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

Chao Zhao[1,2], Yijing Wu[1], Xiaoyan Liu[1], Bin Liu[1], Hui Cao[3], Hua Yu[3], Satyajit D. Sarker[4], Lutfun Nahar[4], Jianbo Xiao[3]*

[1]College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China

[2]Department of Chemistry, University of California, Davis 95616, USA

[3]Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macau

[4]Medicinal Chemistry and Natural Products Research Group, School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, UK

*To whom correspondence should be addressed: Prof. Jianbo Xiao, Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macau, E-mail: jianboxiao@yahoo.com

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

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.

References

Albermann, C., Piepersberg, W., & Wehmeier, U.F. (2001). Synthesis of the milkoligosaccharide 2'-fucosyllactose using recombinant bacterial enzymes. *Carbohydrate Research*, *334*, 97–103.

Anastyuk, S.D., Shevchenko, N.M., Nazarenko, E.L., Dmitrenok, P.S., & Zvyagintseva, T.N. (2009). Structural analysis of a fucoidan from the brown alga Fucus evanescens by MALDI-TOF and tandem ESI mass spectrometry. *Carbohydrate Research*, *344*, 779–787.

Antoine, T., Priem, B., Heyraud, A., Greffe, L., Gilbert, M., Wakarchuk, W.W., et al. (2003). Large-scale *in vivo* synthesis of the carbohydrate moieties of gangliosides GM1 and GM2 by metabolically engineered *Escherichia coli*. *ChemBioChem*, *4*, 406–412.

Apolinario, A.C., Damasceno, B.P., Beltrao, N.E., Pessoa, A., Converti, A., & da Silva, J.A. (2014). Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, *101*, 368–378.

Arfelli, G. & Sartini, E. (2014). Characterisation of brewpub beer carbohydrates using high performance anion exchange chromatography coupled with pulsed amperometric detection. *Food Chemistry*, *142*, 152–158.

Astray, G., Gonzalezbarreiro, C., Mejuto, J.C., Rial-Otero, R., & Simal-Gandara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloids*, *23*, 1631–1640.

Balogh, R., Szarka, S., & Béni, S. (2015). Determination and quantification of 2'-O-fucosyllactose and 3-O-fucosyllactose in human milk by GC-MS as O-trimethylsilyl-oxime derivatives. *Journal of Pharmaceutical and Biomedical Analysis*, *115*, 450–456.

Bao, Y., Chen, C., & Newburg, D.S. (2013). Quantification of neutral human milk oligosaccharides by graphitic carbon high-performance liquid chromatography with tandem mass spectrometry. *Analytical Biochemistry*, *433*, 28–35.

Bartolozzi, A., & Seeberger, P.H. (2001). New approaches to the chemical synthesis of bioactive oligosaccharides. *Current Opinion in Structural Biology*, *11*, 587–592.

Basu, A., Mutturi, S., & Prapulla, S.G. (2016). Production of isomaltooligosaccharides (IMO) using simultaneous saccharification and transglucosylation from starch and sustainable sources. *Process Biochemistry*, *51*, 1464–1471.

Baumgärtner, F., Jurzitza, L., Conrad, J., Beifuss, U., Sprenger, G.A., & Albermann, C. (2015). Synthesis of fucosylated lacto-N-tetraose using whole-cell biotransformation. *Bioorganic and Medicinal Chemistry*, *23*, 6799-6806.

Bharti, S.K., Krishnan, S., Kumar, A., Gupta, A.K., Ghosh, A.K., & Kumar, A. (2015). Mechanism-based antidiabetic activity of Fructo- and isomalto-oligosaccharides: Validation by *in vivo*, in silico, and *in vitro*, interaction potential. *Process Biochemistry*, *50*, 317–327.

Bland, E.J., Keshavarz, T., & Bucke, C. (2004). The influence of small oligosaccharides on the immune system. *Carbohydrate Research*, *339*, 1673–1678.

Bode, L. (2015). The functional biology of human milk oligosaccharides. *Early Human Development*, *91*, 619–622.

Boehm, G., & Stahl, B. (2003). Oligosaccharides. In Functional dairy products. Mattila-Sandholm, T., Saarela, M. Eds. Woodhead Publishing Ltd., Cambridge, UK and CRC Press, Boca Raton, US, pp. 203–243.

Boehm, G., & Stahl, B. (2007). Oligosaccharides from milk. *Journal of Nutrition*, *137*, 847S–849S.

Cataldi, T.R.I., Campa, C., & De Benedetto, G.E. (2000). Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: the potential is still growing. *Fresenius Journal of Analytical Chemistry*, *368*, 739–758.

Cederkvist, F.H., Mormann, M., Froesch, M., Eijsink, V.G.H., Sørlie, M., & Peter-Katalinić, J. (2011). Concurrent enzyme reactions and binding events for chitinases interacting with chitosan oligosaccharides monitored by high resolution mass spectrometry. *International Journal of Mass Spectrometry*, *305*, 178–184.

Chen, C., Zhang, Y., Xue, M., Liu, X., Li, Y., Chen, X., et al. (2015). Sequential one-pot multienzyme (OPME) synthesis oflacto-N-neotetraose and its sialyl and fucosyl derivatives. *Chemical Communications*, *51*, 7689–7692.

Chen, M., Zhu, X., Li, Z., Guo, X., & Ling, P. (2010). Application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in preparation of chitosan oligosaccharides (COS) with degree of polymerization (DP) 5–12 containing well-distributed acetyl groups. *International Journal of Mass Spectrometry*, *290*, 94–99.

Choi, Y.H., Kim, J.H., Park, B.S., & Kim, B.G. (2016). Solubilization and iterativesaturation mutagenesis of α1,3-fucosyltransferase from *Helicobacter pylori* toenhance its catalytic efficiency. *Biotechnology and Bioengineering*, *113*, 1666–1675.

Concha Olmosa, J., & Zúñiga Hansen, M.E. (2012). Enzymatic depolymerization of sugar beet pulp: Production and characterization of pectin and pectic-oligosaccharides as a potential source for functional carbohydrates. *Chemical Engineering Journal*, *192*, 29–36.

Côté, G.L. (2009). Acceptor products of alternansucrase with gentiobiose. Production of novel oligosaccharides for food and feed and elimination of bitterness. *Carbohydrate Research*, *344*, 187–190.

Crittenden, R.G., & Playne, M.J. (1996). Production, properties and applications of food-grade oligosaccharides. *Trends in Food Science and Technology*, *71*, 353–361.

Dumon, C., Bosso, C., Utille, J.P., Heyraud, A., & Samain, E. (2006). Production of LewisX tetrasaccharides by metabolically engineered *Escherichia coli*. *ChemBioChem*, *7*, 359–365.

Dumon, C., Priem, B., Martin, S.L., Heyraud, A., Bosso, C., & Samain, E. (2001). *In vivo* fucosylation of Lacto-N-neotetraose and Lacto-N-neohexaose by heterologous expression of *Helicobacter pylori* α1,3-fucosyltransferase in engineered *Escherichia coli*. *Glycoconjugate Journal*, *18*, 465–474.

Dumon, C., Samain, E., & Priem, B. (2004). Assessment of the two *Helicobacter pylori* α-1,3-fucosyltransferase ortholog genes for the large-scale synthesis of Lewis^{x} human milk oligosaccharides by metabolically engineered *Escherichia coli*. *Biotechnology Progress*, *20*, 412–419.

Endo, T., Koizumi, S., Tabata, K., & Ozaki, A. (2000). Large-scale production of CMP-NeuAc and sialylated oligosaccharides through bacterial coupling. *Applied Microbiology and Biotechnology*, *53*, 257–261.

315 Fei, B.B., Ling, L., Hua, C., & Ren, S.Y. (2014). Effects of soybean oligosaccharides on antioxidant enzyme
316 activities and insulin resistance in pregnant women with gestational diabetes mellitus. *Food Chemistry*,
317 *158*, 429–432.
318 Fu, Y., Cheng, L., Meng, Y., Li, S., Zhao, X., Du, Y., et al. (2015). *Cellulosimicrobium cellulans* strain E4-5
319 enzymatic hydrolysis of curdlan for production of (1→3)-linked β-D-glucan oligosaccharides.
320 *Carbohydrate Polymers*, *134*, 740–744.
321 Fujimoto, Y., Hattori, T., Uno, S., Murata, T., & Usui, T. (2009). Enzymatic synthesis of
322 gentiooligosaccharides by transglycosylation with beta-glycosidases from Penicillium multicolour.
323 *Carbohydrate Research*, *344*: 972–978.
324 Gómez, B., Gullón, B., Yáñez, R., Schols, H., & Alonso, J.L. (2016). Prebiotic potential of pectins and pectic
325 oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. *Journal*
326 *of Functional Foods*, 20, 108–121.
327 Gopal, P.K., & Gill, H.S. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. *British*
328 *Journal of Nutrition*, *84*, S69–S74.
329 Goulas, A., Tzortzis, G., & Gibson, G.R. (2007). Development of a process for the production and purification
330 of a- and b-galactooligosaccharides from *Bifidobacterium bifidum* NCIMB 41171. *International Dairy*
331 *Journal*, *17*, 648–656.
332 Gullón, B., Gómez, B., Martínez-Sabajanes, M., Yáñez, R., Parajó, J.C., & Alonso, J.L. (2013). Pectic
333 oligosaccharides: Manufacture and functional properties. *Trends in Food Science and Technology*, *30*,
334 153–161.
335 Gwendolyn, G.R., Philip, N.R., Li, X., & Anita, M. (2013). Short-chain milk oligosaccharide levels in human
336 milk and infant plasma. *The FASEB Journal*, *27*, 629.16.
337 Hanson, S., Best, M., Bryan, M.C., & Wong, C.H. (2004). Chemoenzymatic Synthesis of Oligosaccharides and
338 Glycoproteins. *Trends in Biochemical Sciences*, *29*, 656–663.
339 He, Y., Liu, S., Kling, D.E., Lawlor, N.T., Huang, Y., Feinberg, S.B., et al. (2016). The human milk
340 oligosaccharide 2'-fucosyllactose modulates CD14 expression in human enterocytes, thereby attenuating
341 LPS-induced inflammation. *Gut*, *65*, 33–46.

342 Hong, P., Ninonuevo, M.R., Lee, B., Lebrilla, C., & Bode, L. (2009). Human milk oligosaccharides reduce
343 HIV-1-gp120 binding to dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN). *British*
344 *Journal of Nutrition*, *101*, 482–486.
345 Hsu, J., Chang, S.J., & Franz, A.H. (2006). MALDI-TOF and ESI-MS analysis of oligosaccharides labeled with
346 a new multifunctional oligosaccharide tag. *Journal of the American Society for Mass Spectrometry*, *17*,
347 194–204.
348 Iliev, I., Vassileva, T., Ignatova, C., Ivanova, I., Haertlé, T., Monsan, P., et al. (2008). Gluco-oligosaccharides
349 synthesized by glucosyltransferases from constitutive mutants of *Leuconostoc mesenteroides* strain Lm
350 28. *Journal of Applied Microbiology*, *104*, 243–250.
351 Jänis, J., Hakanpää, J., Hakulinen, N., Ibatullin, F.M., Hoxha, A., Derrick, P.J., et al. (2005). Determination of
352 thioxylo-oligosaccharide binding to family 11 xylanases using electrospray ionization Fourier transform
353 ion cyclotron resonance mass spectrometry and X-ray crystallography. *Febs Journal*, *272*, 2317–2333
354 Jin, S.M., Joo, W., Li, L., Choi, H.S., & Han, N.S. (2016). *In vitro*, digestion and fermentation of sialyllactoses
355 by infant gut microflora. *Journal of Functional Foods*, *21*, 497–506.
356 Johnson, K.F. (1999). Synthesis of oligosaccharides by bacterial enzymes. *Glycoconjugate Journal*, *16*,
357 141–146.
358 Kim, K.J., Kim, Y.W., Park, H.G., Hwang, C.H., Park, I.Y., Choi, K.Y., et al (2017). A MALDI-MS-based
359 quantitative glycoprofiling method on a 96-well plate platform. *Journal of Industrial and Engineering*
360 *Chemistry*, *46*, 150–156.
361 Kim, S. K., & Rajapakse, N. (2005). Enzymatic production and biological activities of chitosan
362 oligosaccharides (COS): A review. *Carbohydrate Polymers*, 62, 357–368.
363 Kim, Y.S., Park, C.S., & Oh, D.K. (2006). Lactulose production from lactose and fructose by a thermostable
364 β-galactosidase from Sulfolobus solfataricus. *Enzyme and Microbial Technology*, *39*, 903–908.
365 Kobata, A. (2010). Structures and application of oligosaccharides in human milk. *Proceedings of the Japan*
366 *Academy, Ser. B, Physical and Biological Sciences*, *86*, 731–749.
367 Koeller, K.M., & Wong, C.H. (2001). Enzymes for chemical synthesis. *Nature*, *409*, 232–239.
368 Koizumi, S., Endo, T., Tabata, K., Nagano, H., Ohnishi, J., & Ozaki, A. (2000). Large-scale production of
369 GDP-fucose and LewisX by bacterial coupling. *Journal of Industrial Microbiology and Biotechnology*,
370 *25*, 213–217.

Kolida, S., & Gibson, G.R. (2007). Inulin and oligofructose: health benefits and claims-a critical review. *Journal of Nutrition, 137*, 2489S–2597S.

Kulinich, A., & Li, L. (2016). Human milk oligosaccharides: The role in the fine-tuning of innate immune responses. *Carbohydrate Research, 432*, 62-70.

Kumagai, Y., Okuyama, M., & Kimura, A. (2016). Heat treatment of curdlan enhances the enzymatic production of biologically active β-(1,3)-glucan oligosaccharides. *Carbohydrate Polymers*, 146, 396–401.

Kumar, V.P., Prashanth, K.V.H., & Venkatesh, Y.P. (2014). Structural analyses and immunomodulatory properties of fructo-oligosaccharides from onion (*Allium cepa*). *Carbohydrate Polymers, 117*, 115–122.

Kunz, C., & Rudloff, S. (2002). Health benefit of milk-derived carbohydrates. *Bulletin of the International Dairy Federation, 375*, 72–79.

Kunz, C., Rudloff, S., Baier, W., Klein, N., & Strobel, S. (2000). Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annual Review of Nutrition, 20*, 699–722.

Lau, K., Thon, V., Yu, H., Ding, L., Chen, Y., Muthana, M.M., et al. (2010). Highly efficient chemoenzymatic synthesis of β1–4-linked galactosides with promiscuous bacterial β1–4-galactosyltransferases. *Chemical Communications, 46*, 6066–6068.

Lee, H., An, H.J., Lerno, L.A., German, J.B., & Lebrilla, C.B. (2011). Rapid profiling of bovine and human milk gangliosides by matrix-assisted laser desorption/ionization fourier transform ion cyclotron resonance mass spectrometry. *International Journal of Mass Spectrometry, 305*, 138–150.

Leijdekkers, A.G., Sanders, M.G., Schols, H.A., & Gruppen, H. (2011). Characterizing plant cell wall derived oligosaccharides using hydrophilic interaction chromatography with mass spectrometry detection. *Journal of Chromatography A, 1218*, 9227–9235.

Li, M., Liu, X.W., Shao, J., Shen, J., Jia, Q., Yi, W., et al. (2008a). Characterization of a novel α1,2-fucosyltransferase of *Escherichia coli* O128:B12 and functional investigation of its common motif. *Biochemistry, 47*, 378–387.

Li, M., Shen, J., Liu, X.W., Shao, J., Yi, W., Chow, C.S., et al. (2008b). Identification of a new alpha 1, 2-fucosyltransferase involved in O-antigen biosynthesis of *Escherichia coli* O86:B7 and formation of H-type 3 blood groupantigen. *Biochemistry, 47*, 11590–11597.

Li, T., Li, S., Du, L., Wang, N., & Guo, M. (2010). Effects of haw pectic oligosaccharide on lipid metabolism and oxidative stress in experimental hyperlipidemia mice induced by high-fat diet. *Food Chemistry*, *121*, 1010–1013.

Li, W., Wang, K., Sun, Y., Ye, H., Hu, B., & Zeng, X. (2015). Lactosucrose and its analogues derived from lactose and sucrose: Influence of structure on human intestinal microbiota *in vitro*. *Journal of Functional Food*, 17, 73–82.

Liu, H.T., He, J.L., Li, W.M., Zhu, Y., Wang, Y.X., Bai X.F., et al..(2010). Chitosan oligosaccharides protect human umbilical vein endothelial cells from hydrogen peroxide-induced apoptosis. *Carbohydrate Polymers*, *80*, 1062–1071.

Liu, X.M., Maziarz, E.P., Heiler, D.J., & Grobe, G.L. (2003). Comparative studies of poly(dimethyl siloxanes) using automated GPC-MALDI-TOF MS and on-line GPC-ESI-TOF MS. *Journal of the American Society for Mass Spectrometry*, *14*, 195–202.

Ma, B., Audette, G.F., Lin, S., Palcic, M.M., Hazes, B., & Taylor, D.E. (2006). Purification,kinetic characterization, and mapping of the minimal catalytic domain and thekey polar groups of *Helicobacter pylori* alpha-(1,3/1,4)-fucosyltransferases. *The Journal of Biological Chemistry*, *281*, 6385–6394.

Ma, B., Simala-Grant, J.L., & Taylor, D.E. (2006). Fucosylation in prokaryotes andeukaryotes. *Glycobiology*, *16*, 158R–184R.

Ma, C., Sun, Z., Chen, C., Zhang, L., & Zhu, S. (2014). Simultaneous separation and determination of fructose sorbitol, glucose and sucrose in fruits by HPLC-ELSD. *Food Chemistry*, *145*, 784–788.

Maier M, Reusch D, Bruggink C, Bulau,P., Wuhrer, M., & Mølhøj,M. (2016). Applying mini-bore HPAEC-MS/MS for the characterization and quantification of Fc N-glycans from heterogeneously glycosylated IgGs. *Journal of Chromatography B Analytical Technologies in the Biomedical & Life Sciences*, *342*, 1033–1034.

Manas, N.H.A., Jonet, M.A., Murad, A.M.A., Mahadi, N.M., & Illias, M. (2015). Modulation of transglycosylation and improved malto-oligosaccharide synthesis by protein engineering of maltogenic amylase from *Bacillus lehensis* G1. *Process Biochemistry*, *50*, 1572–1580.

Mehra, R., & Kelly, P. (2006). Milk oligosaccharides: Structural and technological aspects. *International Dairy Journal*, *16*, 1334–1340.

Mills, S., Ross, R.P., Hill, C., & Stanton, C. (2011). Milk intelligence: Mining milk for bioactive substances associated with human health. *International Dairy Journal*, *21*, 377–401.

Miyazaki, T., Sato, T., Furukawa, K., & Ajisaka, K. (2010). Enzymatic synthesis of lacto-N-difucohexaose I which binds to *Helicobacter pylori*. *Methods in Enzymology*, *480*, 511–524.

Monti, L., Cattaneo, T.M., Orlandi, M., & Curadi, M.C. (2015). Capillary electrophoresis of sialylated oligosaccharides in milk from different species. *Journal of Chromatography A*, *1409*, 288–291.

Morales, V., Corzo, N., & Sanz, M.L. (2008). HPAEC-PAD oligosaccharide analysis to detect adulterations of honey with sugar syrups. *Food Chemistry*, *107*, 922–928.

Moure, A., Gullón, P., Domínguez, H., & Parajó, J.C. (2006). Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochemistry*, *41*, 1913–1923.

Murata, T., Inukai, T., Suzuki, M., Yamagishi, M., & Usui, A.T. (1999). Facile Enzymatic Conversion of Lactose into Lacto-N-tetraose and Lacto-N-neotetraose. *Glycoconjugate Journal*, *16*, 189–195.

Mussatto, S.I., & Mancilha, I.M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, *68*, 587–597.

Muthana, S., Yu, H., Cao, H.Z., Cheng, J.S., & Chen, X. (2009). Chemoenzymatic Synthesis of a New Class of Macrocyclic Oligosaccharides. *ChemInform*, *74*, 2928–2936.

Na, L., Mao, W., Xue, L., Shuyao, W., Zheng, X., Sujian, C., et al. (2016). Sequence analysis of the pyruvylated galactan sulfate-derived oligosaccharides by negative-ion electrospray tandem mass spectrometry. *Carbohydrate Research*, *433*, 80–88.

Neu, U., Maginnis, M.S., Palma, A.S., Ströh, L.J., Nelson, C.D., Feizi, T., et al. (2010). Structure-function analysis of the human JC polyomavirus establishes the LSTc pentasaccharide as a functional receptor motif. *Cell Host and Microbe*, *8*, 309–319.

Okada, H., Fukushi, E., Yamamori, A., Kawazoe, N., Onodera, S., Kawabata, J., et al. (2010). Novel fructopyranose oligosaccharides isolated from fermented beverage of plant extract. *Carbohydrate Research*, *345*, 414–418.

Park, E., Yang, H., Kim, Y., & Kim, J. (2012). Analysis of oligosaccharides in beer using MALDI-TOF-MS. *Food Chemistry*, *134*, 1658–1664.

Patel, S., & Goyal, A. (2011). Functional oligosaccharides: production, properties and applications. *World Journal of Microbiology and Biotechnology*, *27*, 1119–1128.

Perret, S., Sabin, C., Dumon, C., Pokorná, M., Gautier, C., Galanina, O., et al. (2005). Structural basis for the interaction between human milk oligosaccharides and the bacterial lectin PA-IIL of *Pseudomonas aeruginosa*. *Biochemical Journal*, *389*, 325–332.

Pettit, N., Styslinger, T., Mei, Z., Han, W., Zhao, G., & Wang, P.G. (2010). Characterizationof WbiQ: An α 1,2-fucosyltransferase from *Escherichia coli* O127:K63(B8), andsynthesis of H-type 3 blood group antigen. *Biochemical and Biophysical Research Communications*, *402*, 190–195

Pfenninger, A., Karas, M., Finke, B., & Stahl, B. (2002). Structural analysis of underivatized neutral human milk oligosaccharides in the negative ion mode by nano-electrospray MS(n) (part 1: methodology). *Journal of the American Society for Mass Spectrometry*, *13*, 1331–1340.

Priem, B., Gilbert, M., Wakarchuk, W.W., Heyraud, A., & Samain, E.A. (2002). New fermentation process allows large-scale production of human milk oligosaccharides by metabolically engineered bacteria. *Glycobiology*, *12*, 235–240.

Qu, J., & Sun, Z. (2014). Feruloylated oligosaccharides: Structure, metabolism and function. *Journal of Functional Foods*, *7*, 90–100.

Radu, C.D., Parteni, O., & Ochiuz, L. (2016). Applications of cyclodextrins in medical textiles - review. *Journal of Controlled Release*, *224*, 146–157.

Reis, A., Coimbra, M. A., Domingues, P., Ferrer-Correia, A. J., & Domingues, M. R. M. (2002). Structural characterisation of underivatised olive pulp xylo-oligosaccharides by mass spectrometry using matrix-assisted laser desorption/ionisation and electrospray ionisation. *Rapid Communications in Mass Spectrometry Rcm*, *16*, 2124.

Rentschler, E., Schuh, K., Krewinkel, M., Baur, C., Claaßen, W., Meyer, S., et al. (2015). Enzymatic production of lactulose and epilactose in milk. *Journal of Dairy Science*, *98*, 6767–6775.

Ruhaak, L.R., Lebrilla, C.B., Weimer, B.C., & Slupsky, C. (2013). Current methods for the analysis of human milk oligosaccharides (HMOs) and their novel applications. *Metabolomics in Food and Nutrition, 2013*, 124–147.

Sako, T., Matsumoto, K., & Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal*, *9*, 69–80.

Samanta, A.K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A.P., Senani, S., et al. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrates and Dietary Fibre*, *5*, 62–71.

Sanches Lopes, S.M., Francisco, M.G., Higashi, B., de Almeida, R.T., Krausová, G., Pilau, E.J., et al. (2016). Chemical characterization and prebiotic activity of fructo-oligosaccharides from *Stevia rebaudiana* (Bertoni) roots and *in vitro* adventitious root cultures. *Carbohydrate Polymers*, *152*, 718–725.

Sastre, F., Ferreira, F., & Pedreschi, F. (2016). MALDI-TOF mass spectrometry and reversed-phase HPLC-ELSD chromatography for structural and quantitative studies of major steroid saponins in commercial extracts of Yucca schidigera Roezl, *Journal of Pharmaceutical and Biomedical Analysis*, *120*, 270–282.

Schmolzer, K., Czabany, T., Luley-Goedl, C., Pavkov-Keller, T., Ribitsch, D., Schwab, H., et al. (2015). Complete switch from α-2,3- to α-2,6-regioselectivity in *Pasteurella dagmatis* β-D-galactoside sialyltransferase by active-site redesign. *Chemical Communications*, *51*, 3083–3086.

Shoaf-Sweeney, K.D., & Hutkins, R.W. (2009). Adherence, anti-adherence, and oligosaccharides preventing pathogens from sticking to the host. *Advances in Food and Nutrition Research*, *55*, 101–161.

Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H.R., et al. (2016). Inulin: Properties, health benefits and food applications. *Carbohydrate Polymers*, *147*, 444–454.

Silvério, S.C., Macedo, E.A., Teixeira, J.A., & Rodrigues, L.R. (2015). Perspectives on the biotechnological production and potential applications of lactosucrose: A review. *Journal of Functional Food*, *19*, 74–90.

Singh, R.D., Banerjee, J., & Arora, A. (2015). Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. *Bioactive Carbohydrates and Dietary Fibre*, *5*, 19–30.

Sørensen, H.R., Pedersen, S., & Meyer, A.S. (2007). Characterization of solubilized arabinoxylo-oligosaccharides by MALDI-TOF MS analysis to unravel and direct enzyme catalyzed hydrolysis of insoluble wheat arabinoxylan. *Enzyme and Microbial Technology*, *41*, 103–110.

Soriano del Amo, D., Wang, W., Besanceney, C., Zheng, T., He, Y., Gerwe, B., et al. 2010. Chemoenzymatic synthesis of the sialyl Lewis X glycan andits derivatives. *Carbohydrate Research*, *345*, 1107–1113

Stein, D.B., Lin, Y.N., & Lin, C.H. (2008). Characterization of *Helicobacter pylori* α1,2-fucosyltransferase for enzymatic synthesis of tumor-associated antigens. *Advanced Synthesis and Catalysis*, *350*, 2313–2321

512 Sumiyoshi, W., Urashima, T., Nakamura, T., Arai, I., Saito, T., Tsumura, N., et al. (2003). Determination of
513 each neutral oligosaccharide in the milk of Japanese women during the course of lactation. *British Journal*
514 *of Nutrition*, *89*, 61–69.
515 Suzuki, T., Maeda, S., Nomura, S., Suzuki, M., Grant, G., & Sporns, P.(2011). Rapid analysis of fructans and
516 comparison offructan profiles in several different types of asparagus storage roots using MALDI–TOF
517 MS. *Journal of Horticultural Science & Biotechnology*, *86*, 210–216.
518 Oursel, S., Cholet, S., Junot, C., & Fenaille, F. (2017). Comparative analysis of native and permethylated
519 human milk oligosaccharides by liquid chromatography coupled to high resolution mass spectrometry.
520 *Journal of Chromatography B Analytical Technologies in the Biomedical & Life Sciences*, DOI:
521 10.1016/j.jchromb.2017.03.028
522 Tarr, A.J., Galley, J.D., Fisher, S., Chichlowski, M., Berg, B.M., & Bailey, M.T. (2015). The prebiotics
523 3′-sialyllactose and 6′-sialyllactose diminish stressor-induced anxiety-like behavior and colonic
524 microbiota alterations: evidence for effects on the gut-brain axis. *Brain, Behavior and Immunity*, *50*,
525 166–177.
526 Tesić, M., Wicki, J., Poon, D.K., Withers, S.G., & Douglas, D.J. (2007). Gas phase noncovalent protein
527 complexes that retain solution binding properties: Binding of xylobiose inhibitors to the beta-1, 4
528 exoglucanase from *cellulomonas fimi*. *Journal of the American Society for Mass Spectrometry*, *18*, 64–73.
529 Tokuoka, M., Honda, C., Totsuka, A., Shindo, H, & Hosaka, M. (2017). Analysis of the oligosaccharides in
530 Japanese rice wine, sake, by hydrophilic interaction liquid chromatographyetime-of-flight/mass
531 spectrometry. *Journal of Bioscience and Bioengineering*, https://doi.org/10.1016/j.jbiosc.2017.03.010
532 Van Laere, K.M., Hartemink, R., Bosveld, M., Schols, H.A., & Voragen, A.G.J. (2000). Fermentation of plant
533 cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. *Journal*
534 *of Agricultural and Food Chemistry*, *48*, 1644–1652.
535 van Leeuwen, S.S., Kuipers, B.J., Dijkhuizen, L., & Kamerling, J.P. (2014). Development of a (1)H NMR
536 structural-reporter-group concept for the analysis of prebiotic galacto-oligosaccharides of the
537 [β-d-Galp-(1→x)]n-d-Glcp type. *Carbohydrate Research*, *400*, 54–58.
538 Veros, C.T., & Oldham, N.J. (2007). Quantitative determination of lysozyme-ligand binding in the solution and
539 gas phases by electrospray ionisation mass spectrometry. *Rapid Communications in Mass Spectrometry*,
540 *21*, 3505–3510.

Wang, B. (2009). Sialic acid is an essential nutrient for brain development and cognition. *Annual Review of Nutrition*, *29*, 177–222.

Wang, D., Li, F.L., & Wang, S.A. (2016). A one-step bioprocess for production of high-content fructo-oligosaccharides from inulin by yeast. *Carbohydrate Polymers*, *151*, 1220–1226.

Wang, H.Y., Chu, X., Zhao, Z.X., He, X.S., & Guo, Y.L. (2011). Analysis of low molecular weight compounds by MALDI-FTICR-MS. *Journal of Chromatography B*, *879*, 1166–1179.

Wang, Y., Zeng, T., Wang, S.E., Wang, W., Wang, Q., & Yu, H.X. (2010). Fructo-oligosaccharides enhance the mineral absorption and counteract the adverse effects of phytic acid in mice. *Journal of Nutrition*, *26*, 305–311.

Westphal, Y., Kühnel, S., De Waard, P., Hinz, S.W., Schols, H.A., Voragen, A.G., et al. (2010). Branched arabino-oligosaccharides isolated from sugar beet arabinan. *Carbohydrate Research*, *345*, 1180–1189.

Wu, S., Tao, N., German, J.B., Grimm, R., & Lebrilla, C.B. (2010). Development of an annotated library of neutral human milk oligosaccharides. *Journal of Proteome Research*, *9*, 4138–4151.

Xu, Q., Chao, Y.L., & Wan, Q.B. (2009). Health benefit application of functional oligosaccharides. *Carbohydrate Polymers*, *77*, 435–441.

Yang, H. J., Lee, A., Lee, M. K., Kim, W., & Kim, J. (2010). Detection of small neutral carbohydrates using various supporting materials in laser desorption/ionization mass spectrometry. *Bulletin of the Korean Chemical Society*, *31*, 35–40.

Yang, Z.Y., Wu, D.T., Chen, C.W., Cheong, K.L., Deng, Y., Chen, L.X., et al. (2016). Preparation of xylooligosaccharides from xylan by controlled acid hydrolysis and fast protein liquid chromatography coupled with refractive index detection. *Separation and Purification Technology*, *171*, 151–156.

Yao, Z., Wu, H., Zhang, S., & Du, Y. (2014). Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity. *Carbohydrate Polymers*, *101*, 359–367.

Yi, W., Liu, X., Li, Y., Li, J., Xia, C., Zhou, G., et al. (2009). Remodeling bacterial polysaccharides by metabolic pathway engineering. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 4207–4212.

Yu, H., & Chen, X. (2016). One-pot multienzyme (OPME) systems for chemoenzymatic synthesis of carbohydrates. *Organic and Biomolecular Chemistry*, *14*, 2809–2818.

Yu, H., Lau, K., Thon, V., Autran, C.A., Jantscher-Krenn, E., Xue, M., et al. (2014). Synthetic disialyl hexasaccharides protect neonatal rats from necrotizing enterocolitis. *Angewandte Chemie International Edition*, *53*, 6687–6691.

Yun, J.W., Choi, Y.J., Song, C.H., & Song, S.K. (1999). Microbial production of inulo-oligosaccharides by an endoinulinase from *Pseudomonas* sp. expressed in *Escherichia coli*. *Journal of Bioscience and Bioengineering*, 87, 291–295.

Zhang, H.T., Zhu, L., Zhang, S., Zhan, X. B., & Lin, C.C. (2014). A new, quick, highly sensitive ultramicro-analysis method for the identification of fructose removed from fructofuranosyl-containing gluco-oligosaccharides by ESI-CID-MS/MS. *Carbohydrate Research*, *398*, 1–7.

Zhang, L., Lau, K., Cheng, J., Yu, H., Li, Y., Sugiarto, G., et al. (2010). *Helicobacter hepaticus* Hh0072 gene encodes a novel 1–3-fucosyltransferase belonging to CAZy GT11 family. *Glycobiology*, *20*, 1077–1088.

Zhang, M., Cai, S.L., & Ma, J.W. (2015). Evaluation of cardio-protective effect of soybean oligosaccharides. *Gene*, *555*, 329–334.

Zhao, C., Wu, Y.J., Yang, C.F., Liu, B., & Huang, Y.F. (2015). Hypotensive, hypoglycemic and hypolipidemic effects of bioactive compounds from microalgae and marine microorganisms. *International Journal of Food Science and Technology*, *50*, 1705–1717.

Zhao, C., Wu, Y.J., Yu, H., Shah, I.M., Li, Y.H., Zeng, J., et al. (2016). One-pot multienzyme (OPME) synthesis of human blood group H antigens and a human milk oligosaccharide (HMOS) with highly active *Thermosynechococcus elongatus* α1–2-fucosyltransferase. *Chemical Communications*, *52*, 3899–3902.

Zou, P., Yang, X., Wang, J., Li, Y., Yu, H., Zhang, Y., et al. (2015). Advances in characterisation and biological activities of chitosan and chitosan oligosaccharides. *Food Chemistry*, *190*, 1174–1181.

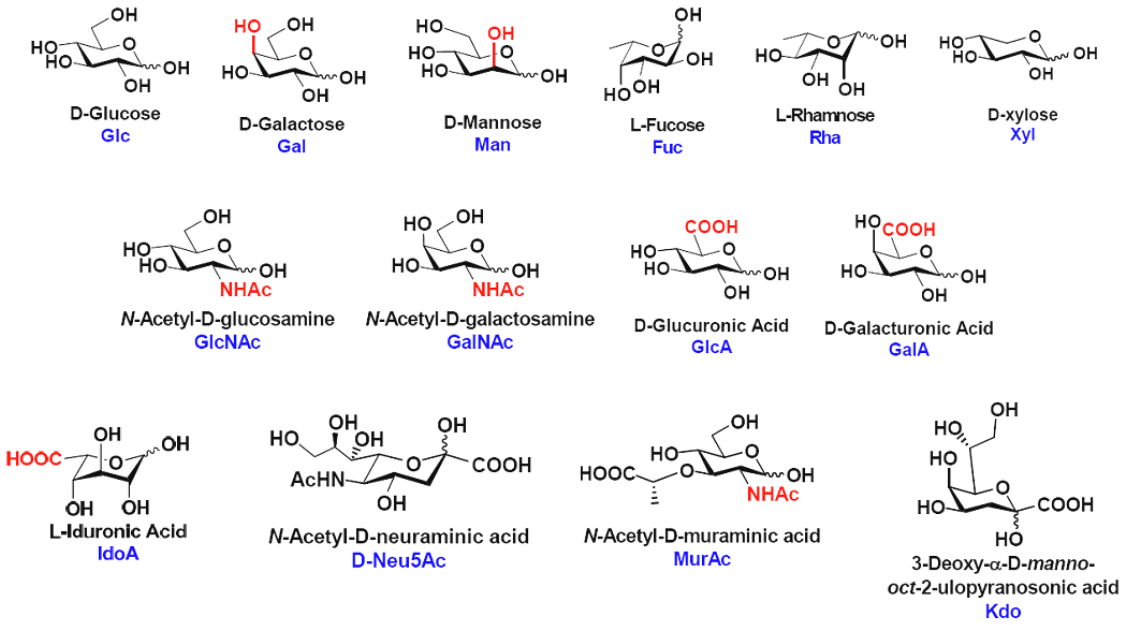

Fig. 1 Common monosaccharides components of the functional oligosaccharides.

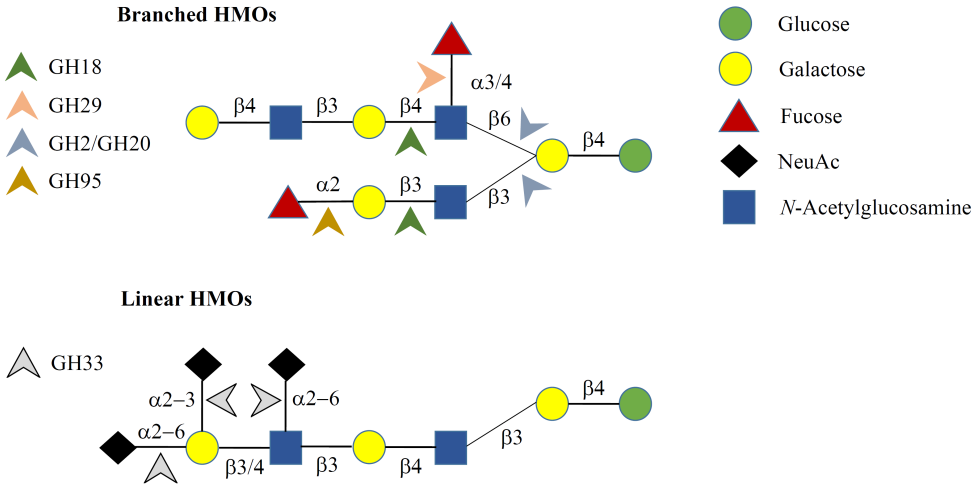

Fig. 2 Schematic of HMOs structures and putative HMOs active glycoside hydrolase families (GH) based on the work of Wu, Tao, German, Grimm, & Lebrilla (2010). GH2, α-galactosidase; GH18, endo-β-N-acetylglycosaminidase; GH20, β-hexosaminidase; GH29, α-1,3/4-fucosidase; GH33, sialidase; GH95, α-1,2-fucosidase.

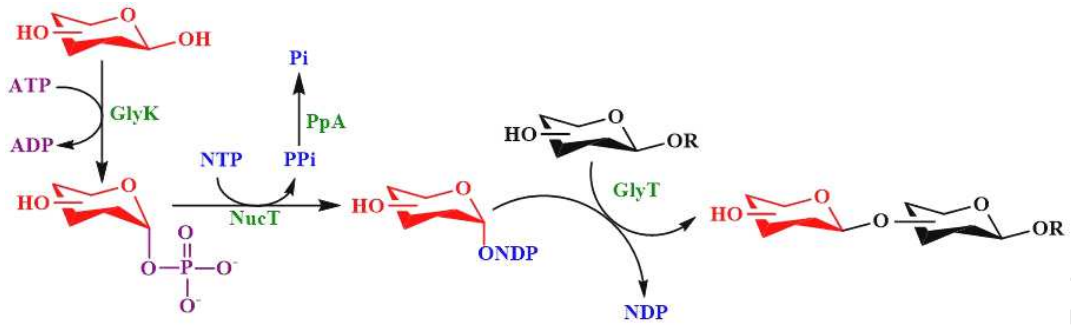

Fig. 3 The simplest routes for glycosyltransferase-catalyzed enzymatic synthesis of mammalian glycans with in situ generation of sugar nucleotides from a monosaccharide. Enzyme abbreviations: GlyK, glycokinase (Yi et al., 2009); NucT, nucleotidyltransferase; GlyT, glycosyltransferase (Zhao et al., 2016);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

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

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,

	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

643

644

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	

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).

656
657

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	

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.

681

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,

				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

	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.

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.