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Schematic overview of oligosaccharides, with survey on their major physiological effects and a focus on milk ones

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ABSTRACT

While Biochemistry textbooks for University degree courses do not deal with oligosaccharides, this subject is now becoming essential in Biomedical Sciences for several reasons. Oligosaccharides are currently used in foods and beverages for their useful physicochemical and prebiotic qualities, albeit their excessive consumption may have side effects. They are also essential components of milk, where they exert a protective role on infants acting as stimulants for the development of immune system and gut microbiota. Oligosaccharides are seen as an alternative to improperly used antibiotics in the diet for monogastric animals and considered promising safe anti-parasite treatments in agriculture. Besides, they are widely used in both cosmetics and pharmaceutics. For these reasons, biotechnological research is hardworking on new enzymes able to synthesize them in a great variety. At the same time, oligosaccharides belong to the so-called FODMAP (Fermentable Oligo-, Di- and Monosaccharides And Polyols) group of compounds, whose decreased use in diet is beneficial to several syndromes of the digestive tract. The scope of this review is to give a general and schematic view of oligosaccharides. We describe the structure, source and use of several of the most known oligosaccharides, focusing on their health implications. Main groups in human milk and their substitutes in infant formulas are also reported. In the end some of the most recent studied oligosaccharides and their promising applications are discussed.

1. Introduction

In general, polysaccharides are molecules with a degree of polymerization (DP) higher than 20–25, while oligosaccharides contain 2–10 sugar units. However, this definition is not so strict since even in the presence of 25 residues they are still called oligosaccharides. Polysaccharides are well described in biology and biochemistry textbooks while the same does not apply for oligosaccharides, despite the increasing interest of the subject in biomedical sciences. Indeed, apart from their essential role as recognition molecules due to glycoconjugation to proteins and lipids, they are also naturally present or artificially added in foods and beverages (Ibrahim, 2018; Varki, 2017). Their beneficial effects on growth and health are typically recognized as milk components. This, together with their physicochemical qualities, has warranted their use as food additives (McKeen et al., 2019; Patel & Goyal, 2011). However, their use is not only limited to dairy and food industry, but also extended to cosmetics, pharmaceutics, farming, agriculture and remediation (Braga Carneiro et al., 2019; Crini, 2014; Falcon Rodriguez, Costales Menendez, Gonzalez-Pena Fundora, & Napoles Garcia, 2015; Hong, Chang, Kim, Jung & Suh, 2017; Ibrahim, 2018; Teng & Kim, 2018; Zdunczyk, Jankowski, Juskiewicz, & Slominski, 2011). Nevertheless, an increasing body of evidence suggests that their excessive consumption is related to several side effects and its use should be avoided in syndromes of the digestive tract (Gibson & Shepherd, 2005; Priyanka, Gayam & Kupec, 2018).

An increasing importance within oligosaccharides is gained by non-digestible oligosaccharides (NDOs) which, together with fiber polysaccharides, belong to prebiotics. By definition, prebiotics are “nondigestible functional ingredients which are selectively fermented and allow specific changes, both in the composition and/or activity of the gastrointestinal microflora that confers benefits upon host well-being and health” (Davani-Davari et al., 2019; Gibson et al., 2010; Roberfroid, 2007). There is growing evidence that an imbalance of microbiota (dysbiosis) correlates with a range of diseases includ-

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Table 1
Some known natural trisaccharides.

Oligosaccharides Family Name or Abbreviation	Trisaccharide Name	Structure	Friendly description
Malto-oligosaccharides	Maltotriose	α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc	Glc(1–4)maltose
Isomalto-oligosaccharides	Panose	α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc	Glc(1–6)maltose
Gentio-oligosaccharides	Gentianose	β-D-Glc-(1→6)-α-D-Glc-(1→2)-β-D-Fru	Glc(1–6)sucrose
FOS, β-(2–1) fructans	1-kestose	β-D-Fru-(2→1)-β-D-Fru-(2→1)-α-D-Glc	Sucrose(1–2)Fru
RFOs, α-D-galactosides	raffinose	α-D-Gal-(1→6)-α-D-Glc-(1→2)-β-D-Fru	Gal(1–6)sucrose
GOS, β-D-galactosides	4′-galactosyl-lactose	β-D-Gal-(1→4)-β-D-Gal-(1→4)-D-Glc	Gal(1–4)lactose

Glucose (Glc), Fructooligosaccharides (FOS), Fructose (Fru), RFOs (Raffinose family of oligosaccharides), Galactooligosaccharides (GOS), Galactose (Gal).

ing intestinal, nervous, immunological and metabolic disorders as well as cardiovascular and kidney diseases, and colorectal cancer (Carding, Verbeke, Vipond, Corfe & Owen, 2015; Davis & Milner, 2009; Jia, Li & Zhou, 2019; Lai et al., 2019; Lenoir-Wijnkoop et al., 2007; Tsigalou, Stavropoulou & Bezirtzoglou, 2018). In many cases, symptoms are alleviated after administration of either probiotics (beneficial bacteria), prebiotics, or a mixture of them (synbiotics) (Tajadadi-Ebrahimi et al., 2014). This has brought to an enormous interest in therapeutic strategies involving diet manipulation and food supplements (Davani-Davari et al., 2019; Liu, Lkhagva, Chung, Kim & Hong, 2018; Saadat, Khosroushahi & Gargari, 2019). Hence, basic, applied and industrial research in this field is running both on physiological effects and on the bulk production of oligosaccharides (Bych et al., 2019; de Borba Gurpilhares, Paes Cinelli, Simas, Pessoa & Duraes Sette, 2019; Le Bourgot, Apper, Blat & Respondek, 2018; Scarpellini et al., 2018; Xiao, Chen, Guang, Zhang & Mu, 2019; Zhu et al., 2018).

Published reviews on oligosaccharides generally focus on a particular class of oligosaccharides and/or their specific roles and applications (like either dietary functional actions and health effects, industrial applications or physiological roles in plants) (Bali, Panesar, Bera & Panesar, 2015; Belorkar & Gupta, 2016; Bering, 2018; Davani-Davari et al., 2019; Ibrahim, 2018; Kruschitz & Nidetzky, 2020; Liu et al., 2020; Xing et al., 2020; Zdunczyk, Jankowski, Juskiewicz, & Slominski, 2011; Bose, Howlader, Wang, & Yin, 2021; Albersheim et al., 1992; da Silva & Correia, 2014; Zipfel & Oldroyd, 2017). A good review on *in vitro* anti-pathogenic effects of non-digestible oligosaccharides has recently been published (Asadpoor et al., 2020). Hopefully more attention can be given to this important class of carbohydrates not only in medicine, science and biotechnology books and journals but also in biochemistry textbooks. We would like to contribute to substantiating this topic as well, giving a comprehensive, schematic view of the most known oligosaccharides, including also the main types present in milk, with their unique protecting qualities. For the most known oligosaccharide groups, chemistry, source, functionalities, application and overridden consumption pitfalls are reported. We are sure that in the very near future, besides the many applications in food and medicinal fields, more employments will be shown for oligosaccharides in other present-day areas, such as fight against viruses, sustainable agriculture and remediation. Really there has been a boom of publications related to the oligosaccharides subject in the last ten years, subject which is strictly linked to the topic of microbiome (Lordan, Thapa, Ross & Cotter, 2020; Pujari & Banerjee, 2020). In the present article the first two sections deal respectively with the natural and the artificial sources of oligosaccharides as a whole. Then the structure, origin and use of several of the most known groups of these carbohydrates are reported, with an additional section on human milk oligosaccharides, pointing on their main types and structures. It follows a section on the physiological effects, of human milk oligosaccharides first, and of other functional oligosaccharides after, with shedding light on their side effects in particular conditions. Lastly, we offer a brief section to introduce some of the most recently studied oligosaccharides and their promising applications.

While classic disaccharides will not be treated in this article (the main disaccharides are typically well characterized in Biochemistry textbooks), in Table 1 and Fig. 1 we show the shorthand notation and structure, respectively, of a selection of trisaccharides belonging to different families. In this paper, glucose (Glc) could also be referred to as glucopyranose and fructose (Fru) as fructofuranose.

2. Natural sources of oligosaccharides

Oligosaccharides are mainly present as glycoconjugates bound to either proteins or lipids, where their structure is more frequently branched rather than linear. Within this context, they play diverse functions including water binding, stabilization of proteins, and biological recognition. Specifically, they can be recognized by specific receptors and other ligands thus regulating several cellular events such as adhesion, differentiation, biomolecules uptake, and pathway activation. Their variegate sequences and structures form the so-called 'saccharidic code' (Gabius, Andrè, Jimenez-Barbero, Romero & Solis, 2011; Lehningher, 2013).

Although they are mainly present attached to biomolecules in mammal cells and yeasts, oligosaccharides can also be found in their free form in plants, where they perform different functions including monosaccharide transport (disaccharides), energy storage and regulatory activity (Albersheim et al., 1992; Larskaya & Gorshkova, 2015; Van den Ende, 2013). In particular oligosaccharins are plant oligosaccharides with regulatory activity named by Albersheim, originating from the plant cell wall after enzymatic lysis activated by various factors including phytohormones. Following their release, they can participate in plant growth and organogenesis regulation. In addition, oligosaccharins can also be produced by enzymatic degradation of polysaccharides deriving from insects, fungi or other pathogens, stimulating the immune responses of plants (Chaliha, Rugen, Field & Kalita, 2018). Both branched and linear β-glucans are present within this family, including sulfated (1→3)-β-D-glucans, mixed (1→3/1→6)-β-D-glucans, acetylated oligoglucuronans and oligosaccharide fragments of xyloglucan containing a cellotetraose backbone (oligomer of four β-D-Glc residues joined with 1→4 glycosidic bonds). Oligosaccharides generated from chitin (a polymer of (1→4) N-acetyl-β-D-glucosamine) and from the deacetylated derivative chitosan (chitosan oligosaccharides, COS), can also act as signals activating symbiosis (Zipfel & Oldroyd, 2017). The cell signaling and physiological responses may be triggered when a carbohydrate-binding protein such as lectin for non-immune cells, or toll-like receptor (TLR) for immune cells, specifically binds to the oligosaccharide in a plant (da Silva & Correia, 2014).

In animals and humans, soluble oligosaccharides can originate from glycogen catabolism and are present in milk and, following dietary intake, in the gut. Furthermore, small amounts are present in blood and urine in the infant (Lis-Kuberska & Orczyk-Pawilowicz, 2019; Ruhaak, Stroble, Underwood & Lebrilla, 2014), more likely due to the transport of human milk oligosaccharides (HMOs) across the intestinal epithelium by receptor-mediated transcytosis or through paracellular

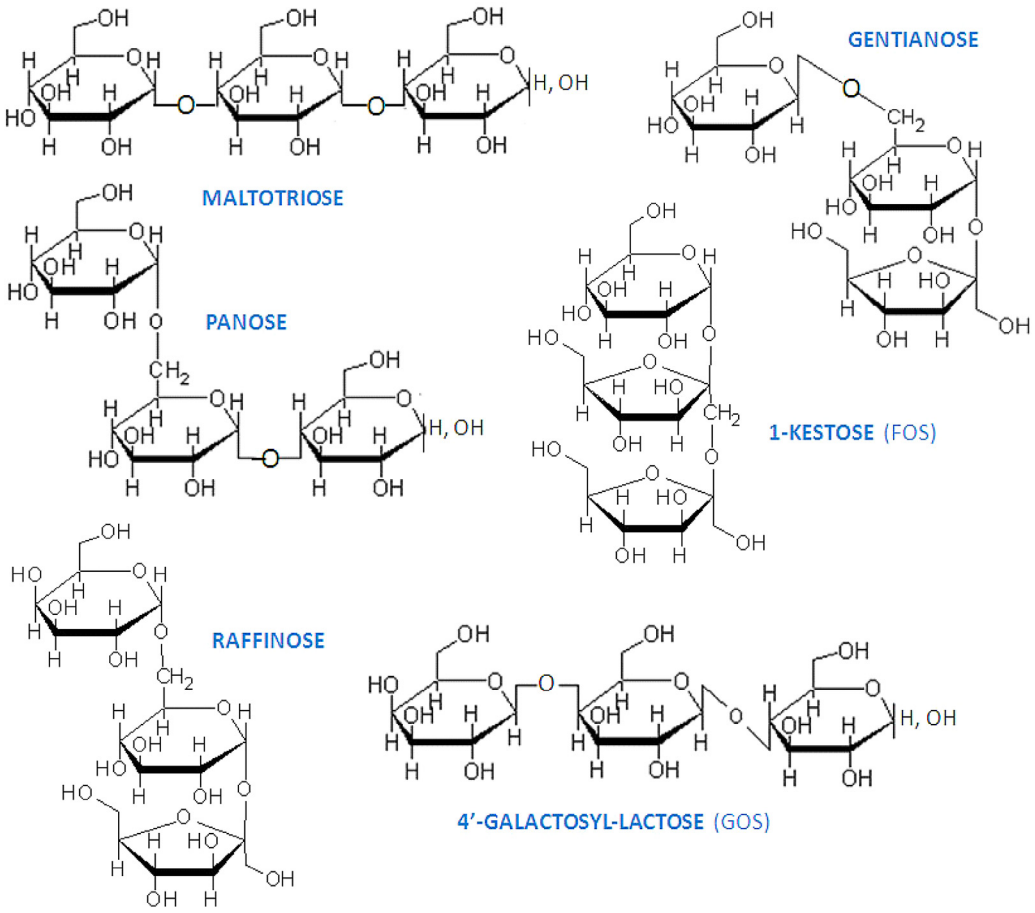


Fig. 1. Structure of some known natural trisaccharides.

routes (Gnoth, Rudloff, Kunz & Kinne, 2001; Goehring, Kennedy, Prieto & Buck, 2014).

Many types of oligosaccharides are produced by food fermentation. For instance, kojibiose (α-D-Glc-(1→2)-D-Glc) and nigerose (also called sakebiose, α-D-Glc-(1→3)-D-Glc) are not only naturally present in honey but also in beer and traditional fermented beverages (e.g., Japanese saké) and foods (Diez-Municio, Herrero, Olano & Moreno, 2014; Ibrahim, 2018; Konishi & Shindo, 1997). Further, in dairy foods such as milk and cheese whey, the disaccharide lactose (β-D-Gal-(1→4)-D-Glc) can be enzymatically transformed into lactulose (β-D-Gal-(1→4)-D-Fru, also called galacto-fructose), and into galacto-oligosaccharides (GOS, characterized by two or more galactose units linked together, Table 1 and Fig. 1) (Khatami, Ashtiani, Bonakdarpour & Mehrdad, 2014; Xiao et al., 2019). Both lactulose and GOS are not digested by monogastric animals such as humans (Diez-Municio et al., 2014; Torres, Gonc¸alves, Teixeira & Rodrigues, 2010). However, several NDOs are fermented by the lower gut microflora and show beneficial effects on microorganisms of the indigenous microbiota, thus being part of dietary prebiotics (Davani-Davari et al., 2019; Gibson et al., 2010; Gibson, Probert, Loo, Rastall & Roberfroid, 2004).

3. Artificial sources of oligosaccharides

Since the low endogenous content in foods, many types of NDOs are industrially produced after chemical, physical or enzymatic hydrolysis of polysaccharides from fibers found in a large number of roots and tubers, and tested for their health benefits (Zeng et al., 2018). Polydextrose, a randomly linked Glc oligomer containing small amounts of sorbitol and citric acid, is instead manufactured by glucose condensation through irradiation-mediated acid catalysis (do Carmo et al., 2016; Wang, Shi & Le, 2014).

In addition to chemical synthesis (Nielsen & Pedersen, 2018), NDOs can also be produced by microbiological or enzymatic synthesis starting from mono- and/or disaccharides (Diez-Municio et al., 2014; Reza, Hossain, Lee, Kim & Park, 2016). Indeed, a wide variety of enzymes from the most diverse sources, mainly microorganisms, are being disclosed. A great part of them have glycosyltransferase activity and some have specific thermal stability (Chaen et al., 2001; Diez-Municio et al., 2014; Ding, Zeng, Zhou, Yu & Chen, 2017). Glycosynthases have also been engineered to improve their catalytic efficiency or other properties such as specificity or solubility or to increase their expression in cells (Benkoulouche, Faure, Remaud-Simeon, Moulis & Andre, 2019). Metabolically engineered whole-cell catalysts can allow large-scale synthesis (Chen, 2018; Raffing & Chen, 2006; Sprenger, Baumgärtner & Albermann, 2017).

The addition of artificially produced oligosaccharides is not limited to infant formula, but also finds applications in functional food production such as soybean–oligosaccharide drinks and yoghurt. By this mean, the nutritional profile of the food is increased to deliver important benefits for disease prevention (Patel & Goyal, 2011). Several types of functional oligosaccharides will be presented in the next section. Various acronyms were introduced for functional foods such as FOSHU (Foods for Specified Health Use) (Nakakuki, 2002). Artificial oligosaccharides added to desserts, ice-creams, jams and bakery products due to their low sweetness, increased viscosity and high moisture-retaining capacity, are considered healthier than sucrose (α-D-Glc-(1→2)-D-β-Fru) (Crittenden & Playne, 1996). However, in some syndromes of the digestive tract, such as irritable bowel syndrome and ulcerative colitis, diets without

NDOs and other fermentable compounds, including fructose and polyols, are beneficial (low FODMAPs diet, where the acronym means Fermentable Oligo-, Di- and Mono-saccharides And Polyols) (Gibson & Shepherd, 2005; Priyanka et al., 2018). This will be discussed later in the review.

In addition to food industry, artificial oligosaccharides are used in other fields such as cosmetics, drug delivery, agriculture and remediation (Braga Carneiro et al., 2019; Falcon Rodriguez, Costales Menendez, Gonzalez-Pena Fundora, & Napoles Garcia, 2015; Hong et al., 2017). The starch derived α-, β- and γ-cyclodextrins composed respectively of 6, 7 and 8 Glc residues linked together through α (1→4) glycosidic bonds in a ring, form cage molecules being a paradigm of such a oligosaccharide type with applications in many areas (Crini, 2014; Ibrahim, 2018).

4. Examples of oligosaccharides

Due to the great variety of oligosaccharides occurring in nature, here we mainly focus on some of the most known types and families, several of them showing importance from the alimentary point of view. Examples of artificial oligomers are reported as well.

4.1. Fructo-oligosaccharides

4.1.1. Natural fructo-oligosaccharides

Fructo-oligosaccharides (FOS) are constituted by relatively short chains (DP < 20) of β–D-Fru units linked together by (2→1) glycosidic bonds with one terminal Glc residue. Hence, the smallest FOS consist of sucrose to which one, two or three additional Fru units are added to produce 1-kestose (Table 1 and Fig. 1), nystose and fructo-nystose, respectively (Martins Meyer, Melim Miguel, Rodriguez Fernandez & Dellamora Ortiz, 2015). They are naturally present in various fruits and vegetables (such as banana, garlic, asparagus, leeks, barley, chicory root, artichokes, wheat, onions, legumes and honey) and can be extracted from many plants (e.g., blue Agarve) (Lambertz, Weiskirchen, Landert & Weiskirchen, 2017).

4.1.2. Artificial fructo-oligosaccharides

FOS are widely used in food industry and can be produced as short chain FOS (scFOS, a mixture with DP 3–5) from beet sugar (sucrose) exploiting fructosyltransferases and other enzymes from molds, yeasts, and a few bacteria (Hidaka, Eida, Takizawa, Tokunaga & Tashiro, 1986; Picazo et al., 2019; Singh, Singh, & Kennedy, 2020). Alternatively, they are generated together with oligofructose (OF) by hydrolysis of inulin, a plant storage fructan polysaccharide present mainly in tuberous roots. Inulin, like FOS, is built up of β–D-Fru units linked by (2→1) glycosidic bonds with one terminal glucose molecule. Native inulins have a very small degree of branching (~1–2%) with a branching pattern consisting in β-D-Fru (2→6) glycosidic bonds (Maguire, 2012).

4.2. Malto-oligosaccharides

4.2.1. Natural malto-oligosaccharides

To this family belong linear polymers of D-Glc units linked with α (1→4) glycosidic bonds, ranging from the disaccharide maltose to the six units malto-oligosaccharide. The trisaccharide maltotriose (Table 1 and Fig. 1) is naturally present in the blood of certain arthropods (Florkin & Scheer, 1971). Maltotriose is a substrate for intestinal mucosal α-glucosidase (Lin et al., 2012) and it is actively transported in the cells through membrane oligosaccharide:H^+ symporters (OHS), which have been characterized in yeast, fungi and bacteria (Pao, Paulsen & Saier, 1998; Trichez et al., 2018). Biotechnological research points to increase maltotriose uptake by the permease in brewer's wort, in order to improve the quality of finished beer (Zastrow, Hollatz, de Araujo & Stambuk, 2001).

4.2.2. Artificial malto-oligosaccharides

They are digestible and nutrient sweeteners industrially produced by multi-enzymatic degradation of starch and marketed for a very high number of applications, from food to drink (beer and wine included), to candies and healthcare products processing. In addition, malto-oligosaccharides are substrates for specific mutases used to produce the disaccharide trehalose (α-D-Glc-(1→1)-α–D-Glc), which alternatively can be extracted from yeast and mushrooms (Elbein, Pan, Pastuszak & Carroll, 2003; Ibrahim, 2018). This symmetrical, non-reducing disaccharide is also present in bacteria, plants and invertebrates, where it performs several roles, including energy production, signaling, and membrane and protein protectant functions. Besides its use in food and cosmetic industries, it also has profitable applications in organ cryopreservation and medicinal stabilization (Teramoto, Sachinvala & Shibata, 2008). Maltotriose (Table 1 and Fig. 1) can be obtained by hydrolysis of the extracellular polysaccharide pullulan (exopolysaccharide) produced by some fungi. Made of maltotriose units linked by α–D-Glc (1→6) glycosidic bonds, pullulan is exploited in food and pharmaceutical industries (e.g., to make resistant coating for pills) (Mohanan and Satyanarayana, 2019); Singh, Saini & Kennedy, 2008).

4.3. Isomalto-oligosaccharides

4.3.1. Natural isomalto-oligosaccharides

Isomalto-oligosaccharides (IMOs) contain Glc residues joined with α (1→6) glycosidic bonds and have a DP mostly within the range of 2 to 6 (Yoo, Kim, Paek & Oh, 2012). The trisaccharide panose (Table 1 and Fig. 1), which is the isomer of maltotriose having one α–D-Glc (1→6) and the other α–D-Glc (1→4) glycosidic bond, is also considered an IMO and can be digested by mucosal α-D-glucosidase (Kaneko, Yokoyama & Suzuki, 1995; Lin et al., 2012). It can be produced by enzymatic hydrolysis of glycogen, amylopectin and by microrganisms (Pan, 1962). Some IMOs are present naturally in low amounts in honey and also in fermented foods.

4.3.2. Artificial isomalto-oligosaccharides

Several companies produce IMOs using starch and a combination of enzymes, giving a mixture of isomaltose, isomaltotriose and panose (Gibson et al., 2010). Unlike malto-oligosaccharides, IMOs present prebiotic activity (see following section) (Ibrahim, 2018; Yen, Tseng, Kuo, Lee & Chen, 2011). Besides, palatinose oligosaccharides, industrially produced from the disaccharide palatinose (α-D-Glc-(1→6)-D-Fru, also called isomaltulose, a low-cariogenic sweetener), are considered IMOs due to their structure which is composed of glucose residues joined together with α (1→6) glycosidic bonds linked to the glucose residue of palatinose (Barea-Alvarez, Benito, Olano, Jimeno & Moreno, 2014).

4.4. Natural gentianose, glycosyl sucrose and its oligomers

Gentianose is a trisaccharide composed of β-D-glucopyranose, α-D-glucopyranose and β-D-fructofuranose units (Table 1 and Fig. 1), which can be hydrolized by the enzyme invertase from yeasts and plants (Reynolds Green, 1901). It is the predominant carbohydrate reserve in gentian roots (Keller & Wiemken, 1982). Glycosyl sucrose is a gentianose analogue differing for the anomeric form and the link of the D-glucose residue to sucrose, being an α (1→4) instead of a β (1→6) glycosidic bond (Crittenden & Playne, 1996). It is also known as "coupling sugar", malto-oligosucrose, sucrosylglucose, erlose and glucosylsucrose, although the last name was also used for the anomer α-D-Glc-(1→2)-α-D-Glc-β-D-Fru in cyanobacteria, where other α-D-Glc residues (up to 8) could be found (1→2) linked to terminal Glc, under heat stress and aging of cells (Fischer, Geyer & Loos, 2006). Analogous oligomers family for erlose (up to pentasaccharide) is also produced by sap-feeding insects in honeydew (Bogo & Mantle, 2000).

4.5. Artificial glycosyl sucrose and gentio-oligosaccharides

Glycosyl sucrose is commercially produced as anticariogenic sweetener, which also inhibits the cariogenicity of sucrose (Nakakuki, 2002). Gentio-oligosaccharides are β-D-Glc-(1→6)-chains with DP of 2 to 6, industrially produced from glucose syrup (hydrolysed starch), particularly by enzymatic transglucosylation or by biocatalytic glycosylation with cultured cells (Crittenden & Playne, 1996; Martins Meyer et al. 2015; Yoo et al., 2012). They are prebiotics and food additives developed in Japan, giving food a unique bitter taste (chocolate, coffee and spices for instance) and vegetal juices flavor increase, beside sweetening and storage improving (Wang, Wu & Chen, 2018).

4.6. Raffinose family of oligosaccharides

The raffinose family of oligosaccharides (RFOs) contains Glc, Gal and Fru together, and belongs to the "superfamily" of the alpha-galactosides. This also includes the galactosyl cyclitols family, where Gal is bound to myo-inositol or a similar compound (Obendorf, Horbowicz & Lahuta, 2012; Piotrowicz Cieslak, Gracia Lopez & Gulewicz, 2003). RFOs are characterized by the presence of α (1→6) bonds between α-D-Gal moieties, which are bonded (1→6) to the glucose residue of the terminal sucrose. Raffinose (Raf, Table 1 and Fig. 1), the trisaccharide progenitor, is synthesized by raffinose synthase through a condensation reaction between sucrose and galactinol (composed of Gal and myoinositol) with the final release of myoinositol. Stachyose (Sta), the tetrasaccharide, consists of two α-D-Gal, one α-D-Glc and one β-D-Fru, whereas verbascose and ajugose are pentasaccharide and esasaccharide, respectively, with three or four α-D-Gal units before the sucrose unit.

There is RFO type specificity for cell compartment and for plant species. In contrast to raffinose, which is commonly found in plants, stachyose and other higher DP RFOs such as verbascose and ajugose, accumulate only in the vacuole of certain plant species (Peters & Keller, 2009). The rate of accumulation of RFOs is different in plant organs and dependent on light, temperature, salinity, water content and pathogen attack (Van den Ende, 2013). They are abundant in seeds and among all legumes, soybean, lupin and pea seeds contain the highest content of these oligosaccharides (Kuo, VanMiddlesworth & Wolf, 1988), the reason why they are also known as soybean oligosaccharides.

RFOs have an osmoprotectant function. For instance, they protect the embryo of plant seeds from desiccation occurring during maturation (Peterbauer & Richter, 2001; Peters, Mundree, Thomson, Farrant & Keller, 2007). In addition, RFOs have a wide range of cellular functions, including transport and storage of carbon, signal transduction, membrane trafficking, and antioxidant properties (Kochhar, Rees & Pollock, 2003; Sengupta, Mukherjee, Basak & Majumder, 2015; Van den Ende, 2013). Given the observation that RFOs accumulate in both generative and vegetative tissues in response to abiotic stresses such as freezing, they are currently emerging as crucial molecules in stress responses in plants (Peters & Keller, 2009, 2015; Chaliha et al., 2018). Raffinose solutions are largely used as cryopreservants, since they are more efficient than mono- and disaccharides in stabilizing the protein–lipid complex of the cell membrane (Salomon & Maxwell, 2000). Being abundant in seeds, RFOs are largely responsible of flatulence upon legumes consumption in monogastric animals like humans, which lack α-galactosidase (Han & Baik, 2006; Kuo et al., 1988). Hence, they were considered as anti-nutritional factors although they possess, as other oligosaccharides, prebiotic potential (Zdunczyk, Jankowski, Juskiewicz, & Slominski, 2011).

4.7. Lactosucrose

Lactosucrose is an analogue of raffinose, differing from the latter for the anomeric form and the bond of the D-Gal residue to sucrose, being β (1→4) instead of α (1→6). It can be naturally present at low concentration in yogurt when sucrose is present (Ibrahim, 2018). It is also artificially made by enzymatic way, starting from lactose and sucrose (Crittenden & Playne, 1996; Yoo et al., 2012). Lactosucrose is a food additive, a prebiotic, non-cariogenic, proposed as excipient in pharmaceutical and cosmetic formulations and as bioactive in prevention of skin diseases (Ibrahim, 2018; Silverio, Macedo, Teixeira & Rodrigues, 2015). In fact, the oligosaccharide should potentiate the growth of the good skin microflora, competitively reducing pathogens. It is present in patented preparations even for oral administration, such as the one used for preventing and treating the fungal infection athlete's foot (Nobuaki, 1998), and in symbiotic formulations (Bustamante et al., 2020).

4.8. Galacto-oligosaccharides

Galacto-oligosaccharides (GOS) are NDOs usually composed of 2–10 molecules of Gal and a terminal Glc unit. They are primarily derived through transgalactosylation reactions of lactose catalyzed by fungal, bacterial or yeast β-D-galactosidases (e.g. 4′-galactosyl-lactose, Table 1 and Fig. 1). This reaction causes the production of oligosaccharides with different glycosidic bonds, which are also called transgalactooligosaccharides (TOS) (Torres et al., 2010). GOS are structurally related to human milk oligosaccharides (HMOs) and both families present prebiotic properties. Although GOS concentration in milk is low, there is a great potential to produce them from by-products of dairy industry (Ibrahim, 2018; Xiao et al., 2019). GOS can be produced from lactulose (GOS-Lu) by enzymes with different features, with seemingly more prebiotic potential than GOS derived from lactose (GOS-La) (Diez-Municio et al., 2014; Hernandez-Hernandez, Montanes, Clemente, Moreno & Sanz, 2012; Xiao et al., 2019). Like FOS, they are often used in foods and dietary supplements as prebiotics, additives to improve food texture and for the low cariogenic properties. Primarily, they can be present in formula milk mixtures used as a substitute of human milk (Ibrahim, 2018). Besides, GOS intra-vaginal gel reduces the recurrence of bacterial vaginosis (BV) by restoring the beneficial vaginal flora (Coste, Judlin, Lepargmeur & Antoun, 2012).

4.9. Human milk oligosaccharides

Human milk oligosaccharides (HMOs) are the third most abundant component in dry weight of human milk (5–20 g/L) after lactose (~70 g/L) and lipids (~40 g/L). A peculiar characteristic of them is the resistance to gastrointestinal hydrolysis and to digestion catalysed by pancreatic and brush-border enzymes within the infant's intestinal lumen. Therefore, they are not absorbed in significant amounts and only up to 1% reaches the systemic circulation, where they are finally excreted intact with the infant's urine (Goehring et al., 2014). They are composed of five building blocks: Glc, Gal, N-acetylglucosamine (GlcNAc), fucose (Fuc), and the sialic acid derivative N-acetylneuraminic acid (Neu5Ac). Up to now, more than 150 different and structurally distinct HMOs have been identified, which are built of 3–22 monosaccharides, but the estimated total number of neutral and acidic HMOs exceeds 1000. Their diversity and abundance are unique to the breast milk from humans and not seen in milk from other mammals (Bode, 2018; Boehm & Stahl, 2007). The highest concentration of HMOs is found in colostrum (20 g/L), and this amount decreases to 16 g/L at day 30 of lactation and to 8 g/L after 3 months (Coppa et al., 1999; Davis, Goonatilleke, Smilowitz, German & Lebrilla, 2017).

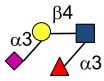
The synthesis of HMOs takes place in the Golgi apparatus of the alveolar cells within the maternal mammary glands (Rudloff et al., 2006). For the majority of HMOs the first step, represented by the lactose core formation, is catalysed by β−4-galactosyltransferase 1 in the presence of α-lactalbumin (Ramakrishnan & Qasba, 2014; Smilowitz, Lebrilla, Mills, German & Freeman, 2014). Lacto-N-tetraose (LNT) and Lacto-N-neotetraose (LNnT) (Table 2) together account for more than 90% of human milk oligosaccharides (Bering, 2018). Structurally LNT is composed of the disaccharide Lacto-N-biose (β-Gal-(1→3)-GlcNAc) added to Gal via β1–3 or β1–6 glycosidic bonds. With the same types of bond

Table 2
Prevalent Human Milk Oligosaccharides (HMOs).

Name	Abbreviation	Friendly Structure	Pattern
Lacto-N-tetraose	LNT	Lacto-N-biose β1–3 lactose	neutral
Lacto-N-neotetraose	LNnT	NAcLactosamine β1–3 lactose	neutral
2′-fucosyllactose	2′-FL	L-Fuc α1–2 lactose	neutral
Lacto-N-fucopentaose-I	LNFP-I	L-Fuc α1–2 Lacto-N-biose β1–3 lactose	neutral
Disialyllacto-N-tetraose	DS-LNT	LNT with sialyl α2–6 and α2–3 bound to GlcNAc and Gal, respectively, of the Lacto-N-biose unit	acidic

Fucose (Fuc), N-acetylglucosamine (GlcNAc), Galactose (Gal).

Table 3
Lewis-X (Le^X) and sialyl Lewis-X (sLe^X) structures.

Abbreviation	IUPAC Structure	Graphical Representation
Le^X	Galβ1–4(Fucα1–3)GlcNAc-R	
sLe^X	Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc-R	

Yellow circle, galactose (Gal); red triangle, fucose (Fuc); blu square, N-acetylglucosamine (GlcNAc), Purple diamond, sialic acid (Neu5Ac), symbols according to CFG guidelines (Varki et al., 2015).

in LNnT it is added to Gal the disaccharide N-Acetyllactosamine (β-Gal-(1→4)-GlcNAc) (Table 2). Further elongations with Lacto-N-biose or N-Acetyllactosamine units give origin to other HMOs of type I or II, respectively (Ayechu-Muruzabal et al., 2018).

The composition of HMOs in human milk varies over the course of lactation as well as between women, as specified by genetic factors, and particularly dependent on the mother's Secretor (Se) and Lewis (Le) blood group characteristics. These determine the expression of specific (1,2)-α-D-fucosyltransferase (FUT2) and (1,3/4)-α-D-fucosyltransferase (FUT3), resulting in the formation of four possible milk groups. The majority of mothers express the FUT2 dominant Se allele, which is responsible for the addition of α–D-Fuc via 1→2 linkage to terminal Gal to form 2-fucosylated structures (Kumazaki & Yoshida, 1984). 2′-fucosyllactose (2′-FL) and lacto-N-fucopentaose I (LNFP-I) (Table 2) are only few examples of the most abundant HMOs produced by the Secretor phenotype in contrast to non-Secretor one, who does not express FUT2. Accordingly, the total HMOs concentration is also influenced by the Secretor phenotype of the mother. In fact, the total concentration of HMOs found in breast milk of Secretor mothers is significantly higher than that of the non-Secretor phenotype mothers (Lis-Kuberka & Orczyk-Pawilowicz, 2019). Other α−(1,3)FUT enzymes can catalyze the formation of the trisaccharide Lewis-X (Le^X, also called CD15) and tetrasaccharide sialyl Lewis-X (sLe^X) structures (Table 3). These fucosylated lactosaminyl structures are present in both HMOs and glycoconjugates bearing key functions in cell migration, development, and immunity (Mondal et al., 2018). Dominant Le allele allows the expression of FUT3 and is present in Types I and III milk groups (Ayechu-Muruzabal et al., 2018). The proportions of fucosylated and sialylated HMOs in human term milk are 60–80% and 10–15%, respectively (Ninonuevo et al., 2006). The simplest sialylated milk oligosaccharides are the trisaccharides 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL). Others are disialyllacto-N-tetraose (DS-LNT, Table 2) and, at lower concentrations, sialyllacto-N-tetraoses (LSTa, LSTb, and LSTc) (Thurl, Munzert, Boehm, Matthews & Stahl, 2017). All milk groups exhibit an almost parallel decrease to one-third of the initial acidic oligosaccharides concentrations during the first three months of lactation (Thurl et al., 2010).

When breastfeeding is not possible, milk formulas are used as substitutes of human milk. Two main types of formula milk are available; either based on bovine milk or on modified composition of isolated and hydrolyzed bovine milk proteins. However, the majority of HMOs and glycoconjugates showing a protective effect are not found in both preparations (Lis-Kuberska & Orczyk-Pawilowicz, 2019; Wang & Brand-Miller, 2003). Therefore, prebiotic mixtures of long chain FOS (from 2 to more than 60 DP, lcFOS) and short chain GOS (from 3 to 10 DP, sc-GOS), with the typical ratio GOS:FOS of 9:1 and either derived from plant or synthesized, can be added to the formulas thus mimicking the prebiotic properties of HMOs (Bakker-Zierikzee et al., 2005; Bode & Jantscher-Krenn, 2012; Kunz, Rudloff, Baier, Klein & Strobel, 2000; Oozeer et al., 2013; Urashima, Hirabayashi, Sato & Kobata, 2018). Some HMOs have now been produced and isolated by food industry through chemical and chemoenzymatic syntheses or biotechnology (Bych et al., 2019), and preclinical studies or clinical trials in healthy infants have been conducted to ascertain the effectiveness of such HMOs-added formulas. The results from these studies showed that the supplementation of 2′-FL and LNnT is tolerable and stimulates normal growth within the first 4–6 months of life, hence commercialization has begun (Lis-Kuberska & Orczyk-Pawilowicz, 2019; Marriage, Buck, Goehring, Oliver & Williams, 2015; Puccio et al., 2017). Furthermore, HMOs concentrations are not affected by pasteurization (Daniels et al., 2017). Currently, specific HMO interventions for special groups of infants, such as preterm infants, are under investigation (Bering, 2018).

5. Physiologic activities of oligosaccharides

5.1. Human milk oligosaccharides

One of the major roles of HMOs is to function as prebiotics, fostering the growth of beneficial bacteria of colon microflora such as *Bifidobacterium, Bacteroides, Lactobacilli* while supressing that of potentially harmful strains, like *Clostridium, Enterococcus, Klebsiella, Enterobacter* (Ayechu-Muruzabal et al., 2018; Belorkar & Gupta, 2016; Shao et al., 2019). In addition, HMOs constitute a host defense system since they can act as receptor analogues for pathogens ("decoy receptors"), thus preventing their adhesion to epithelium and consequent infections (Asadpoor et al., 2020; Bode, 2012; McKeen et al., 2019). In fact, fucosylated and sialylated molecules of human milk can be recognized and bound by lectin receptors of sialic acid- and fucose-dependent pathogens (Bode, 2012). Moreover, HMOs have been re-

ported as antimicrobials that directly kill bacteria or at least reduce bacterial proliferation (Bode, 2018). This last aspect has also been related to an effect on the expression of glycosyltransferases in enteropathogenic *E. coli* (EPEC), which reduces sialylation ultimately leading to an impaired adhesion of pathogens (Angeloni et al., 2005; Bode, 2018). *In vitro* the anti-pathogenic properties of single HMOs were found to be strain-specific; for instance, LSTa exhibit a strong antimicrobial activity against *Group B Streptococcus* (GBS) (Craft, Thomas & Townsend, 2018). Glycoconjugates and free oligosaccharides unique to human milk are also classified as essential elements of the innate immunity delivered by the mother to the newborn, with the effect of strengthening their immune system (Lis-Kuberska & Orczyk-Pawilowicz, 2019). The presence of Fuc and sLe^x allows the binding of HMOs to selectin, C-type lectins and TLRs, thus interfering with selectin-mediated cell-cell interactions. This leads to a reduction in neutrophil rolling on activated endothelial cells, adhesion and transmigration as well as a reduction in neutrophil activation (Rudloff, Stefan, Pohlentz & Kunz, 2002). Moreover, sialylated HMOs have the *ex vivo* ability to reduce the formation of platelet-neutrophil complexes, which can be more easily activated than circulating neutrophils (Ayechu-Muruzabal et al., 2018; Bode, Rudloff, Kunz, Strobel & Klein, 2004; Hill & Newburg, 2015). Other glycan-binding receptors like galectins or siglecs play major roles in facilitating and regulating immune responses and represent potential DS-LNT targets (Triantis, Bode & van Neerven, 2018). To this end, HMOs derived from human colostrum have demonstrated anti-inflammatory properties with the ability to negatively affect phagocytosis and oxidative burst (Bode, 2018; Patel & Kim, 2018). In addition, they can modulate the production of chemokines and interleukins related to the maturation of lymphoid tissue, ultimately regulating the Th1/Th2 lymphocyte balance (He, Liu, Leone & Newburg, 2014). Therefore, HMOs can be considered essential for the development of the immune system in early life (Ayechu-Muruzabal et al., 2018).

Both *in vitro* and *ex vivo* data demonstrated that HMOs and human milk glycoconjugates are able to modulate microbiota and the responses and maturation of intestinal epithelial cells. In particular they contribute to the integrity of epithelial barrier of the gastrointestinal tract of neonates and protect them against necrotizing enterocolitis (NEC) (Ayechu-Muruzabal et al., 2018; He et al., 2014; Patel & Kim, 2018). Indeed, a low secretor phenotype was associated with the onset of NEC in premature infants (Morrow et al., 2011). This causality was confirmed by the observation of a lower concentration of sialylated HMOs in human milk administered to infants who developed NEC or suffered from a poorer health compared to healthy infants (Bode, 2018; Charbonneau et al., 2016). Yet, a non-secretor genotype was associated with gram-negative sepsis (Morrow et al., 2011) and it has been proposed that fucosylation in preterm milk may not be as well-regulated as in term milk (Bering, 2018; De Leoz et al., 2012). Some studies also suggest an association between human milk feeding and long-term benefits in neurodevelopment (Belfort, 2018).

As reported before, prebiotic mixtures of long chain FOS (from 2 to more than 60 DP, lcFOS) and short chain GOS (from 3 to 10 DP, scGOS), with the typical ratio GOS:FOS of 9:1, can be added to the milk formulas thus mimicking the prebiotic properties of HMOs (Bakker-Zierikzee et al., 2005; Bode & Jantscher-Krenn, 2012; Kunz et al., 2000; Oozeer et al., 2013; Urashima et al., 2018). In support of this, it has been reported that GOS- and FOS-added milk formulas positively influence the health condition of the infant by decreasing the occurrence of atopic dermatitis, allergic manifestations and infections (Lis-Kuberska & Orczyk-Pawilowicz, 2019; Vandenplas, De Greef & Veereman, 2014). In addition, it has also been observed that such glyco-added milk formula influences the microbiota composition of the infant's feces providing softer stools, despite these oligosaccharides do not contain Fuc and sialic acid residues, which are so beneficial for the infant (Bode, 2012; Lis-Kuberska & Orczyk-Pawilowicz, 2019). Addition of pectin-derived acidic oligosaccharides to come closer to the functional composition of human milk has also been suggested (Grüber et al., 2010).

5.2. FOS and other prebiotic oligosaccharides

Oligosaccharides are all considered as soluble fiber. FOS and GOS were the first oligosaccharide families extensively studied as prebiotics, enhancing beneficial microorganisms of the indigenous microbiota such as *Bifidobacteria* and *Lactobacilli* which can out-compete potential pathogenic bacteria (Gibson et al., 2004; Roberfroid, 2007; Lordan et al., 2019). They are NDOs since cannot be digested in the upper intestine of monogastric animals, although they are easily fermented by the lower gut microflora, giving 2 kcal of energy/g of material (half of digestible carbohydrates). A meta-analysis performed in humans indicated a statistically significant effect of FOS supplementation on reduction of both postprandial Glc and insulin concentrations (Kellow, Coughlan & Reid, 2014). Mechanistically, soluble dietary fiber swells in the stomach and increases the viscosity of the digesta. This leads to a decreased absorption of nutrients by the intestinal mucosa mirrored by an increase in satiety, with a lower postprandial blood Glc and a reduced insulin response. Hence, FOS and other NDOs are frequently used to enrich foods with dietary fibers and replace sugars in low-sugar foods, in order to lower blood Glc and energy content (Respondek et al., 2014).

FOS, GOS, and other prebiotic oligosaccharides are selectively fermented to lactic acid and short-chain fatty acids (SCFA) acetate, propionate and butyrate in the large intestine by a limited number of bacteria, especially *Bifidobacteria* and *Lactobacilli* (Gibson et al., 2010; Le Bourgot et al., 2018). Moreover, metabolites produced by some strains are often consumed by other beneficial species (cross-feeding) (Davani-Davari et al., 2019). In addition to the energetic role, these SCFA exert positive actions on human intestinal health, metabolism and immunity. For instance, several studies found that, SCFA provoke the acidification of the intestinal tract favouring growth and metabolism of specific species (Flint, Duncan, Scott & Louis, 2007) and stimulating the production of mucin (Schley & Field, 2002; Vogt et al., 2015; Willemsen, Koetsier, van Deventer & van Tol, 2003). Mucin is in turn consumed as energy source by newly identified health-promoting bacteria as *Akkermansia muciniphila, Roseburia hominis* and *Roseburia intestinalis*, and notably, the abundance of *A. muciniphila* is inversely correlated with obesity and type-1 diabetes (Lordan et al., 2020). The intake of some prebiotics has further been found to increase the absorption of minerals (e.g. calcium, magnesium and iron) thereby positively modulating bone mineral density, aspects that have been connected with SCFA production (Camara-Martos & Amaro-Lopez, 2002; Gibson et al., 2010; Qiang, YongLie & QianBing, 2009; Yoo et al., 2012). Moreover, several SCFA can exert direct effects by binding to the G protein-coupled receptors GPR41 and GPR43, which are expressed in several types of gastrointestinal cells, thereby stimulating the secretion of hormones with glucoregulatory actions such as glucagon-like peptide-1 (GLP-1), potentiating insulin release, satiation and satiety (Liu et al., 2020; Tolhurst et al., 2012). Besides, the activation of GPR41 and GPR43 receptors on neutrophils leads to decreased production of proinflammatory cytokines (Macfarlane & Macfarlane, 2011). However, not only decrease in "inflammaging" but also immunity reinforcing, shown as increased NK cell activity, were seen in elderly persons consuming a specific GOS mixture produced in *Bifidobacterium bifidum* (Vulevic et al., 2015). Butyrate, produced by genera as *Faecalibacterium prausnitzii*, demonstrated also other specific beneficial effects: 1) it is able to decrease the permeability of the gastrointestinal tract by a variety of mechanisms, such as upregulating the expression of tight junction proteins between gastrointestinal cells (Yan & Ajuwon, 2017; Zheng et al., 2017); 2) it showed protective effects against the risk of colorectal cancer and its progression (Davis & Milner, 2009).

The prebiotic effects of oligosaccharides extend to all body since microbiota-derived metabolites can enter blood circulation. For instance, oral GOS administration increased the water-holding capacity of the skin and prevented transepidermal water loss in UV irradiated hairless mice (Hong et al., 2015). Other protective effects on skin and other organs like brain are due to immune response-mediated pathways

(Bailey et al., 2011; Liu, Cao & Zhang, 2015). It has also been shown that SCFA can epigenetically affect genic expression in multiple tissues (Krautkramer et al., 2016). Moreover, GPCRs activation by SCFA brings neurochemicals secretion such as serotonin, and further, oligosaccharide modulation of brain-derived neurotrophic factor, neurotransmitters and synaptic proteins in rat are attributed to direct SCFA vagal stimulation (Savignac et al., 2013). A real complex gut–brain neural circuit does exist (De Vadder et al., 2014; Liu et al., 2015). Prebiotics enhanced recall and memory processes in healthy middle-aged adults (Best, Howe, Bryan, Buckley & Scholey, 2015) and in mice attenuated the accumulation of dementia-related glial protein (Han et al., 2010). Due to the shown positive SCFA effects against induced psychosocial stress in mice (Van de Wouw et al., 2018) and association between autism and dysbiosis (Adams, Johansen, Powell, Quig & Rubin, 2011), there is an expectation of prebiotic therapeutic effects in mood and stress-related diseases and autism (Davani-Davari et al., 2019).

Nowadays, FOS are used in a variety of conditions ranging from constipation relief due to their ability to absorb water and increase intestinal peristalsis to diarrhea (for the nourishment of beneficial intestinal bacteria at the expense of pathogens), after antibiotic therapy, and in hypertriglyceridemic and hypercolesterolemic conditions (Gibson et al., 2010; Mussatto & Mancilha, 2007; Singh & Singh, 2010). Studies on animal models of metabolic diseases showed that NDOs supplementation decreased inflammation markers such as haptoglobin and LPS, with further a change in the bile acid profile (Liu et al., 2020). At the same time, bile acids can activate receptors such as TGR5 and FXR, bringing beneficial effects on glucose metabolism and diet-induced obesity. However, there are also contrasting results in literature on the possible lipid lowering effects of FOS supplementation; therefore, their role as lipid profile modulator is still debated (Davani-Davari et al., 2019; Le Bourgot et al., 2018). Nevertheless, NDOs administration improves intestinal ecology in a way favouring host health, as mainly suggested from animal studies. For instance, mannan-oligosaccharides (MOS) supplementation in mice decreased the ratio of the abundance of *Firmicutes/Bacteroidetes*, a modification linked to inhibition of obesity development (Turnbaugh et al., 2006). MOS could alleviate metabolic syndrome caused by high-fat diet, with decreased weight gain, insulin resistance, and plasma lipids. GOS in mice were also able to reduce body adipocyte tissue and liver steatosis, and in addition, they decreased bile acids pool in the small intestine (Liu et al., 2020; Xiao et al., 2019). GOS proposed mechanism foresees stimulation of bacterial bile salt hydrolase producing the secondary bile acids, whose receptors are present in the gut, in white (WAT) and brown (BAT) adipose tissues, in skeletal muscles, brain and other tissues. If so, the ultimate effects should be increased WAT beiging and thermogenesis, increased tissue energy expenditure through cAMP-dependent thyroid hormone activator type 2 iodothyronine deiodinase and promoted satiety (Liu et al., 2020; Watanabe et al., 2006). In fact, the positive prebiotic effects are extremely related to counter obesity and its complications such as cardiovascular diseases, hypertension, type 2 diabetes and other chronic diseases (Liu et al., 2020). Also, there are contrasting studies regarding the prebiotic benefits in gastrointestinal disorders including irritable bowel syndrome (IBS, see following section) and Crohn's disease (Davani-Davari et al., 2019 and refs therein).

6. Side effects of oligosaccharides

The FOS intake should not exceed 20 g/day for adults and 4.2 g/day for age less than one year (Ibrahim, 2018), although the use of FOS and similar compounds is discouraged in several gastrointestinal diseases (Molodecky et al., 2012). In fact, oligosaccharides belonging to the so-called FODMAP group of compounds including fructans, FOS and GOS, lactose, fructose, and polyols are poorly assimilated and thus highly fermented within the intestine. Among polyols, sorbitol is used as sweetener and is present in stone fruits; others are mannitol, maltitol, xylitol, polydextrose, and isomalt, often used by the food industry as thickeners. A diet low in FODMAPs has been shown to improve symptoms in patients with IBS and non-celiac gluten sensitivity (NCGS) and it has also been recommended for diverticulitis, exercise-induced gastrointestinal symptoms, and inflammatory bowel diseases such as ulcerative colitis (Durchschein, Petritsch & Hammer, 2016; Jayanthi, Probert, Pinder, Wicks & Mayberry, 1992; Molodecky et al., 2012; Priyanka et al., 2018). The acronym FODMAP was first coined in 2005 by Gibson and Shepherd, who suggested a connection between western lifestyle, FODMAP-rich foods intake, and susceptibility to Crohn's disease (Gibson & Shepherd, 2005). FODMAPs might worsen IBS symptoms by increasing either small intestinal water volume or colonic gas production and intestinal motility in patients with perturbed intestinal microbiota (Catassi, Lionetti, Gatti & Catassi, 2017). Nonetheless, the British Dietetic Association (BDA) recommends this diet as the second-line intervention in IBS patients after the conventional IBS dietary intervention, e.g., the restriction of high-fiber food, resistant starch, fresh fruit, coffee, tea, alcohol, fizzy drinks and sorbitol. The original proponents recommended an "all FODMAP-free period" of 6–8 weeks, followed by a gradual reintroduction of a single FODMAPs to identify specific dietary triggers and limits of tolerance (Shepherd & Gibson, 2013; Tuck, Muir, Barrett & Gibson, 2014). In fact, a so restrictive diet excluding several cereals, all legumes, many vegetables and fruits, milk and milk-derivatives can lead to deprivation of minerals, vitamins, other antioxidant and beneficial bioactive compounds. Furthermore, the lack of FOS and GOS can cause a decrease in commensal microorganisms that in normal conditions exert beneficial effects on host metabolism, physiology and pathogen protection (actions discussed previously). Thus, nutritional supplements are suggested when following this diet (Catassi et al., 2017).

7. Other oligosaccharides and their applications

As reported previously, besides oligosaccharides naturally present in living organisms, a major part of them are produced by industry (Diez-Municio et al., 2014; Ibrahim, 2018; Martins Meyer et al., 2015; Nakakuki, 2002). A further example is represented by oligosaccharides obtained from carrageenan, linear sulfated polysaccharides consisting of D-Gal with alternating α-D-(1→3) and β-D-(1→4) bonds, extracted from red edible seaweeds (Duan, Yu, Liu, Tian & Mou, 2016). Due to its gel-forming ability and chemical stability, carrageenan has been widely applied as thickening, clarification, and gelling agents by the food, beverage, and cosmetic industries (de Borba Gurpilhares et al., 2019). Although they possess anti-inflammatory, anti-tumor, anti-coagulant, anti-hyperlipidemic and anti-thrombotic activities (Panlasigui, Baello, Dimatangal & Dumelod, 2003) their application in medicine has been limited due to their high molecular weight and low solubility. Nonetheless, the degradation of sulfated polysaccharides operated by carrageenases is a promising tool to employ for the exploitation of such novel compounds in medical field (Zhu et al., 2018).

Other complex oligosaccharides derived from plant cell walls, such as xylo-oligosaccharides (XOS) from the main hemicelluloses component of cereal fiber xylan and its derivatives, the wheat bran arabinoxylan and glucuronoarabinoxylan, demonstrated to be effective modulators of metabolism and useful prebiotics (Gibson et al., 2010; Ibrahim, 2018; Swennen, Courtin, Lindemans & Delcour, 2006; Teng & Kim, 2018). XOS found applications in food industry and pharmaceutics, further showing beneficial effects in the treatment of atopic dermatitis, a role played by the galacturonic acid-rich pectic-oligosaccharides (POS) as well (Grüber et al., 2010). POS *in vitro* inhibit proliferation and binding to gut epithelial cells of several pathogens, as well as adherence of *E. coli* on uroepithelial cells (Asadpoor et al., 2020). Modified citrus pectin (MCP) is a pectin fiber from citrus fruit peels which has been treated with a specific pH and heat-controlled hydrolysis to give a more soluble product, marketed as a dietary supplement under the PectaSol name (Yan & Katz, 2010). PectaSol-C has a lower average molecular weight of 5000–10,000 and consists of a mixture of galacturonic acid-, galactose- and arabinose-rich oligosaccharides (Courts, 2013). In clin-

ical trials both slowdowned prostate cancer and gave clinical benefits with far advanced solid tumors. Besides, in preclinical studies MCP has shown promising results in several types of tumor, including gastrointestinal, ovarian and breast cancer, and reduces fibrosis to the kidney, liver, and adipose tissue (Eliaz & Raz, 2019 and refs therein). These pleiotropic effects are greatly due to neutral POS tight binding to Gal-3 (Maxwell, Belshaw, Waldron & Morris, 2011), that in this way inhibits Gal-3 promotion of metastasis, inflammation and extracellular matrix cross-linking (Eliaz & Raz, 2019). Absorption only of galactans and arabinogalactans of MCP from the small intestinal epithelium has been elucidated (Courts, 2013). Additionally, immunomodulatory effects (Eliaz & Raz, 2019) and lowering of liver cholesterol accumulation in high-cholesterol diet are reported after POS administration in mice (Yamaguchi, Shimizu & Hatanaka, 1994). Furthermore, POS are used for chelation of toxic metals, then efficiently excreted with urine (Zhao et al., 2008; Eliaz & Raz, 2019).

Prebiotics are now increasingly used as alternatives to banned antibiotics in non-ruminant animal diets. To this end, MOS originating from yeast cell walls, and galactoglucomanno-oligosaccharides from wood, are being tested (Teng & Kim, 2018; Zdunczyk, Jankowski, Juskiewicz, & Slominski, 2011). A further new promising field of application of oligosaccharides is agriculture, where they may find a role as germination promoters as well as safe anti-parasite treatments (Falcon Rodriguez, Costales Menendez, Gonzalez-Pena Fundora, & Napoles Garcia, 2015; Mukhtar Ahmed, Khan, Siddiqui & Jahan, 2020). In addition, potential for phytoremediation has been suggested (Kollarova, Kamenicka, Vatehova & Liskova, 2018). Finally, cyclodextrins and the derived material based on these useful oligosaccharides, are also used in remediation, being effective in complexing pollutants present in soils, air and water (Crini, 2014; Topuz & Uyar, 2017).

Conclusions

While in nature oligosaccharides are mainly present as branched glycoconjugates mediating signal functions, they can also be linear and in free form, in plants, vegetables, fruits, honey and milk, where they play several physiological functions including regulatory activity. The latter group of oligosaccharides present properties that can be exploited in food, cosmetic and pharmaceutical industries. Further they are part of prebiotics that positively affect gut microflora giving benefits on human and animal health. Increasing number of studies report their effects on prevention against colorectal cancer, inflammatory bowel disease, cardiovascular diseases and metabolic disorders. The beneficial effects are mostly due to selective fermentation of the oligosaccharides to SCFA, leading to stimulation of many protective physiological functions. Besides, these carbohydrates are a fundamental part of human milk, regulating both infant bowel and immunity development. Due their low endogenous content in food, they are industrially produced and added to nutrients as functional ingredients. While chemical synthesis and engineered biocatalysis are making a big progress, bulk production is by fiber polysaccharides hydrolysis. Enzymatic processing of polysaccharides additionally produces oligosaccharide types of exceptional value, such as cyclodextrins that are useful in many fields, including nanomedicine and remediation.

This review arose with the aim to give a general and schematic view on a family of compounds largely used in everyday life. We treated the most common families such as FOS, IMOs, RFOs and GOS and dedicated a focus on HMOs which provide incomparable value to breast milk. A section mentions other classes, including marine and newly discovered/synthesized oligosaccharides. Starting from their basic structure we then described oligosaccharide derivation and pointed mainly to their functions and applications. We highlighted that in some syndromes, not exclusively of the digestive tract (autoimmune, migraine and eczema are some other examples), elimination of oligosaccharides and other fermentable compounds is beneficial. It is thus important to know their vast use.

Declaration of Competing Interests

None.

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