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Functional properties, structural studies and chemo-enzymatic synthesis of oligosaccharides

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The authors declare that there is no conflict of interest regarding the publication of this article.

Abstract: Oligosaccharides offer beneficial effects on immune system and gut health, such as anticancer activity, immunomodulatory activity, and complement activation. Functional oligosaccharides are widely found in plants, algae, bacteria and higher fungi. Milk oligosaccharides, especially human milk oligosaccharides, have considerable health benefits, such as the growth-promotion of the beneficial bacterial flora in the intestines, and developing resistance to bactertial and viral infections. Recent developments in high performance liquid chromatography, mass spectrometry, nuclear magnetic resonance and capillary electrophoresis techniques contribute to the analysis of the oligosaccharide identification and mixture quantification. Synthesis of oligosaccharides is becoming increasingly important to pharmaceutical industries, in which chemo-enzymatic synthesis is considered as an effective method. This article gives a brief summary of structures, accessible sources, physiological and chemical characteristics, and potential health benefits of functional oligosaccharides.

Keywords: Oligosaccharides; Functional properties; Structural analysis; Milk oligosaccharides; Chemo-enzymatic synthesis

1. Introduction

Personal health has become an ever-increasing important issue for consumers. Identification and characterization of functional food components have advanced nutrition science. Non-digestible dietary fibers and functional oligosaccharides are functional carbohydrates with various benefits (Bland, Keshavarz, & Bucke, 2004). According to the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature, naturally occurring carbohydrates that consist of 3–10 monosaccharide units, linear or branched, connected by α- and/or β-glycosidic linkages, are defined as oligosaccharides (or glycans). However, the physiological or rational chemical reasons for setting these limits remains unclear. Carbohydrates, whose monosaccharide units are fructose, galactose, glucose and/or xylose, are recognized as the main classes of functional oligosaccharides available at present or under development (Mussatto & Mancilha, 2007) (Fig. 1). These molecules are well-known as prebiotics, because they promote the growth of beneficial bacteria, particularly *Bifidobacteria* species. These functional oligosaccharides have shown advantageous physicochemical and physiological properties that contribute to the improvement of consumer health. Thus, application of oligosaccharides as ingredients in functional foods has great potential for improving the quality of foods in relation to consumers' health.

2. Health benefit of functional oligosaccharides

Functional oligosaccharides have been applied for many purposes, such as nutrients, pharmaceuticals, feeds, cosmetics, immunostimulating agents and prebiotic compounds (Patel & Goyal, 2011; Sako, Matsumoto, & Tanaka, 1999), which incorporate 13 classes of commercially produced non-digestible oligosaccharides showing bifidogenic functions. In addition, known functional oligosaccharides also include arabino-oligosaccharides, arabinogalacto-oligosaccharides, arabinoxylo-oligosaccharides, galacturono-oligosaccharides, rhamnogalacturonoligosaccharides, and human milk oligosaccharides (HMOs) (Table 1). In particular, cyclodextrins produced from starch through enzymatic conversion in nature is a family of macrocyclic oligosaccharides (Astray, Gonzalezbarreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009; Radu, Parteni, & Ochiuz, 2016). Cyclodextrins (α, β, and γ) are cyclic α-(1→4)-glucans with degrees of polymerization of 6, 7, and 8 monosacharide units, respectively. Macrocyclic carbohydrates have been widely applied as building-blocks in supramolecular chemistry, drug carriers, molecular reactors, and artificial receptors (Muthana, Yu, Cao, Cheng, & Chen, 2009). The use of functional oligosaccharides improves the balance of the intestinal microflora and greatly decreases the gastrointestinal

infections (Xu, Chao, & Wan, 2009). Additionally, the consumption of functional oligosaccharides can reduce the risk of lifestyle-related diseases, such as cardiovascular disease, cancer, obesity and type 2 diabetes, which are related to obesity (Mussatto & Mancilha, 2007) (Table 2). Thus, functional oligosaccharides are widely cited to be important dietary fibers in nutritional advice for metabolic syndromes induced specific disorders.

3. Sources of functional oligosaccharides

Plants and algae are the richest sources of functional oligosaccharides (Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000) (Table 2). Depolymerization of suitable raw materials or partial enzymatic hydrolysis of purified pectins can produce the pectic oligosaccharides (Gullón, Gómez, Martínez-Sabajanes, Yáñez, Parajó, & Alonso, 2013). Some typical feruloylated oligosaccharides could be prepared from plant sources, e.g., wheat, maize bran, sugarcane bagasse and rice (Qu & Sun, 2014). Particularly, marine oligosaccharides have attracted attention in drug development (Zhao, Wu, Yang, Liu, & Huang, 2015). Carrageenans, extracted from marine red algae, belong to an anion polymers family and share a common backbone of alternating (1→3)-linked β-D-galactopyranose and (1→4)-linked α-D-galactopyranose (Yao, Wu, Zhang, & Du, 2014). Carrageenans are well-known for their valuable biological activities, mainly attributed to the presence of sulphates (Kim & Rajapakse, 2005). Chitosan and its derivatives show potential in various fields such as food, cosmetics, biomedicine and agriculture. Chitosan oligosaccharides have low viscosity and high solubility in water, particularly at neutral pH. Recent studies have focused on the health benefits of chitosan oligosaccharides, such as decreasing blood cholesterol, controlling of high blood pressure, protecting from infections, and improving the antitumor properties (Zou et al, 2015).

4. Milk oligosaccharides

Milk has evolved as a complete food for mammalian nourishment during infancy. Milk oligosaccharides are the most relevant prebiotic components (Mills, Ross, Hill, & Stanton, 2011; Boehm & Stahl, 2007). The concentration of total oligosaccharides in the milk of most mammals is much too low. Moreover, the major type of oligosaccharides in human milk, fucosylated oligosaccharides, was not detected in mammalian milk (Mehra & Kelly, 2006). The concentration of human milk oligosaccharides (HMOs) in mature human milk is 10–15 g/L, which is 100- to 1000-fold higher than that in bovine milk, and the content of HMOs

often exceeds the total amount of protein in mature human milk (Table 3). Complex oligosaccharides, particularly unconjugated complex glycans, HMOs, make up a high percentage of the total solids in human milk. Nearly 200 HMOs have been identified, among which more than 80 have been fully characterized from a structural perspective. The biological functions of HMOs are closely associated with their structural conformation (Bode, 2015). Galactose, glucose, fucose, *N*-acetylglucosamine, and the sialic acid derivative, *N*-acetyl-neuraminic acid are the five monosaccharide building blocks that can constitute HMOs (Kobata, 2010). These glycans can be fucosylated and/or sialylated (Fig. 2). All HMOs carry lactose (Galβ1–4Glc) at the reducing end, which can be elongated in a β-1,3 or β-1,6-linkage by two different disaccharides, either type 1 carbohydrate structures (containing Galβ1–3GlcNAc units) or type 2 structures (containing Galβ1–4GlcNAc units). HMOs with more than 15 disaccharide units can form complex structural backbones and be further modified by adding fructose and/or sialic acid. Studies have demonstrated that HMOs can induce increased levels of bifidobacteria in the colonic flora of breast-fed infants, accompanied by a great reduction in pathogenic potential bacteria, by the bifidogenic activity of HMOs (Jin, Joo, Li, Choi, & Han, 2016). HMOs were shown to greatly affect the composition of the gut microflora. The HMOs lacto-N-fucopentaose I could be selectively utilized by *Bifidobacterium longum* subsp. *infantis*, but not *B. animalis* subsp. *lactis*, making it a promising potential prebiotic (Zhao et al., 2016). HMOs protect from viral, bacterial, or protozoan pathogens and affect fungal–host interactions (Hong, Ninonuevo, Lee, Lebrilla, & Bode, 2009; Shoaf-Sweeney & Hutkins, 2009).

5. Structural analysis of glycan oligosaccharides

Because of the complexity and heterogeneity of oligosaccharides, characterization technologies for oligosaccharides are not as advanced as the technologies for characterizing nucleic acids and proteins. Moreover, oligosaccharides are particularly difficult to separate, analyze and obtain detail structural information due to the coexisting isomeric structures and multiple connectivity sites. Many techniques have been developed to elucidate oligosaccharide structural characterization in order to understand their specific functions, however there is no legal method for analyzing and quantifying oligosaccharides (Table 4). The sensitive method of high-resolution mass spectrometry (HR-MS), which can provide a good breadth of information, has become a main tool for oligosaccharide analysis (Bao, Chen, & Newburg, 2013). Oligosaccharides fractionation attained by gel permeation chromatography (GPC) followed by analysis of high-molecular mass fractions by

matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) and electrospray ionization ion trap mass spectrometry (ESI-ITMS) indicated that complex oligosaccharides have a larger mass range compared to previous techniques (Hsu, Chang, & Franz, 2006). Although MALDI-MS has been used successfully to characterize underivatised oligosaccharides, MALDI-TOF/MS and MALDI post-source decay TOF/MS analysis are ten-fold more sensitive than MALDI-MS (Park, Yang, Kim, & Kim, 2012). Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) can be used to analyze oligosaccharides directly or with analytical derivatization (Wang, Chu, Zhao, He, & Guo, 2011). A combination of negative-ion electrospray tandem mass spectrometry (ES-MS/MS), methylation analysis, and ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR) has been applied to identify new oligosaccharides. ^{13}C- and ^{1}H-NMR, together with ES-MS, have been applied to determine the structures of complex sulfated oligosaccharides isolated from human milk (Balogh, Szarka, & Béni, 2015). FT-ICR has been used to detect oligosaccharides recently (Lee, An, Lerno, German, & Lebrilla, 2011). Additionally, the efficient method of nano-electrospray ionization mass spectroscopy (nESI-MS) with quadruple ion trap has been used to identify the position of fucose, types of linkages, and differentiation of linear and branched structures of isomeric oligosaccharides from a complex mixture of native underivatised neutral oligosaccharides (Pfenninger, Karas, Finke, & Stahl, 2002). Detect interactions of proteins with glycans or glycoconjugates by nESI-MS. The development of additional techniques may result in structural characterization of isolated oligosaccharides. The structures of HMOs are quite complex and novel techniques such as porous graphitic carbon (PGC) LC-MS are now available to perform the separation and identification of most isomers (Ruhaak, Lebrilla, Weimer, & Slupsky, 2013). Oligosaccharides studies will benefit from the application of the most advanced analytical methods, such as high performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) or capillary electrophoresis (CE), which can be used to measure samples at picomole and femtomole levels, respectively (Monti, Cattaneo, Orlandi, & Curadi, 2015; Morales, Corzo, & Sanz, 2008).

6. Chemo-enzymatic synthesis of bioactive oligosaccharides

Since the nature cannot always provide enough amounts of such functional carbohydrates for scientific research or clinical applications, development of new techniques to improve the production of such carbohydrates has become a new challenge in glycoscience. The

development of automated methods can meet the demand for molecular tools for rapid analysis of glycobiology. A few major advances in carbohydrate synthesis have been observed in recent years (Bartolozzi & Seeberger, 2001). Current methods for obtaining synthetic oligosaccharides are chemical synthesis or chemo-enzymatic synthesis (Muthana, Yu, Cao, Cheng, & Chen, 2009). The chemical synthetic pathway is challenging due to the numerous protection and deprotection steps; the large amounts of reagents and organic solvents that are often toxic; and carrying out the reactions under harsh conditions. The laborious chemical synthetic pathway frequently results in low yields. The chemo-enzymatic method involves glycosyltransferases (GT) and glycosidases (GH), which are the enzymes naturally involved in oligosaccharide synthesis in prokaryotes and eukaryotes (Yu & Chen, 2016). By exploiting these enzymes in the laboratory for the synthesis of oligosaccharides, many of the challenges faced when using chemical synthesis could be overcome. Some diferent complex oligosaccharides and derivatives with 3–11 monosaccharides units have been reported by preparative-scale and improved large-scale productions (Table 5).

Chemo-enzymatic methods can be applied in the synthesis of virtually any complex oligosaccharide (Hanson, Best, Bryan, & Wong, 2004). Enzymatic coupling has some advantages over its chemical counterpart. The use of enzymes in the synthesis of oligosaccharides has attracted growing interest as an alternative to chemical synthesis (Koeller & Wong, 2001). Glycosyltransferase-catalyzed enzymatic and chemo-enzymatic syntheses are widely considered to be effective ways for oligosaccharides production (Fig. 3). Enzymatic glycosylation occurs stereo- and regioselectively under mild conditions without protecting group manipulation. Functional enzymes enable the large-scale synthesis of difficult-to-produce saccharide linkages and complex molecules. An efficient one-pot multienzyme fucosylation system used for the gram-scale synthesis of lacto-*N*-fucopentaose I has been reported recently (Zhao et al., 2016). The development of one-pot multienzyme-catalysed syntheses reduces the substrate costs for *in vitro* production of fucosylated carbohydrates (Yu & Chen, 2016). Additionally, cost-effective large-scale production of HMOs may be conducted using whole cell systems; thus *in vitro* synthesis offers the unique advantage of flexibility. Moreover, small-scale enzymatic synthesis of structurally complex HMOs, which cannot currently be produced in engineered cells, is an invaluable tool for supporting studies on biological function and possible applications of these oligosaccharide structures.

GT's and GH's are stereo- and regioselective, therefore circumventing the tedious protection/deprotection steps. The reaction conditions are generally mild and can be carried

out in physiological buffers and temperatures eliminating the use of harsh conditions and toxic chemicals. In addition, enzymes are highly efficient and have flexibility on the substrates, which often result in great yields of oligosaccharide products. A disadvantage to the chemo-enzymatic method is identifying active GT and GH using recombinant expression systems and determining the enzyme's preferred substrate. Obtaining good expression levels in recombinant expression systems can be challenging. For example, proteins expressed in *Escherichia coli* systems may aggregate due to misfolding and many eukaryotic proteins require post-translational modifications for activities. With the advent of whole genomic sequences across species, putative GT's and GH's have been inferred by sequence homology studies.

7. Conclusions

In summary, the functional oligosaccharides are associated with a variety of biological processes such as resistance against the infection of bacteria and virus, antioxidant, antimutagenicity, cancer metastasis inhibition, blood-clotting cascade and many other pharmacological activities. However, the synergistic effect of a mixture of more structurally oligosaccharides from the nature sources should also be investigated as, most likely, one single will not provide the desired function. More efforts need to be applied for the production of more complex oligosaccharides, especially the ones that are branched. The large-scale production of oligosaccharides using multiple OPME systems or engineered *E. coli* living-cell fermentation approaches would promote a new era for oligosaccharides synthesis. The development of universal sequencing tools for oligosaccharides with comparable speed and throughput still remains a challenge, and the most advanced analytical techniques are promising to be useful tools. The identification, production and commercialization of new functional oligosaccharides with enhanced bioactive properties offer new research and business opportunities. They are good candidates for various applications in food and pharmacological industry.

Abbreviations

IUPAC	International Union of Pure and Applied Chemistry
IUBMB	International Union of Biochemistry and Molecular Biology
CDs	Cyclodextrins
HMOs	Human milk oligosaccharides
GlcNAc	*N*-acetyl-glucosamine

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Neu5Ac	*N*-acetyl-neuraminic aicd
MS	Mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
GT	Glycosyltransferases
GH	Glycosidases

Acknowledgments: This work was financially supported by National Natural Science Foundation of China (2016J06009) and Science and Technology Major Project (2014NZ0002-1) of Fujian Province, China. The project was also supported by FAFU grants KXb16011A and 612014042.

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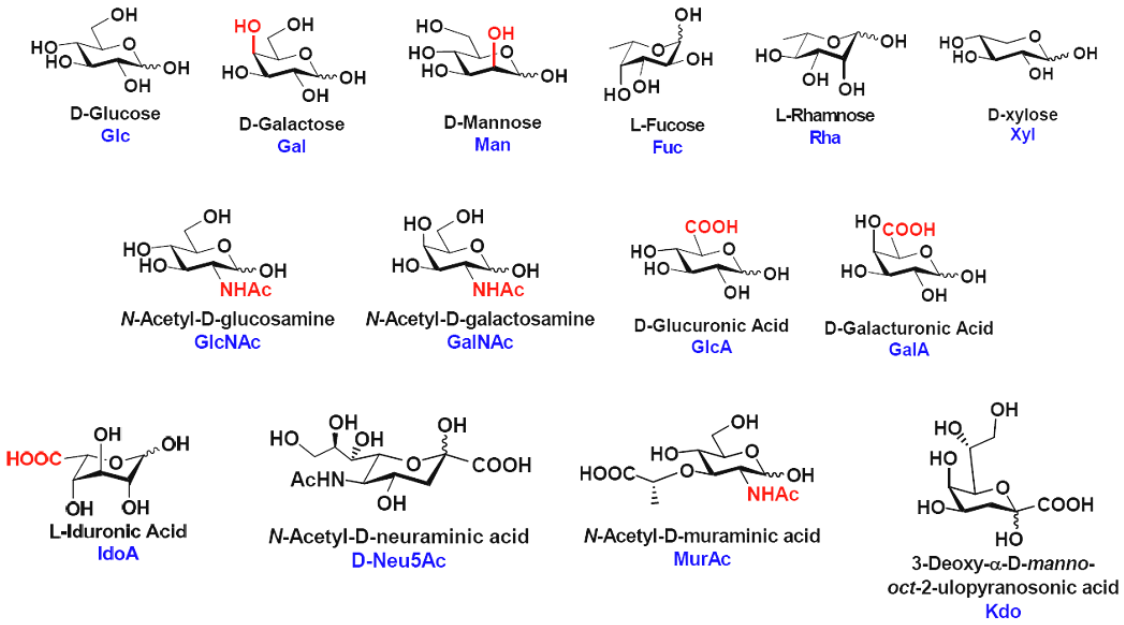

Fig. 1 Common monosaccharides components of the functional oligosaccharides.

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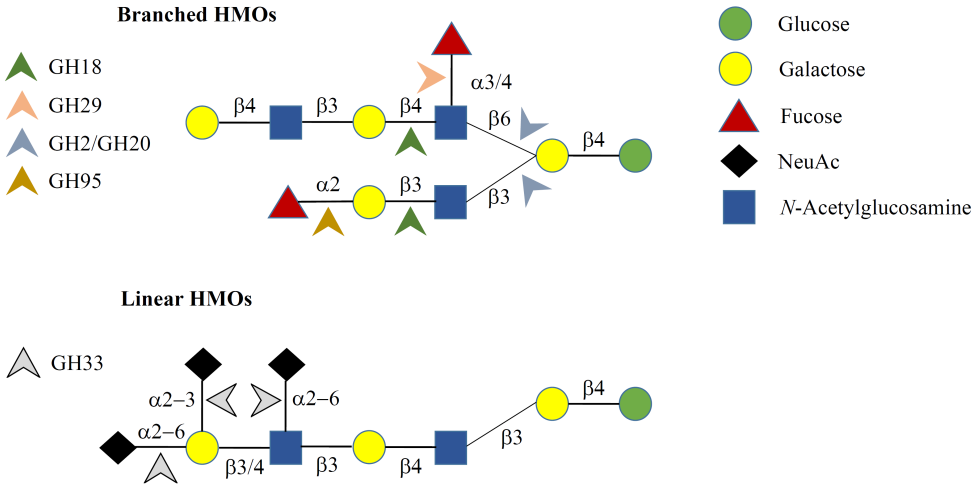

Fig. 2 Schematic of HMOs structures and putative HMOs active glycoside hydrolase families (GH) based on the work of Wu, Tao, German, Grimm, & Lebrilla (2010). GH2, α-galactosidase; GH18, endo-β-N-acetylglycosaminidase; GH20, β-hexosaminidase; GH29, α-1,3/4-fucosidase; GH33, sialidase; GH95, α-1,2-fucosidase.

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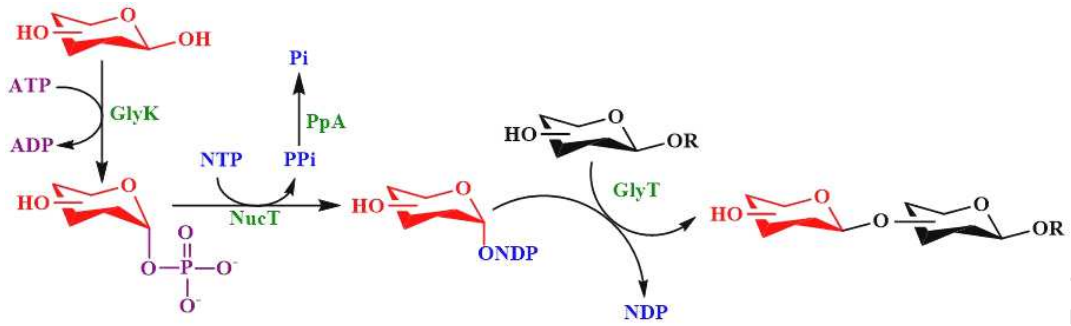

Fig. 3 The simplest routes for glycosyltransferase-catalyzed enzymatic synthesis of mammalian glycans with in situ generation of sugar nucleotides from a monosaccharide. Enzyme abbreviations: GlyK, glycokinase (Yi et al., 2009); NucT, nucleotidyltransferase; GlyT, glycosyltransferase (Zhao et al., 2016); PpA, inorganic pyrophosphatase (Lau et al., 2010).

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641 **Table 1** Natural functional oligosaccharides and their glycosidic linkages.

Type	Monosaccharides	Number of monosaccharides	Bonds indicative of functions
Arabino-oligosaccharides	Arabinose	2-8	α-1,5
Arabinogalacto-oligosaccharides	Arabinose, galactose	2-9	β-1,4
Arabinoxylo-oligosaccharides	Xylose, arabinose	5-10	α-1,2, α-1,3, β-1,4
Clycosylsucrose	Glucose, fructose	3	α-1,2, β-1,4
Cyclodextrins (CDs)	D-glucopyranose	6 (α-CD),7 (β-CD),8 (γ-CD)	α-1,4
Fructo-oligosaccharides	Sucrose, fructose	2–5	β-1,2
Galacto-oligosaccharides	Galactose	2–5	β-1,2, α-1,4
Galacturono-oligosaccharides	Galactosamine	2-9	α-1,4
Gentio-oligosaccharides	Glucose	2–10	β-1,6
Glucose-oligosaccharides	Glucose	2–10	α-1,2, β-1,3, β-1,6
Human milk oligosaccharides	Glucose, galactose, GlcNAc	2-8	α-1,2, α-1,3, α-1,4, α-2,3, β-2,6, β-1,3, β-1,4
Isomalto-oligosaccharides	Glucose	2–5	α-1,4
Lactosucrose	Galactose, fructose	2–3	β-1,4
Lactulose	Galactose, fructose	2	β-1,4
Malto-oligosaccharides	Mannitose, glucose	2–10	α-1,2, α-1,4
Palatinose	Glucose, fructose	2	β-1,6
Raffinose	Galactose, fructose, glucose	3	β-1,2, α-1,4
Rhamnogalacturon-oligosaccharides	Rhamnose, galactose	4-8	α-1,2, α-1,4, β-1,4
Soybean oligosaccharides	Fructose, galactose, glucose	2–4	α-1,6
Stachyose	Galactose, fructose, glucose	4	α-1,4
Xylo-oligosaccharides	Xylose	2–7	α-1,4

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Table 2 Sources and applications of natural and glycan oligosaccharides based on the work of Patel & Goyal (2011).

Oligosaccharides	Natural occurrence	Applications	References
Isomalto-oligosaccharide	Starch from wheat, barley, potato, rice, cassava, honey, maltose, sucrose, dextran	Antidiabetic, prevent dental caries, stimulate the growth of colonic *Bifidobacterium* and *Lactobacilli*	Basu, Mutturi, & Prapulla, 2016; Bharti et al., 2015
Soybean oligosaccharides	Soybean seed	Competitive exclusion against potential pathogenic bacteria, reduction of oxidative stress, cardio-protective, chronic diseases prevention, amelioration insulin resistance	Fei, Ling, Hua, & Ren, 2014; Zhang, Cai, & Ma, 2015
Fructo-oligosaccharides	Antichoke, garlic, onion, asparagus, chicory, fermented beverage of plant extract, *Aspergillus*, *Fusarium*, *Arthrobacter*, *Aureobasidum*, *Gluconacetobacter*, *Bacillus*, *Saccharomyces*	Prebiotic activity, prevent urogenital infections, sweetener in beverages, acariogenic quality, effect on lipid metabolism, reduce risk of colon cancer, immunomodulatory property, antidiabetic activity	Kumar, Prashanth, & Venkatesh, 2014; Okada et al., 2010; Sanches Lopes et al., 2016; Wang et al., 2010; Wang, Li, & Wang, 2016
Lactulose	Cow milk	Used in treatment of hyperammonemia and portosystemic encephalopathy	Mussatto & Mancilha, 2007; Rentschler et al., 2015
Inulin	Chicory roots, onion, asparagus, antichoke	Function as dietary fiber, effect on lipid metabolism, reduction in risk of gastrointestinal diseases; absorption of calcium, magnesium and iron increased, stimulation of immune system	Apolinario et al., 2014; Shoaib et al., 2016; Yun, Choi, Song, & Song, 1999
Galacto-oligosaccharides	*Bifidobacterium bifidum*, *Kluyveromyces lactis*, *Sulfolobus solfataricus*; Human milk, cow milk	Prebiotic	Goulas, Tzortzis, & Gibson, 2007; Kim, Park, & Oh, 2006
Gluco-oligosaccharides	*Leuconostoc mesenteroides* NRRL B-1299	Promote beneficial cutaneous flora	Iliev et al., 2008
Lactosucrose	*Pseudomonas aurantiaca*	Increase in *Bifidobacteria* population	Crittenden, & Playne, 1996; Kolida, & Gibson, 2007; Li et al., 2015; Silvério, Macedo, Teixeira, & Rodrigues, 2015
Malto-oligosaccharides	From starch by the action of pullulanase, isoamylase and amylases	Reduce the levels of *Clostridium perfringens* and family *Enterobacteriaceae*	Manas, Jonet, Murad, Mahadi, & Illias, 2015
Xylooligosaccharides	*Aspergillus*, *Trichoderma*, *Penicillium*, *Bacillus*, *Streptomyces*, hardwood, corncob,	Prebiotic, antioxidant, gelling agent, treatment of diabetes, arteriosclerosis and colon cancer	Moure, Gullón, Domínguez, & Parajó, 2006; Samanta et al., 2015; Singh, Banerjee, & Arora,

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	wheat straw, rice hull, barley straw		2015; Yang, 2016
Chitosan oligosaccharides	Depolymerised products of chitosan or chitin	Antioxidant, anti-tumor, anti-hypertensive, anti-microbial, fat-binding and hypocholesterolemic effects	Liu et al., 2010; Zou et al., 2015
Human milk oligosaccharides	Human milk	Facilitate preferential growth of *Bifidobacteria* and *Lactobacilli*, inhibition of lippolysaccharide-mediated inflammation, enhancement of brain development	He et al., 2016; Wang, 2009
β-glucan oligosaccharide	Curdlan	Induction of monocytes to produce tumor necrosis factor alpha, stimulation of the secretion of interleukin 1b	Fu et al., 2015; Kumagai, Okuyama, & Kimura, 2016
Gentio-oligosaccharides	By digestion of starch; gentiobiose; *Penicillium multicolor*	Prebiotic	Côté, 2009; Fujimoto et al., 2009
Pectin-derived oligosaccharides	Higher plants; Sugar beet pulp	Prebiotic properties, amelioration diarrhoea, adsorption of calcium ions increased, antibacterial, antihyperlipidemic and antioxidant effects	Concha Olmosa & Zúñiga Hansen, 2012; Gómez, Gullón, Yáñez, Schols, & Alonso, 2016
Cyclodextrins	Transformation of starch by certain bacteria such as *Bacillus macerans*	Stabilization of deliquescent or volatile compounds in foods and chemicals, improvement poor aqueous solubility of drug compounds	Astray, 2009; Li et al., 2010; Radu, Parteni, & Ochiuz, 2016
Arabino-oligosaccharides	Sugar beet arabinan	Prebiotic	Westphal et al., 2010

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Table 3 Distribution of oligosaccharides in human milk and bovine milk.

Abbreviation	Trivial name	Human milk (g L^{-1})*	Bovine milk (g L^{-1})*	**Structure**	Reference(s)
Neutral oligosaccharides					
2'-FL	2'-Fucosyllactose	0-3.8	-	Fucα1–2Galβ1–4Glc	Baumgärtner et al., 2015; Boehm & Stahl, 2003; Kulinich & Li, 2016; Kunz, Rudloff, Baier, Klein, & Strobel, 2000; Miyazaki, Sato, Furukawa, & Ajisaka, 2010; Perret et al., 2005
3'-FL	3'-Fucosyllactose	0.04-1.1	-	Galβ1–4(Fucα1–3)Glc	
DF-L	Difucosyllactose			Fucα1–2Galβ1–4(Fucα1–3)Glc	
LNT	Lacto-*N*-tetraose	0.5-1.5	Trace	Galβ1–3GlcNAcβ1–3Galβ1–4Glc	
LNnT	Lacto-*N*-neotetraose			Galβ1–4GlcNAcβ1–3Galβ1–4Glc	
LNFP I	Lacto-*N*-fucopentaose I	1.2-1.7	-	Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc	
LNFP-II	Lacto-*N*-fucopentaose II	0.3-1.0	-	Galβ1–3(Fucα1–4)GlcNAcβ1–3 Galβ1–4Glc	
LNFP-III	Lacto-*N*-fucopentaose III	0.01-0.2	-	Galβ1–4(Fucα1–3)GlcNAcβ1–3 Galβ1–4Glc	
LNFP-V	Lacto-*N*-fucopentaose V			Galβ1–3GlcNAcβ1–3 Galβ1–4(Fucα1–3)Glc	
LNFP-VI	Lacto-*N*-fucopentaose VI			Galβ1–4GlcNAcβ1–3 Galβ1–4(Fucα1–3)Glc	
LNDFH-I	Lacto-*N*-difucohexaose I	0.1-0.2	-	Fucα1–2Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4Glc	
LNDFH-II	Lacto-*N*-difucohexaose II			Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1–4(Fucα1–3)Glc	
LNnDFH	Lacto-*N*-neodifucohexaose			Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4(Fucα1–3)Glc	
Para-LNnH	Para-Lacto-*N*-neohexaose			Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	
LNnO	Lacto-*N*-neooctaose			Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	
LNnFP V	Lacto-*N*-neofucopentaose V			Galβ1–4GlcNAcβ1–3Galβ1–4(Fucα1–3)Glc	
LNH	Lacto-*N*-neohexaose			Gal(β1,3)GlcNAc(β1,3)[Gal(β1,4)GlcNAc(β1,6)]Gal(β1,4)Glc	
Acidic oligosaccharides					
F-SL	3'Sialyl-3fucosyllactose			Neu5Acα2–3Galβ1–4(Fucα1–3)Glc	Boehm & Stahl, 2003; Jin, Joo, Li, Choi, & Han,
6'-SL	6'Sialyllactose	0.3-0.5	0.03-0.06	Neu5Acα2–6Galβ1–4Glc	
3'-SL	3'Sialyllactose	0.1-0.3		Neu5Acα2–3Galβ1–4Glc	
LSTa	LS-Tetrasaccharide a	0.03-0.2	Trace	Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	

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LSTc	LS-Tetrasaccharide c	0.1-0.6	Trace	Neu5Acα2–6Galβ1–4GlcNAcβ1–3Galβ1–4Glc	2016; Neu et al., 2010; Tarr et al., 2015
Minor human milk oligosaccharides					
PI	BGA tetraose type 5			GalNAcα1–3(Fucα1–2)Galβ1–4Glc	Kobata, 2010
PII	BGA hexaose type 1			GalNAcα1–3(Fucα1–2)Galβ1–3GlcNAcβ1–3Galβ1–4Glc	

BGA: Blood group A antigen; * The concentrations were compiled from previous studies (Bao, Chen, & Newburg, 2013; Gopal & Gill, 2000; Gwendolyn, Philip, Li, & Anita, 2013; Kunz & Rudloff, 2002; Sumiyoshi et al., 2003).

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Table 4 The main properties and applications with different methods for oligosaccharide analysis.

Technique	Main Properties	Applications	References
HR-MS	Eelucidate molecular species without standard samples; Multiple stages of isolation and dissociation (MS^n); Limited ionization capacity and low sensitivity for oligosaccharide analysis.	Chitosan oligosaccharides; Native and permethylated human milk oligosaccharides	Cederkvist et al., 2011; Oursel, Cholet, Junot, & Fenaille, 2017
ESI-MS-MS or MS^n	Obtain information about the sequence, branching pattern and localization of modifications on oligosaccharides; Be valuable in the evaluation of isomeric oligosaccharides; Characterization of sulfated oligosaccharides ES-CID-MS/MS in the negative-ion mode.	Fructofuranosyl-containing gluco-oligosaccharides	Leijdekkers, Sanders, Schols, & Gruppen, 2011; Na et al, 2016; Tesić, Wicki, Poon, Withers, & Douglas, 2007; Veros, & Oldham, 2007; Zhang, Zhu, Zhang, Zhan, & Lin, 2014
NMR-ES-MS	Primary method for structural analysis of sulfated polysaccharide and derived oligosaccharides by NMR; Gain information about possible non-ionizable constituents; Large amounts of sample at milligram scale, quite time consuming, and a high level of expertise for NMR data interpretation.	Galactooligosaccharides	van Leeuwen, Kuipers, Dijkhuizen, & Kamerling, 2014
FT-ICR-MS	Ultra-high mass resolution and mass accuracy, non-destructive detection, high sensitivity and multistage MS^n; Identification at molecular-level analyses of organic mixtures without prior extraction or separation steps.	Thioxylo-oligosaccharide	Cederkvist et al., 2011; Jänis et al, 2005
MALDI-MS	Short analysis time, low fragmentation, wide mass range, salt and impurity tolerance of oligosaccharide analysis; Be difficult in sulfated oligosaccharides analysis due to the labile nature of the sulfate group.	Olive xylo-oligosaccharides	Reis, Coimbra, Domingues, Ferrer-Correia, & Domingues, 2002;, Kim et al, 2016
MALDI-TOF-MS	Determination of the molecular masses of neutral and acidic oligosaccharides; Process of soft-ionization causes little or no fragmentation of analytes; A qualitative profile of the solubilized oligosaccharides; Not directly distinguish anomerity or branching configuration of oligosaccharides.	Arabinoxylo-oligosaccharides; Fructans; Chitosan oligosaccharides	Yang, Lee, Lee, Kim, & Kim, 2010; Sørensen, Pedersen, & Anastyuk, Shevchenko, Nazarenko, Dmitrenok, & Zvyagintseva, 2009; Chen, Zhu, Li, Guo, & Ling, 2010; Meyer, 2007; Park, Yang, Kim, & Kim, 2012; Suzuki et al., 2011
GPC-MALDI-TOF-MS	Determining the molecular weight of polymers by GPC; Less accurate molecular weight results for cationic polymers due to aggregation and ion exclusion.	High molecular weight oligomers	Liu, Maziarz, Heiler, & Grobe, 2003
GPC-ESI-ITMS	Detection of mono-disperse oligomers; Higher chromatographic resolution compared to GPC-MALDI-TOF-MS.	Low molecular weight oligomers	Liu, Maziarz, Heiler, & Grobe, 2003
HILIC-ELSD-MS^n	Suitable for separation of highly polar carbohydrates; Detection of optical properties or functional groups of the analytes and compounds lacking chromophores.	Maltooligosaccharides; Labelled xyloglucans and xylan-derived oligosaccharides;	Leijdekkers, Sanders, Schols, & Gruppen, 2011
HILIC-TOF-MS	Faster separations with high fraction of organic solvent used in HILIC mobile phases, and higher desolvation within the MS source.	Xylo-oligosaccharides; Sake oligosaccharides	Ma, Sun, Chen, Zhang, & Zhu, 2014; Sastre, Ferreira, & Pedreschi, 2016; Tokuoka, Honda, Totsuka, Shindo, & Hosaka, 2017
HPAEC-PAD	Hydroxyl groups deprotonated to oxyanions under high pH for normal phase separation of oligosaccharides; Compatibility with gradient elution and picomolar sensitivity for oligosaccharide detection.	*N*-linked oligosaccharides	Arfelli, & Sartini, 2014; Cataldi, Campa, & De Benedetto, 2000; Maier et al., 2016
HPSEC-RI	Characterize the physicochemical properties of the interacting biopolymer fractions in detail; Be unapplied in gradient elution and sensitive to temperature by RI detector.	Xylo-oligosaccharide	

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HPAEC-CE	Obtain the high resolution under physiological conditions; Minute quantities of samples with short analysis time; Require simple preparation and fluorescent derivatization of sample.	Sialylated oligosaccharides; Glycoprotein-derived oligosaccharides	Monti, Cattaneo, Orlandi, & Curadi, 2011

HR-MS, high-resolution mass spectrometry; ES-MS-MS, electrospray tandem mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; FT-ICR-MS, fourier transform-ion cyclotron resonance-mass spectrometry; GPC, gel permeation chromatography; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; nESI-MS nano-electrospray ionization mass spectroscopy; ITMS, ion trap mass spectrometry; HPAEC, high performance anion exchange chromatography; PAD, pulsed amperometric detection; CE, capillary electrophoresis, HILIC, hydrophilic interaction liquid chromatography; HPSEC, high performance size exclusion chromatograph.

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Table 5 Chemoenzymatic synthesis of oligosaccharides.

Product	Enzyme(s)	Yield (mg)	Yield (%)	Reference
One-pot chemoenzymatic synthesis of α1–2-linked fucosides				
Fucα1–2Galβ1–3GlcNAcβProN$_3$	α1–2Te2FT, Fkp, PmPpA	50.5	96	Zhao et al., 2016
Fucα1–2Galβ1–3GlcNAcαProN$_3$	α1–2Te2FT, Fkp, PmPpA	51.3	95	Zhao et al., 2016
Fucα1–2Galβ1–3GalNAcαProN$_3$	α1–2Te2FT, Fkp, PmPpA	35.7	95	Zhao et al., 2016
Fucα1–2Galβ1–3GalNAcβProN$_3$	α1–2Te2FT, Fkp, PmPpA	43.8	98	Zhao et al., 2016
LNFP I, Fucα1–2LNT	α1–2Te2FT, Fkp, PmPpA	1146	95	Zhao et al., 2016
2'-Fucosyllactose, Fucα1–2Galβ1–4Glc	GST-α1–2-HpFucT	18	65	Albermann, Piepersberg, & Wehmeier, 2010
2'-Fucosyllactose-N$_3$	GST-WbsJ	5.2	78	Li et al., 2008a,b
Fucα1–2Galβ-OMe	GST-WbsJ	4.4	71	Li et al., 2008a,b
T-antigen-OMe, β-D-Gal-(1–3)-α-D-GalNAc-OMe	GST-WbiQ	19	100	Pettit et al., 2010
Lewis^y-tetrasaccharide	α1–2-HpFucT, α1–3-HpFucT$^{1–433}$	4	45	Stein, Lin, & Lin, 2008
One-pot chemoenzymatic synthesis of α1–3/4-linked fucosides				
Lewis^a-O-(CH$_2$)$_8$CO$_2$CH$_3$ or Lewis^x-O-(CH$_2$)$_8$CO$_2$CH$_3$	α1–3/4-HpFUCT$^{1–428}$, α1–3-FucT$^{1–441}$	-	87–94	Ma et al., 2006; Ma, Simala-Grant, & Taylor, 2006
Lewis^x	α1–3-HpFucT Δ52 FutA	-	95	Choi, Kim, Park, & Kim, 2016
3'-Fucosyllactose, Galβ1–4-(Fucα1–3-)Glc	α1–3-HpFucT Δ52 FutA	-	96	Choi, Kim, Park, & Kim, 2016
Lewis^x-ProN$_3$	HhFT1, Fkp	25	63	Zhang et al., 2010
Sialyl Lewis^x-ProN$_3$	α1–3-HpFucT$^{1–433}$, Fkp, iPPase	18.6	83	Soriano del Amo et al., 2010
LNFPIII-ProN$_3$	α1–3-HpFucT Δ52 FutA, FKP	109	92	Chen et al., 2015
LNDFH I, lacto-N-difuco-hexoase I	Commercial fucosyltransferase III (FUT3)	1.7	85	Miyazaki, Sato, Furukawa, Ajisaka, 2010
One-pot chemoenzymatic synthesis of carbohydrates				
LNT, Galβ1–3GlcNAcβ1–3Galβ1–4Glc	*Aureobacterium* sp. L-101 lacto-N-biosidase	7.1	19-26	Murata, Inukai, Suzuki,

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				Yamagishi, & Usui, 1999
LNT2, GlcNAcβ1–3Galβ1–4Glc	NmLgtA, NmLgtB	1360	95%	Johnson, 1999
LNnT, Galβ1–4GlcNAcβ1–3Galβ1–4Glc	NmLgtA, NmLgtB	1190	92	Johnson, 1999
LSTd, Neu5Acα2-3LNnT	*Trypanosoma cruzi* α2–3-trans-sialidase	138	98	Yu et al, 2014
3'-SL, Neu5Acα2–3Galβ1–4Glc	EcNanA, NmCSS, PmST1	68	68	Schmolzer, et al, 2015
DSLNnT	NmCSS, Pd2,6ST	236	99	Yu, et al, 2014
DSLac, Neu5Acα2–3(Neu5Acα2–6)Galβ1–4Glc	NmCSS, Pd2,6ST	112	93	Yu, et al, 2014
DS'LNT, Neu5Acα2–6Galβ1–3GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4Glc	NmCSS, Pd2,6ST	268	98	Yu, et al, 2014
Gb3 trisaccharide, Neu5Acα2–8Neu5Acα2–3Galβ1–4Glc	NgLgtC	5000	75	Johnson, 1999
Gb4 tetrasaccharide	NgLgtD	1500	60	Johnson, 1999
A whole-cell approach or engineered E. coli living-strategy				
3'-SL, Neu5Acα2–3Galβ1–4Glc	*Corynebacterium ammoniagenes* DN510 cells, *E. Coli* K12 CTP synthetase, *E. coli* K1 CMP-Neu5Ac synthetase, *N. gonorrhoeae* α2–3-sialyltransferase	72,000	44	Endo, Koizumi, Tabata, & Ozaki, 2000
LNT-2, GlcNAcβ1–3Galβ1–4Glc	*E. coli* JM109 (lacY+ lacZ–) with lgtA gene	6000	73	Priem, Gilbert, Wakarchuk, Heyraud, & Samain, 2002
LNnDFH, lacto-N-neodifucohexaose	NmLgtA, NmLgtB, *H. pylori* 26695 α1–3-fucosyltransferase *FutA* and *RcsA*	1700	70	Dumon, Priem, Martin, Heyraud, Bosso, & Samain, 2001
LNFP II, lacto-N-neofucopentaose II	NmLgtA, NmLgtB, *H. pylori* 26695 α1–3-fucosyltransferase *futB* gene	260	-	Dumon, Samain, & Priem, 2004
LNnFP V, Lacto-N-neofucopentaose V Gal-(β1–4)GlcNAc(β1–3)Gal(β1–4)[Fuc(α1-3)]Glc	NmLgtA, NmLgtB, *H. pylori* 26695 α1–3-fucosyltransferase *futB*	280	-	Dumon, Samain, & Priem, 2004
Lewis^{x} trisaccharide	*Helicobacter pylori* α1–3-fucosyltransferase	2100	32	Koizumi, Endo, Tabata, Nagano, Ohnishi, & Ozaki, 2000
GM2, GalNAcβ1–4(NeuAcα1–3)Galβ1–4Glc	CMP-NeuAc synthase, α2–3-sialyltransferase, UDP-GlcNAc C4	1250	-	Antoine, et al, 2003

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	epimerase, β1–4-GalNAc transferase			
GM1, Galβ1–3GalNAcβ1–4(NeuAcα1–3)Galβ1–4Glc	β1–3-galactosyltransferase	890	-	Antoine, et al, 2003
Galβ1–4(Fucα1–3)GlcNAcβ1–4GlcNAc	*Rhizobium leguminosarum* chitin-synthase NodC and *Bacillus circulans* chitinase A1	620	-	Dumon, Bosso, Utille, Heyraud, & Samain, 2006
Galβ1–4(Fucα1–3)GlcNAcβ1–3Gal	NmLgtA	1840	-	Dumon, Bosso, Utille, Heyraud, & Samain, 2006

EcNanA, *E. coli* sialic acid aldolase; FucT, fucosyltransferase; NmCSS, *Neisseria meningitidis* CMP-sialic acid synthetase; Pd2,6ST, *Photobacterium damselae* α2–6-sialyltransferase; PmPpA, *Pasteurella multocida* inorganic pyrophosphatase; PmST, *Pasteurella multocida* α2–3-sialyltransferase; PmST1, *Pasteurella multocida* α2–3-sialyltransferase 1; Psp2,6ST, *Photobacterium* sp. JT-ISH-224 α2–6-sialyltransferase; NmLgtA, *Neisseria meningitidis* β1–3-N-acetylglucosaminyltransferase ; NmLgtB, Neisseria meningitidis β1–4GalT; NgLgtC, *Neisseria gonorrhoeae* α1–4-galactosyltransferase; NgLgtD, *Neisseria gonorrhoeae* β1–3-Nacetylgalactosaminyltransferase; Pd2,6ST, *Photobacterium damselae* α2–6- sialyltransferase; HhFT1, Helicobacter hepaticus α1-3-fucosyltransferase.

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1. The biological functions of milk oligosaccharides, especially human milk oligosaccharides.
2. Developments in techniques for analysis of the oligosaccharide.
3. Advances in the oligosaccharides synthesis.

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