructose, galactose, glucose and/or xylose are the monosaccharides units presents. This property makes the NDOs suitable for use in sweet, low-caloric diet foods, and for consumption by individuals with diabetes (Crittenden & Playne, 1996; Rivero-Urgell & Santamaria-Orleans, 2001).

Most oligosaccharides are quantitatively hydrolyzed in the upper part of the gastrointestinal tract. The resulting monosaccharides are transported via the portal blood to the liver and, subsequently, to the systemic circulation. Such carbohydrates are essential for health as they serve both as substrates and regulators of major metabolic pathways. Nevertheless, some oligosaccharides present specific physicochemical properties and resist to the digestive process, reaching the caeco-colon as they have been eaten. In the caeco-colon, most (but not necessarily all) of the non-digestible oligosaccharides are hydrolyzed to small oligomers and monomers, which are further metabolized by one, a few, or most of the anaerobic bacteria. Such a metabolic process, know as fermentation, not only serves the bacteria by providing energy for proliferation, but it also produces gases (H_2, CO_2, CH_4), which are metabolically useless to the host, and small organic acids (short-chain fatty acids – SCFA) such as acetate, propionate, butyrate and L-lactate. Even though they do not provide the body with monosaccharides, the non-digestible oligosaccharides are indirect energy substrates and metabolic regulators (Delzenne & Roberfroid, 1994). The amounts and types of SCFA produced in the colon depend on the type of NDO substrate as well as on the composition of the intestinal flora (Sako et al., 1999).

The gastrointestinal tract is very heavily populated with bacteria, mainly of strictly anaerobic bacteria. The numerically predominant colonic anaerobes are given in Table 2. This microbial community is extremely complex, both in terms of the number of organisms, approximately 10^{13} in total, and in its diversity, with over 400–500 different species reported. Most of these organisms are benign to the host; however, certain gut species are pathogenic and may be involved in the onset of acute and chronic disorders (Isoulari, Salminen, & Ouwehand, 2004; Manning & Gibson, 2004; Ziemer & Gibson, 1998). Due to their chemical structure, the non-digestible oligosaccharides are substrates that can only be consumed by a limited number of bacteria, stimulating thus their growth. Among the group of bacteria present in the gastrointestinal tract, the bifidobacteria and lactobacilli are those that most utilize oligosaccharides being considered as the only microorganisms able to beneficially affect the host's health (Bielecka, Biedrzycka, Majkowska, Juskiewicz, & Wróblewska, 2002).

Table 2
The numerically predominant anaerobic microorganisms in the human colon[a]

Microbial group	Range in log counts (g dry wt^{-1})
Bacteroides	9.2–13.5
Eubacteria	5.0–13.3
Bifidobacteria	4.9–13.4
Clostridia	3.3–13.1
Lactobacilli	3.6–12.5
Ruminococci	4.6–12.8
Peptostreptococci	3.8–12.6
Peptococci	5.1–12.9
Streptococci (anaerobic)	7.0–12.3
Methanobrevibacter	7.0–10.3
Desulfovibrios	5.2–10.9

[a] From cited by Ziemer and Gibson (1998).

The rate at which oligosaccharides are fermented depending on the degree of polymerization, sugar and glycosidic linkage and degree of branching, synergy between bacteria during fermentation, relationship between substrate bacteria and fermentation products, nature of the fermentations and saccharolytic capacity (Voragen, 1998). According to Sangeetha, Ramesh, and Prapulla (2005), Manning and Gibson (2004), Gibson (2004), Bielecka et al. (2002), Rivero-Urgell and Santamaria-Orleans (2001), Roberfroid and Slavin (2000), Sako et al. (1999), Ziemer and Gibson (1998), Younes, Demigné, and Rémésy (1996), and Delzenne and Roberfroid (1994), the NDOs fermentation in the caeco-colon by the bacteria there existent may cause the following effects on the health:

1. *A significant modification of the colonic microflora*, because these oligosaccharides serve as substrate for growth and proliferation of anaerobic bacteria, mainly the bifidobacteria, which inhibit the growth of putrefactive and pathogenic bacteria present in the caeco-colon. For example, the establishment of a bifidus microflora in the intestines of breast-fed-infants has been attributed to the presence of galactose-containing oligosaccharides in human milk.

2. *A decrease of pH in the colon* and consequently, in faeces, resulting from the production of SCFA. Lower pH values inhibit the growth of certain pathogenic bacterial species while stimulating the growth of the bifidobacteria and other lactic acid species.
3. *Nutrient production*, such as vitamins of the B complex (B1, B2, B6 and B12), nicotinic and folic acids.
4. *An increase in fecal dry weight excretion*, which is related to the increased number of bacteria resulting from the extensive fermentation of NDOs.
5. *Constipation relief due to fecal bulking and possibly effects on intestinal motility*. The indigestible quality of NDOs means that they have effects similar to dietary fiber, and thus prevent constipation. The end products of NDOs fermentation by colonic bacteria, the SCFA, are efficiently absorbed and utilized by the human colonic epithelial cells, stimulating their growth as well as the salt and water absorption, increasing thus the humidity of the fecal bolus through osmotic pressure, and consequently improving the intestinal motility.
6. *Inhibition of diarrhea*, especially when it is associated with intestinal infections. This may be directly related to the possible inhibitory effect of bifidobacteria both on gram+ and gram− bacteria.
7. *A protective effect against infection in the gastrointestinal, respiratory and urogenital tracts*, due to their capacity to inhibit the adhesion of bacteria to the epithelial surfaces (initial stage of the infective process).
8. *An increase in absorption of different minerals*, such as iron, calcium, and magnesium, due to the binding/sequestering capacity of the NDOs. The minerals that are bound/sequestered and, consequently, are not absorbed in the small intestine reach the colon, where they are released from the carbohydrate matrix and absorbed. The increase on calcium absorption, in particular, reduces the risk of osteoporosis since this mineral promotes an increase in the bone density and bone mass. The hypotheses most frequently proposed to explain this enhancing effect of NDOs on mineral absorption are the osmotic effect, acidification of the colonic content due to fermentation and production of SCFA, formation of calcium and magnesium salts of these acids, hypertrophy of the colon wall.
9. *A benefic effect on the carbohydrates and lipids metabolism*, leading to a decrease in the cholesterol, triglycerides and phospholipids concentration in the blood, reducing thus the risk of diabetes and obesity. Changes in the concentration of serum cholesterol have been related with changes in the intestinal microflora. Some strains of *Lactobacillus acidophilus* assimilate the cholesterol present in the medium, while others appear to inhibit the absorption of cholesterol through the intestinal wall. On the other hand, the changes in lipid metabolism were suggested to be a consequence of a metabolic adaptation of the liver that might be induced by SCFA.
10. *A reduction of cancer risk*, mainly the gut cancer. This anticarcinogenic effect appears to be related to an increase in cellular immunity, the components of the cell wall and the extra-cellular components of bifidobacteria. Fecal physiological parameters such as pH, ammonia, *p*-cresol, and indole are considered to be risk factors not only for colon cancer development but also for systemic disorders. It has been demonstrated in a human study that the intake of transgalactosylated disaccharides reduces the fecal pH as well as ammonia, *p*-cresol and indole concentrations with an increase in bifidobacteria and lactobacilli and a decrease in *Bacteroidaceae* populations. These alterations may be considered to be beneficial in reducing the risk of cancer development. A low colonic pH may also aid in the excretion of carcinogens.

All the effects above mentioned beneficially affect the host health, and for this reason, the NDOs are considered as functional food (Rivero-Urgell & Santamaria-Orleans, 2001; Roberfroid & Slavin, 2000), which can be defined as "a food ingredient which affects physiological function (s) of the body in a targeted way so as to have positive effect (s) which may, in due course, justify health claims" (Roberfroid, 1996). In addition, most of the NDOs are also classified as prebiotics because they selectively stimulate the growth and/or metabolic activity of bacteria species benefic for health, proportioning an improvement in the composition of the colonic microflora, and thus improving the host health (Crittenden & Playne, 1996; Roberfroid & Slavin, 2000; Voragen, 1998; Ziemer & Gibson, 1998).

Some authors consider that the beneficial effects for health caused by the oligosaccharides ingestion are similar to the effect of dietary fiber since they increase the fecal bolus and reduce the gastrointestinal transit times (enhancing the healthy gastrointestinal tract), improve the glucose control and modulate the metabolism of triglycerides (Delzenne & Roberfroid, 1994; Roberfroid & Slavin, 2000; Santos, 2002). Moreover, the NDOs present some advantages when compared to the fibers, because they require a lower daily dose, and do not cause diarrhea if consumed in recommended doses. They are slightly sweet, do not have unpleasant texture and taste, are totally soluble in water, physically stable and easy to be incorporated in processed food and beverages (Tomomatsu, 1994).

According to Roberfroid and Slavin (2000) the evaluation of an acceptable dose is difficult because each individual has his own feeling about acceptable and non-acceptable intestinal discomfort. However, excessive consumption doses of NDOs may cause intestinal discomfort, flatulence or even diarrhea because of their osmotic effect, which may transfer water into the large bowel (an effect which is inversely related to chain length), and because of their high fermentation rate and production of gases. For example,

galactooligosaccharides consumption higher than 20 g/day, and fructooligosaccharides consumption higher than 40 g/day are reported to cause diarrhea (Sako et al., 1999; Spiegel, Rose, Karabell, Frankos, & Schmitt, 1994). On the other hand, in studies with healthy as well as diabetic subjects, high doses of isomaltulose, up to 50 g/day, were tolerated without signs of intestinal discomfort (Lina, Jonker, & Kozianowsky, 2002). A daily intake of 8.8 g/day of γ-cyclodextrin was considered enough for examining gastrointestinal tolerance (Munro, Newberne, Young, & Bär, 2004).

The effective bifidogenic doses appear to vary among the different oligosaccharide types. Nevertheless, most oligosaccharides have been demonstrated to increase bifidobacteria numbers in the colon at doses of <15 g/day (Crittenden & Playne, 1996). Some authors have suggested that an intake of 10 g/day of galactooligosaccharides is sufficient to cause a bifidogenic effect. However, when the subjects' initial number of indigenous bifidobacteria is low, which is often the case in middle-aged and elderly people, daily intake of 2.5 g/day is enough to lead to an increase in fecal bifidobacteria population (Gibson, 2004; Sako et al., 1999). The doses of other NDOs necessary to show the bifidogenic effects are comparable to that of galactooligosaccharides. For xylooligosaccharides, for example, 2 g/day has been considered enough to show bifidogenic effect (Sako et al., 1999). Rivero-Urgell and Santamaria-Orleans (2001) reported that the fructooligosacharides ingestion required to act as bifidogenic agents is between 2 and 10 g/day in adults. Nevertheless, Manning and Gibson (2004) considered that at least 4 g/day, but preferentially 8 g/day of fructooligosacharides, would be needed to significantly elevate the bifidobacteria cell number in the human gut. The daily dosage required for isomaltooligosaccharides is of 8–10 g per day (Goulas, Fisher, Grimble, Grandison, & Rastall, 2004).

3. Natural sources of non-digestible oligosaccharides

NDOs of various types can be found as natural components in milk, honey, fruits and vegetables such as onion, Jerusalem artichoke, chicory, leek, garlic, artichoke, banana, rye, barley, yacon and salsify. For most of these sources, concentrations range between 0.3% and 6% of fresh weight; for chicory and salsify these values are between 5% and 10% while in Jerusalem artichoke and yacon they can reach up to 20%. Other examples of naturally occurring non-digestible oligosaccharides are the galactosylsucroses raffinose and stachyose in soyabean and other pulses and leguminous seeds, xylooligosaccharides in bamboo shoots and galactose-containing oligosaccharides in milk, particularly colostrums either in free form or as glycoconjugates (Voragen, 1998).

Specifically, asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato and rye are special sources of fructooligosaccharides (Sangeetha et al., 2005; Yun, 1996; Ziemer & Gibson, 1998). Isomaltulose naturally occurs in honey, sugarcane juice, and products derived thereof such as treacles or food-grade molasses (Lina et al., 2002). Xylooligosaccharides appear naturally in bamboo shoots, fruits, vegetables, milk and honey (Vázquez et al., 2000). Galactooligosaccharides are found naturally in human milk and to a smaller extent in cow's milk (Alander et al., 2001). Cyclodextrins are naturally occurring water-soluble glucans (Singh, Sharma, & Banerjee, 2002). Seeds of legumes, lentils, peas, beans, chickpeas, mallow, composite, and mustard are rich in raffinose oligosaccharides (Johansen, Glitso, & Knudsen, 1996; Sánchez-Mata, Peñuela-Teruel, Cámara-Hurtado, Díez- Marqués, & Torija-Isasa, 1998).

4. Commercial production of non-digestible oligosaccharides

Industrial production processes have been established to extract the NDOs from natural sources, by hydrolyzing polysaccharides, and by enzymatic and chemical synthesis from disaccharide substrates. With the exception of soybean oligosaccharides and raffinose (which are produced by direct extraction) and lactulose (which is produced by isomerization reaction), the NDOs are manufactured using enzymatic processes. They are either "built up" from simple sugars, such as sucrose or lactose, by enzymatic transglycosylation reactions, or formed by controlled enzymatic hydrolysis of polysaccharides, such as starch or xylan (Fig. 2) (Sako et al., 1999). These processes usually produce a range of oligosaccharides differing in their degree of polymerization and sometimes in the position of the glycosidic linkages. Unreacted substrates and monosaccharides are also present after oligosaccharide formation. Such contaminating sugars are often removed by membrane or chromatographic procedures to form higher-grade products that contain purer oligosaccharides (Crittenden & Playne, 1996).

Raffinose oligosaccharides can be directly extracted from plant materials using water or aqueous methanol or ethanol solutions (Johansen et al., 1996).

Galactooligosaccharides are commercially produced from lactose by the action of β-galactosidases, which have transgalactosylation activity. In the industrial production process of galactooligosaccharides, a highly concentrated solution of lactose, which is usually purified from cow's milk whey, is used as a substrate solution in this reaction. The main products are trisaccharides, namely 4′- or 6′-galactosyllactose, and longer chain oligosaccharides consisting of 4 or more monosaccharides units (Sako et al., 1999).

Like galactooligosaccharides, the lactulose is also manufactured from lactose, but in this case, an alkali isomerization process is used to convert the glucose moiety of lactose to a fructose residue. The resulting compound is the lactulose disaccharide. Lactulose is relatively expensive to produce, not only because of the low product yield (20–30%) from the reaction, but also due to the high cost of purification, since galactose, isosacharic acids and colored products are also generated by the partial degradation of lactulose (Villamiel, Corzo, Foda, Montes, & Olano, 2002).

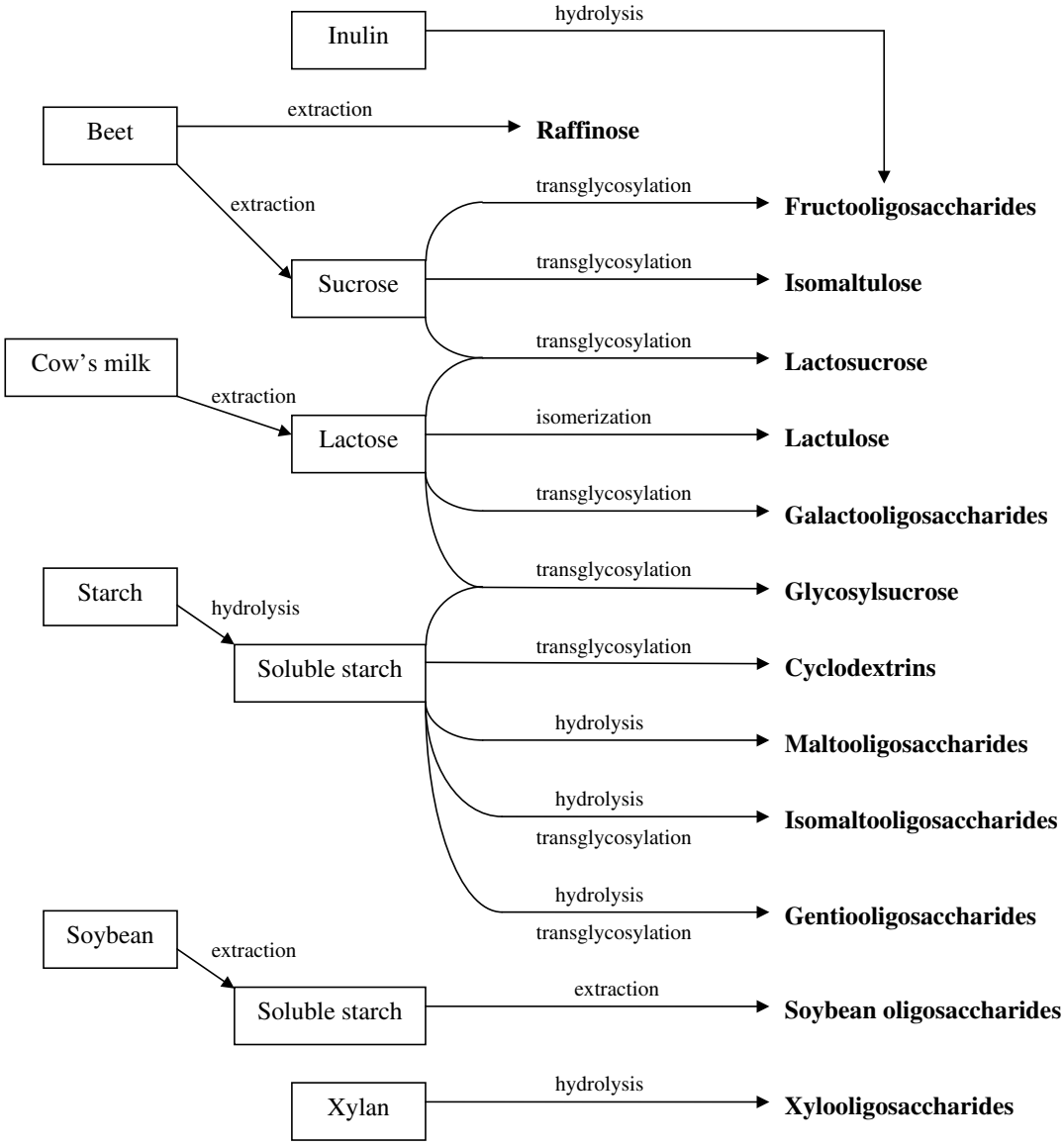

Fig. 2. Schematic representation of production processes of non-digestible oligosaccharides (adapted from Sako et al., 1999).

Lactosucrose is the third NDO that is produced using lactose as raw material. This trisaccharide is produced in a reversible transfer reaction starting from lactose and sucrose, using the transfructosylation activity of the enzyme β-fructofuranosidase. In this reaction, the fructosyl moiety of sucrose is transferred to lactose thus forming lactosucrose. However, the enzyme not only catalyzes the transfer reaction, but also catalyzes the hydrolysis of sucrose and lactosucrose. For this reason, the maximum attainable lactosucrose yield in a batch process assuming equimolar initial reactant concentration, and the absence of any parallel and consecutive reaction, is around 52%, at 50 °C (Kawase, Pilgrim, Araki, & Hashimoto, 2001).

The industrial processes for fructooligosaccharides production can be divided into two classes: in the first one, they are produced from the disaccharide sucrose using the transfructosylation activity of the enzyme β-fructofuranosidase. Similar to the production of galactooligosaccharides, a high concentration of the starting material is required for efficient transglycosylation (Park & Almeida, 1991). According to Yun (1996) it is recommended that a sucrose concentration ranging from 600 to 850 g/l should be used as a substrate in order to save evaporation cost for final processing. The second method used for fructooligosaccharides production is the controlled enzymatic hydrolysis of the polysaccharide inulin (inulin oligofructose), which can be extracted from chicory roots, for example (Crittenden & Playne, 1996). For this reason, fructooligosaccharides are easily understood as inulin-type oligosaccharides of D-fructose attached by β (2 → 1) linkages that carry a D-glycosyl residue at the end of the chain (Yun, 1996).

Isomaltulose, also referred to as palatinose, is a natural occurring disaccharide manufactured from sucrose by enzymatic rearrangement of the glycosidic linkage from a

(1,2)-fructoside to a (1,6)-fructoside followed by crystallization (Lina et al., 2002).

Glycosylsucrose is a trisaccharide manufactured from the disaccharides maltose and sucrose through the transglycosylation action catalysed by the enzyme cyclomaltodextrin glucanotransferase (Crittenden & Playne, 1996).

Maltooligosaccharides contain α-D-glucose residues linked by $\alpha(1 \rightarrow 4)$ glycosidic linkages. They are produced commercially from starch by the action of debranching enzymes such as pullulanase and isoamylase, combined with hydrolysis by various α-amylases (Crittenden & Playne, 1996).

Like maltooligosaccharides, isomaltooligosaccharides are also produced using starch as the raw material, but they require a combination of immobilized enzymes in a two-stage reactor. In the first stage, starch is liquefied using α-amylase. The liquefied starch is then processed in a second-stage that involves reactions catalysed by both β-amylase and α-glucosidase. The β-amylase first hydrolyses the liquefied starch to maltose. The transglucosidase activity of α-glucosidase then produces isomaltooligosaccharides (Kaneko et al., 1994).

Cyclodextrins are non-reducing cyclic $\alpha(1 \rightarrow 4)$-linked maltooligosaccharides. The three most common types of cyclodextrin are designed, α-, β-, and γ-, which are composed of 6, 7, and 8 glucose units, respectively. On a commercial scale, they are produced from starch using cyclodextrin glucosyltransferases, a group of amylolytic enzymes produced naturally by different strains of Bacilli and other species of bacteria. The cyclodextrin glycosyltransferase enzyme catalyses intramolecular (cyclizing) and intermolecular (coupling, disproportionation) transglycosylation as well as having a hydrolytic action on starch (Hamilton, Kelly, & Fogarty, 2000; Munro et al., 2004).

Gentiooligosaccharides consist of several glucose residues linked by $\beta(1 \rightarrow 6)$ glycosidic bounds. They are produced from acid or enzymatic hydrolysis of starch and subsequent transglycosylation action of the obtained glucose syrup catalyzed by β-glycosidase enzyme (Crittenden & Playne, 1996).

Soybean oligosaccharides are extracted directly from the raw material and do not require enzymatic manufacturing processes. Soybean whey, a by-product from the production of soy protein isolates and concentrates, contains the oligosaccharides raffinose, stachyose, and verbascose, which consist of 1, 2, or 3 α-1–6 linked units of galactose linked through α-1–3 bonds to a terminal sucrose. The oligosaccharide found in the highest concentration is stachyose, followed by raffinose, followed by verbascose (Karr-Lilienthal, Kadzere, Grieshop, & Fahey Jr, 2005).

The xylooligosaccharides production at an industrial scale is carried out from the polysaccharide xylan, which is extracted from lignocellulosic materials. Typical raw materials for xylooligosaccharides production are hardwoods, corn cobs, straws, bagasses, hulls, malt cakes and bran. Three different approaches have been used for xylooligosaccharides production from these feedstocks: (a) Enzyme treatments of native xylan-containing lignocellulosic material; (b) Chemical fractionation of a suitable lignocellulosic material to isolate (or to solubilize) xylan, with further enzymatic hydrolysis of this polymer to xylooligosaccharides; and (c) Hydrolytic degradation of xylan to xylooligosaccharides by steam, water or dilute solutions of mineral acids. For enzymatic production process, enzyme complexes with low exo-xylanase and/or β-xylosidase activity are desired, in order to avoid the xylose production (Vázquez et al., 2000).

Some NDOs are produced from two raw materials. For instance, lactosucrose is produced using lactose and sucrose, and glycosylsucrose is produced using sucrose and liquid starch (Sako et al., 1999).

In general, food grade oligosaccharides are not pure products, but are mixtures containing oligosaccharides of different degrees of polymerization, the original polysaccharide or disaccharide, and monomeric sugars. Most manufacturers produce several classes of products; higher grades contain purer oligosaccharide mixtures with lower levels of contaminating monosaccharides and reactant di- or polysaccharides. For NDOs, the absence of simple sugars lowers cariogenicity and calorific value, and allows the oligosaccharides to be included in diabetic foods (Crittenden & Playne, 1996, 2002).

5. Applications of non-digestible oligosaccharides

A number of NDOs have been introduced as functional food ingredients during the last few decades, and their industrial applications are continuously increasing. Major uses focus in beverages (fruit drinks, coffee, cocoa, tea, soda, health drinks and alcoholic beverages), milk products (fermented milk, instant powders, powdered milk and ice cream), probiotic yogurts (based on live microorganisms that exert beneficial effects for the host via improvement of the microbiological balance in the intestine) and synbiotic products (containing a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare (Gibson & Roberfroid, 1995).

Other current applications of NDOs in the food industry include desserts such as jellies, puddings and sherbets; confectionary products such as candy, cookies, biscuits, breakfast cereals; chocolate and sweets; breads and pastries; table spreads and spreads such as jams and marmalades; and meat products such as fish paste and tofu (Voragen, 1998). Nevertheless, since the specific physicochemical and physiological properties of NDOs products vary depending on the type of mixture prepared, the most appropriate oligosaccharide for a particular food application also vary (Crittenden & Playne, 1996). Bread, for example, is a suitable food for galactooligosacharides inclusion because during the fermentation with yeast and the baking of bread,

they are not broken down, and render bread excellent in taste and texture. Infant-food and food special for old-aged or hospitalized people are promising examples of products for galactooligosaccharides inclusion, since these people are more susceptible to modifications in the intestinal microflora (Sako et al., 1999).

Some non-food applications have also been proposed for oligosaccharides including drug delivery, cosmetics and mouth washes (Crittenden & Playne, 1996). The NDOs can also be employed in feed, pharmaceutical, products for diabetics, and in cosmetics as stabilizers, bulking agents, immunostimulating agents or prebiotic ingredients (Remaud-Simeon, Willemot, Sarçabal, Montalk, & Monsan, 2000). Lactulose, for example, is currently used predominantly as a pharmaceutical product controlling constipation and portosystemic encephalopathy (Villamiel et al., 2002). Isomaltooligosaccharides have been used for treatment of chronic constipation and hyperlipidemia occurring as complications of maintenance haemodialysis (Goulas et al., 2004).

Cyclodextrins are used in food, pharmaceuticals, cosmetics, environment protection, packing and textile industry. In pharmaceutical field, they are capable of alleviating the undesirable properties of drug molecules in various rotes of administration including oral, rectal, nasal, ocular, transdermal, and dermal. In environmental aspects, they play a major role in terms of solubilization of organic contaminants, enrichment and removal of organic pollutants and heavy metals from soil, water and atmosphere. They are also applied in water treatment to increase the stabilizing action, encapsulation and adsorption of contaminants. In cosmetic preparations (toothpaste, skim creams, liquid and solid fabric softeners, paper towels, tissues and underarm shields) they control the release of fragrances (Del Valle, 2004; Singh et al., 2002).

6. Novel oligosaccharides with prebiotic potential

A new source of NDOs is plant cell wall polysaccharides. Similar enzymatic hydrolysis processes could also be applied for the production of a whole array of oligosaccharides from this source. Such plant polysaccharides are often present in large amounts in fiber-rich by-products and wastes (e.g., cereal bran, fruit pomace, sugar-beet pulp, potato fiber and press cakes of oleaginous seeds or pulses). The availability of well-defined enzymes or enzyme combinations for the tailored production of NDOs from these substrates is a prerequisite (Voragen, 1998).

The potential of plant cell wall polysaccharides as sources of novel prebiotic oligosaccharides has started to receive some attention recently. Specific glycanases can be used to generate several novel oligosaccharides from plant cell wall polysaccharides (Oosterveld, Beldman, & Voragen, 2002; Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000). Arabinogalacto-oligosaccharides can be made from soybeans by endogalactanases, arabino-oligosaccharides can be made from sugar beet by endoarabinanases, rhamnogalacturono-oligosaccharides can be made from apple by rhamnogalacturonases, arabinoxylo-oligosaccharides can be made from wheat by xylanases, and galacturono-oligosaccharides can be made from polygalacturonic acid by endogalacturonases. All these oligosaccharides, except the oligo-rhamnogalacturonans and the oligogalacturonans were fermented by bifidobacteria and shown prebiotic potential (Rastall & Maitin, 2002). Cinnamoyl-oligosaccharides can be produced by hydrolysis of wheat bran and straw by an endoxylanase (Lequart, Nuzillard, Kurek, & Debeire, 1999).

Feruloylated- and *p*-coumaroylated- oligosaccharides can be isolated from *Gramineae* or from certain members of the genus *Caryophyllales* by mild acid hydrolysis or by treatment with polysaccharides hydrolyzing enzymes (Ishii, 1997). Glucooligosaccharides coming from cellulose, arabinooligosaccharides coming from arabinose, besides xylooligosaccharides, can be obtained by autohydrolysis of rice husks (Vegas, Alonso, Domínguez, & Parajó, 2004) and barley husks (Garrote, Domínguez, & Parajó, 2004). Cellooligosaccharides, which are composed of 1,4-linked β-D-glucopyranose moieties can be prepared by acid catalyzed hydrolysis of cellulose, followed by fractionation/purification of the resulting liquid phase (Akpinar, McGorrin, & Penner, 2004).

Cereal bioprocessing through enzymatic reactions or through fermentation can also produce a large range of oligosaccharides with potential prebiotic properties. The α-amylase present in the cereal grain can hydrolyse the gelatinized starch granules, and the different fractions of the oligosaccharides obtained could then be separated and their functionality could be tested (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Glucooligosaccharides can also be produced from cellobiose by transglucosylation reactions catalyzed by the β-glycosidase enzyme derived from yeast cell walls (Onishi & Tanaka, 1996). β-gluco-oligosacharides can be produced by enzymatic hydrolysations of the oat bran-β-D-glucan, by action of the endo-β-glucanase II enzyme. These oligosaccharides have been shown to enhance the growth of lactic acid bacteria and their use as prebiotics has been suggested (Kontula, von Wright, & Mattila-Sandholm, 1998).

Oligosaccharides from plants and algae have also been widely studied during the last few years. Such oligosaccharides include oligoglucans, oligochitosans and oligogalacturonates, and are called oligosaccharins (Delattre, Michaud, Lion, Courtois, & Courtois, 2005). Among these, chitosan oligosaccharides can be produced by partial hydrolysis of chitosan, from which pentamers and hexamers are obtained as reaction intermediates. There are two methods for hydrolysis of chitosan: chemical and enzymatic. Chemical hydrolysis is performed at high temperatures under highly acidic conditions and produces a large amount of glucosamine (chitosan monomer), owing to difficulties in controlling the progress of the reaction. Therefore, this method produces low yields of pentamers and hexamers. Enzymatic hydrolysis has some advantages for

the production of chitosan oligosaccharides in that some chitosanases can catalyze the hydrolysis under mild conditions and do not produce monosaccharides. Recent advances have insighted into the health benefits of chitosan oligosaccharides, including lowering of blood cholesterol, lowering of high blood pressure, protective effects against infections, controlling arthritis and enhancing antitumor properties. They are expected to be utilized as functional foods, medical supplies, and biologically active substances (Kim & Rajapakse, 2005; Ming, Kuroiwa, Ichikawa, Sato, & Mukataka, 2006).

Agar oligosaccharides can be attained by many methods, including the water extraction, ethanol extraction, acid hydrolysis and enzyme hydrolysis, which lead to different products with different activities. A special enzyme hydrolyzing the agar, agarase (agarose 4-glycanohydrolase, E.C.3.2.1.81), has been found in certain marine mollusks. However, the most was reported from several bacterial genera, including *Cytophaga*, *Vibrio*, *Streptomyces*, *Alteromonas*, *Pseudoalteromonas, and Pseudomonas*. Most of these bacteria was isolated from marine environments, while a few species isolated from rivers, hot spring, soil and sewage have also been described (Wang, Jiang, Mou, & Guan, 2004).

Further characterization of these oligosaccharides regarding their effect in several biological activity tests and their fermentability by the human intestinal flora would be helpful in verifying if they are suitable to be used as prebiotics.

7. Concluding remarks

Oligosaccharides are functional food ingredients that have great potential to improve the quality of many foods. In addition, many of these compounds possess properties that are beneficial to the health of consumers. For these reasons, the interest on the oligosaccharides use in food and pharmaceutical compounds has strongly increased in the last few years. This fact consequently has stimulated the development of researches regarding the discovery of new oligosaccharides. Recently, advances in the enzyme technology have being used to synthesize novel oligosaccharides. By enzymatic synthesis oligosaccharides can be produced in large scale, because the regio- and stereo-specificity of the reaction can be controlled (Crout & Vic, 1998). The recent advent of glycosynthases-specifically mutated glycosidases, that efficiently synthesize oligosaccharides but do not hydrolyze them-, represents a promising alternative for synthesis of new oligosaccharides (Perugino, Trincone, Rossi, & Moracci, 2004). The field of solid-phase oligosaccharide synthesis has also gained much attention in recent years because immobilization of the sugar significantly improves the recovery of product (Seeberger & Haase, 2000).

The large-scale production of oligosaccharides using either glycosyltransferases isolated from engineered microorganisms or whole cells as an enzyme source could promote a new era in the field of carbohydrate synthesis (Endo & Koizumi, 2000). Recent advances in the cloning and expression of bacterial glycosyltransferase genes have enabled the production of several oligosaccharides in large quantities. Further advances in the genetic engineering of mammalian glycosyltransferases and the exploration of novel bacterial glycosyltransferase genes should expand the diversity of oligosaccharides that can be produced on an industrial scale (Endo & Koizumi, 2000).

Another promissory area for oligosaccharides production that might be explored focus on lignocellulosic materials. Some researches demonstrated that it is possible to obtain different types of oligosaccharides using these materials as raw-material. Nevertheless, the effects for the health proportioned by ingestion of these oligosaccharides also need to be evaluated, because when metabolized, the different groups in the structure may cause different effects to the human organism.

The discovery of new food-grade oligosaccharides is of great interest because it is continuously increased the amount of products containing oligosaccharides introduced in the market, in pharmaceutical compounds and mainly in food, where they can act as prebiotic ingredients, alone or in synbiotic preparations. The development of new functional ingredients has the advantage that food manufacturers can add extra value to products the consumer is already familiar with.

In fact, nowadays, NDOs are recognized as important food ingredients to keep and improve our health, and as many consumers depend on processed foods as the mainstay of their diets, the increased NDOs content of popular foods assist consumers in obtaining recommended levels of unavailable carbohydrate. Moreover, as is known that the bifidobacterial number in the human gut tend to decrease with age, the ingestion of bifidobacteria-containing preparations or foods, or food supplemented with substrates (bifidogenic factors or prebiotics) that specifically promote the growth of endogenous bifidobacteria in the gut, is very useful for the solution of this problem. In this sense, synbiotic health-food products containing both probiotic bifidobacteria and prebiotic oligosaccharides are the more recent novelty in the food industry. The advantages provided to food manufacturers by the physicochemical properties of oligosaccharides, combined with the growing consumer interest in preventive health reinforce the expectancies that the dietary supplement industry will continue to exhibit strong growth, and the oligosaccharides production and use will continue to expand.

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