Recent approaches for the quantitative analysis of functional oligosaccharides used in the food industry: A review

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Highlights

- Quantitative analysis developed for the determination of functional oligosaccharides in food matrices are discussed (2015 – 2020).
- Structural properties of different oligosaccharides are highlighted.
- Sample preparation techniques for specific oligosaccharides and sample matrices are reviewed.
- Applications of functional OS in food products are generalized.

Abstract

Functional oligosaccharides (OS) are diverse groups of carbohydrates that confer several health benefits stemming from their prebiotic activity. Commonly used oligosaccharides, fructooligosaccharides and galactooligosaccharides, are used in a wide range of applications from food ingredients to mimic the prebiotic activity of human milk oligosaccharides (HMOs) in infant formula to sugar and fat replacers in dairy and bakery products. However, while consumption of these compounds is associated with several positive health effects, increased consumption can cause intestinal discomfort and aggravation of intestinal bowel syndrome symptoms. Hence, it is essential to develop rapid and reliable techniques to quantify OS for quality control and proper assessment of their functionality in food and food products. The present review will focus on recent analytical techniques used for the quantification of OS in different matrices such as food and beverage products.

1 Introduction

Functional oligosaccharides (OS) are diverse groups of carbohydrates consisting of monomers present in varying degrees of polymerization (DP) ranging between 3 to 10 units (Ibrahim, 2018). Currently, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are the most utilized functional OS in the food industry, however other OS groups such as agro-residue derived xylooligosaccharides (XOS), and pectic oligosaccharides are also being considered due to their relatively inexpensive production cost (Babbar et al., 2015; Moreno et al., 2017; Samanta et al., 2015). Technological properties of OS, such as gel-forming ability, water holding capacity, and thickening ability can improve sensory and physicochemical characteristics of food products hence, leading to increased application of OS in the food industry (Balthazar et al., 2017, 2015; Farias et al., 2019). They are commonly used as a partial replacement for fat and sugar, and as bulking agents in dairy and bakery products; they are also incorporated as food fortifying agents in various food products such as infant formulas, ice cream, cereal products, etc., to improve their nutritional functionality (Ibrahim, 2018; Nobre et al., 2015; Zhao et al., 2017).

The study of functional OS has been a growing field of interest in both the food and pharmaceutical industry mainly due to their activity as prebiotics – commonly defined as "non-digestible compounds that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon" (Cezar et al., 2017; Samanta et al., 2015; R. D. Singh et al., 2015; Vandenplas et al., 2015). Selective promotion of the growth of *Bifidobacteria spp.* and *Lactobacillus spp.* by prebiotics allows the liberation of metabolites such as short-chain fatty acids (acetate, propionate, butyrate, lactate, etc.) which are associated with reduced risk of hypercholesterolemia (Bali et al., 2015), and control of several physiological processes such as mucosal proliferation, inflammation, colorectal carcinogenesis, mineral absorption and elimination of nitrogen compounds (Flores-Maltos et al., 2016). Furthermore, several studies have reported a positive effect of consumption of prebiotic OS with increased absorption of Ca, Mg, and Fe, and against diseases such as cardiovascular disease, cancer, obesity, and type 2 diabetes (Bali et al., 2015; Gómez et al., 2016; Sánchez-Martínez et al., 2020; R. D. Singh et al., 2015; Zhao et al., 2017). Toxicity studies of FOS and GOS have shown that consumption of up to 9 g/kg body weight and 2g/kg body weight per day for FOS and GOS, respectively, had no adverse effects in Wister rats (Jain et al., 2019; Y. Zhou et al., 2017). However, several studies have been published on the association of increased consumption of OS, specifically FOS, with aggravation of irritable bowel syndrome symptoms (Charoenwongpaiboon et al., 2019; B. Chen et al., 2017; Huazano-garcía & López, 2017; Mellado-Mojica et al., 2016). Consumption of FOS of greater than 15 g/day was reported to cause intestinal discomfort, bloating, and flatulence (Kumar et al., 2018). Hence, the development of rapid and sensitive techniques for quantitation of functional OS, along with their increasing application in the food industry, is warranted. In this review, recently developed techniques over the last five years for the quantitative determination of functional OS in different food matrices will be discussed.

2 Analysis of Oligosaccharides

2.1 Fructooligosaccharides

Fructooligosaccharides (FOS) are composed of fructosyl units linked together via β(2 – 1) or β(2 – 6) glycosidic bonds with a glucose residue linked via α(1 – 2) glycosidic bond at the reducing end; commonly used FOS in fortified food products are 1-kestose (GF2), nystose (GF3), and fructofuranosyl nystose (GF4) (S. P. Singh et al., 2017; Vega & Zuniga-Hansen, 2015). FOS are naturally present as reserve phytochemicals in a variety of plants such as Jerusalem artichoke, bananas, tomatoes, onion, asparagus, chicory, leek, garlic, asparagus, wheat, honey, and yacon (Caetano et al., 2016; Flores-Maltos et al., 2016) with the highest concentrations reported in Jerusalem artichoke, chicory, and yacon (Caetano et al., 2016; Guo et al., 2016).

Currently, high-performance liquid chromatography (HPLC) methods often coupled with refractive index detector (RID) and evaporative light scattering detector (ELSD) are commonly used for the routine analysis of OS in food products and for monitoring OS synthesis (Bersaneti et al., 2017; Ko et al., 2019; Prokopov et al., 2018; Trollope et al., 2015; Vega & Zuniga-Hansen, 2015; Weiß & Alt, 2017). In a study conducted by dos Santos Lima et al. (2019), FOS present in wine and grape juices such as GF2, GF3, and raffinose was quantified using reversed-phase HPLC-RID. A RP-C18 column with polar endcapping was used to provide better separation and stability for highly polar compounds such as oligosaccharides. Before injection, wine samples were diluted with ultra-pure water and then filtered through a 0.45µm PTFE membrane. The method showed high specificity with minimal interference from common refraction detection interferences such as sugar, ethanol, and organic acids. Also, the method provided good linearity, recovery (GF2: 90.3% and GF3: 108.8), and sensitivity with LOD values 0.090 g/L (GF2) and 0.074 g/L (GF3), hence is a promising method for the routine detection of FOS in wine and grape juices. In another study, a simplified sample treatment of milk and milk-related products before the detection of FOS using HPLC-RID was developed and evaluated (Rodríguez-Gómez et al., 2015). A precipitating solution (495 mM zinc acetate dihydrate, 18 mM phosphotungstic hydrate, and 5.8% (v/v) glacial acetic acid) was added into homogenized samples in a 5:1 sample/precipitation solution ratio resulting in the precipitation of protein and fat. The results of the analysis demonstrated high precision (%RSD < 3.5%) and high sensitivity with LOD values of 0.5, 0.4, and 0.6 µg/mL for GF2, GF3, and GF4, respectively. Hence, the sensitivity of the method coupled with short analysis time (< 20 min) makes it a suitable technique for the routine analysis of common FOS in milk and milk products.

However, RID can be susceptible to baseline drift because of temperature variation and gradient elution. Subsequently, detection by ELSD provides better sensitivity characterized by great baseline stability due to gradient elution compatibility and temperature independence (Costa & Conte-Junior, 2018; Duan et al., 2018; Zhuang et al., 2019). Zhuang et al. (2019) developed a technique for simultaneous quantification of FOS (DP 3-15) present in Atractylodis rhizome using HPLC-ELSD. Due to a lack of available standards, the authors used FOS standards that were extracted and purified in their laboratory. A reflux extraction method using 60% ethanol optimized at a 20 fold solvent to sample ratio for 2.5 h was

employed. Results showed excellent linearity ($R^2 > 0.9993$) for all FOS of interest, as well as high sensitivity with reported LOD values ranging from 0.04 – 0.14 mg/mL. Simultaneous quantification of 13 FOS in *Morinda officinalis* was also published in another study using UHPLC-ELSD using am ethylene-bridged hybrid (BEH) amide column with gradient elution (Hao et al., 2019). The authors demonstrated that using a weakly basic (pH < 12) mobile phase improves analyte separation by reducing peak tailing, increasing peak resolution, maintaining a neutral charge on the analytes, and eliminating co-elution of salts. The method was validated and presents several advantages in that it is rapid (< 10 min), sensitive (LOD: 10.78-33.44 µg/mL; LOQ: 35.94-124.81 µg/mL), and precise (%RSD < 4.76).

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was also used for the routine analysis of FOS (Charoenwongpaiboon et al., 2019; Huazano-garcía & López, 2017; Mellado-Mojica et al., 2016; Menéndez et al., 2019; Porras-domínguez et al., 2017). Pöhnl et al. (2017) compared the performance of HPAEC-PAD and UHPLC-ELSD for the determination of FOS in *Allium cepa* (onion). Results of the study showed that HPAEC-PAD was superior to UHPLC-ELSD in that it has better sensitivity, a wider range of quantifiable FOS (up to DP of 18) and was able to baseline separate constitutional isomers. Also, the UHPLC-ELSD method was limited by the solubility of higher molecular weight FOS in organic solvents, while solubility issues are insignificant with HPAEC-PAD. These results were in agreement with another study for the detection of FOS in milk and milk products (Rodríguez-Gómez et al., 2015). However, UHPLC-ELSD still presents suitable results for low to medium molecular weight FOS analysis with shorter run time compared to HPAEC-PAD; hence, it could still be applied for routine analysis in the food industry.

Other cited methods for FOS analysis include gas-liquid chromatography mass spectrometry (GLC-MS), high-performance capillary electrophoresis with laser-induced fluorescence detection (HPCE-LIFD), and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (Rodríguez-Gómez et al., 2015; Zhao et al., 2017) however, recent studies focused on the evaluation, and further development of these methods were not available.

2.2 Galactooligosaccharides

Galactooligosaccharides are carbohydrates that are commonly composed of two to five galactose units linked via various glycosidic bonds (i.e. β(1 – 2), β(1 – 3), β(1 – 4), β(1 – 6)) with an N-glucose residue at the end forming a lactose terminal within the molecule (Azcarate-peril et al., 2016; Figueroa-Lozano et al., 2020; Solaiman et al., 2020). GOSs are found naturally in foods like banana, artichoke, onion, garlic, and honey; however, industrial GOS are commonly derived from industrial by-products (i.e. cheese whey and whey permeate) through the enzymatic transgalactosylation of lactose by β-galactosidase (EC 3.2.1.23) (Aburto et al., 2018; Chanalia et al., 2018; Rad et al., 2018; Vera et al., 2016). While they are structurally less complex, GOSs are reported to have similar structure and function with human milk oligosaccharides (HMO) hence they are commonly used to mimic the function of HMO in infant formulas and dairy products (Aburto et al., 2018; X. Y. Chen & Michael, 2017).

Recent studies on GOS were focused on their synthesis, purification and production (Chanalia et al., 2018; Sabater et al., 2019; G. Wang et al., 2020) while quantitation studies have been limited. Currently, the AOAC 2001.02 method is the only validated method for the determination of GOS in raw materials and food products (Blanco-morales et al., 2018; Kaczynski et al., 2019; Lans et al., 2018). This method describes an indirect measurement of GOS by enzymatic hydrolysis of GOS to glucose and galactose, which are then quantified using HPAEC-PAD (X. Wang & Rastall, 2018). However, the presence of high levels of free lactose in the sample matrix dramatically reduces the accuracy of the method; thus, it is not suitable for GOS analysis in high-lactose products (Gill et al., 2016; Yang & Xu, 2018). Subsequently, this method was improved by Lin et al. (2018). Galactose and glucose were measured in the same chromatographic run, thus eliminating the need to determine a correction factor required in the AOAC 2001.02 method. Also, the degree of conversion of lactose was taken into consideration and applied to the formula for calculating GOS content. However, while this method was validated for GOS raw materials, further evaluation is required for its suitability in GOS analysis in high lactose food products.

Quantification of GOS by gas chromatography – flame ionization detector (GC-FID) in infant formula was reported (Sabater et al., 2016). Prior to analysis, GOS samples were derivatized to trimethyl silylated oximes (TMSO). Furthermore, the authors demonstrated that the hydrolysis of maltodextrins with α-amyloglucosidase reduced interferences and improved peak resolution of GOS peaks, thus allowing quantification. This method showed good repeatability (%RSD < 12.3%) and allowed the quantification of GOS with a degree of polymerization (DP) up to 7. The same method was recently used to analyze the composition of the reaction mixture from the synthesis of GOS by propionibacteria from lactose and lactulose alongside HPLC-RID (Sabater et al., 2019). For HPLC-RID, GOS were quantified through an external calibration method using universal standards (i.e. raffinose for trisaccharides and stachyose for tetrasaccharides), and their concentration was expressed as the percentage of the total carbohydrate content. Hence, the previous methods presented several advantages over the AOAC 2001.02 method in that GOSs were quantified without prior hydrolyzation to glucose and galactose, thus simplifying GOS content calculation and minimizing lactose interference. However, quantitation was not the primary aim of these studies; hence these methods were not validated.

2.3 Milk Oligosaccharides

Milk oligosaccharides (MOS) are a group of carbohydrates composed of monomers glucose, galactose, N-acetylglucosamine, fucose, sialic acid, and N-acetylneuraminic acid with a fructose residue at the reducing end (Grabarics et al., 2017; Wei et al., 2018). Milk oligosaccharides (MOS), especially those from human milk, are novel bioactives associated with several physiological processes, including modulation of the gut microbiota, immune regulation, and anti-pathogenic response in the gastrointestinal tract (Grabarics et al., 2017; Walsh et al., 2020). Efforts have been placed into mimicking the composition of human milk oligosaccharides (HMOs) through the addition of FOS and GOS into infant formulas (Ma et al., 2019; Vandenplas et al., 2018) however, Walsh et al. (2020) recognized that these efforts alone are insufficient in simulating the health benefits gained from actual human MOS. Hence, to facilitate a better understanding of the effects of HMOs in infant health, and to properly

develop methods for synthesis of HMOs, various techniques for the accurate quantitation of HMOs have been developed (Grabarics et al., 2017; Yan et al., 2017).

Simultaneous quantification of 16 acidic and neutral HMOs was achieved by Tonon et al. (2019) using graphitized carbon liquid chromatography-electrospray ionization mass spectrometry (GCLC-EIMS) with simple sample preparation. Briefly, centrifugation and ultra-filtration were applied for the removal of lipids and proteins, respectively, and followed by a reduction reaction to prevent the separation of anomers in the porous graphitized carbon phase. The method showed promising results with excellent linearity ($R^2 > 0.9999$), high sensitivity (LOQ = 0.039), and suitable recovery (89-110%). Also, the reported method presents a couple of advantages in that it was able to extract and quantify both neutral and acidic HMOs simultaneously. Furthermore, the simultaneous extraction and analysis of HMOs in the same chromatographic run minimized the loss of HMOs due to lactose precipitation and provided higher recovery values (89-110%) compared to another study using graphitized-carbon solid phase extraction (GC-SPE) (19.5-20%) (Robinson et al., 2018). Absolute quantitation HMOs were also reported by Xu et al. (2016) using ultraperformance liquid chromatography tandem triple quadrupole mass spectrometry (UPLC-QqQMS) under multiple reaction monitoring (MRM). The study also evaluated the feasibility of utilizing pooled human milk samples for the construction of calibration standards. Briefly, pooled milk samples were defatted and deproteinated by centrifugation and ethanol extraction, respectively. Half of the extracted samples were serially diluted and injected into the system, and the total response of HMOs in each diluted pool was determined by summing the peak areas of all of the transitions. The other half was cleaned by PGC-SPE, lyophilized, and weighed to calculate the concentration of HMOs in each diluted pool. Absolute concentrations of total, sialylated, fucosylated and non-fucosylated neutral HMOs were determined using the universal calibration curve constructed from pooled milk samples. The validity of the method was evaluated using commercially available HMO standards and showed suitable results in terms of sensitivity (LOD: 0.6 – 6.8 fmol) and linearity ($R^2 > 0.99$). The same method was applied by Zhang et al. (2019) for the separation and quantitation of 12 HMOs in human milk. Under MRM mode in a triple quadrupole mass spectrometer, sensitivity is enhanced by applying two stages of mass selectivity to minimize interferences from the background matrix. The method was able to successfully quantify and separate 12 HMOs, including four HMO isomers without the need for intricate sample pretreatment such as lactose removal and derivatization. The same instrumental technique was also successfully applied for the investigation of variation in HMO concentration due to lactational changes (Ma et al., 2018), and the analysis of major milk OS content in formulated milk powders (Ma et al., 2019).

Bovine milk oligosaccharides (BMO) are also potential alternatives for HMOs. Several BMOs have been identified to be structurally similar to HMOs (Vicaretti et al., 2018). Sialylated milk oligosaccharides, present in both BMOs and HMOs, were analyzed and quantified by Yan et al. (2018) via on-line solid-phase extraction – hydrophilic interaction chromatography tandem mass spectrometry (SPE-HILIC-MS) in mammalian milk (i.e. goat, sheep, buffalo, cow, donkey etc.). The authors utilized a dual gradient pump system used for purification (clean-up column) and analysis (analytical column), respectively, allowing direct injection of defatted and deproteinated samples into the system. Sialylated OSs were separated from neutral OSs and lactose based on their electrostatic differences using a zwitterionic SPE matrix.

The technique successfully separated 30 mono- and di-sialylated HMOs and was used for profiling of other mammalian milk. Method validations on the established conditions showed excellent linearity (R^2 > 0.99) and average recovery for 3-sialyllactose (90%) and 6-sialyllactose (106%). Sialylated OS was also determined in donkey milk using a fluorescent detector (FLD) equipped UHPLC-MS (Licitra et al., 2019) by derivatizing samples using 2-AB labelling agent (Dimethylsulfoxide/acetic acid (70/30) containing 1.05M 2-aminobenzamide and 3M sodium cyanoborohydride). A calibration curve (R^2 > 0.999; linear range over 0.1 – 102.4 µg/mL) for 3-SL was used for quantifying other sialylated OS present in donkey milk. The same method was applied for the detection of significant HMOs with a few modifications, namely 1M of 2-methylpyridine borane was used instead of sodium cyanoborohydride, and detection was done solely by FLD (Huang et al., 2019). A HILIC column was also used. Labelling OSs with 2-AB increased hydrophobicity and enhanced fluorescence absorption by adding a hydrophobic fluorophore group at the reducing end. Furthermore, direct injection into the HILIC column eliminated a clean-up step that is often required in milk OS analyses. The technique allowed the simultaneous quantitation of major HMOs with the use of simple sample treatment, minimal sample volume (10µL) with decent recovery rate (88-107%), and high sensitivity (LOD < 10 pg) for neutral and acidic HMOs. Huang et al. (2019) also noted that centrifugation is appropriate for the determination of HMOs occurring at high levels while ultrafiltration, such as membrane separation, provided satisfactory results for the quantitation of trace BMOs. Suitability of capillary electrophoresis for MOS quantitation was also evaluated (Monti et al., 2015). Micellar electrokinetic chromatography – CE (MEKC-CE) showed promising results for the quantitation of sialylated OS in milk samples with good linearity (R^2 > 0.999). However, further method validation such as recovery studies, and LOD and LOQ determination are warranted. Also, long analysis time (< 1 hour) and time consuming sample preparation (extraction with ethanol for 24 h and drying with N_2 at room temperature) can be regarded as main disadvantages of this technique. CE with laser-induced fluorescent detection (LIF) is also used (Difilippo et al., 2016; Vicaretti et al., 2018). Normally, lactose-free OS are labelled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS) to establish a linear correlation between peak area and mole concentration (Difilippo et al., 2016). Also, this allows efficient separation and resolution of both neutral and acidic OS with response factors independent of structure (Vicaretti et al., 2018).

Furthermore, commercially available HMOs, 2-fucosyllactose (2-FL) and 3-fucosyllactose (3-FL) were detected in milk, UHT milk, yogurt, ground cereal bar and infant formula using HPLC-RID (Christensen et al., 2020). Notable sample preparation steps include centrifugation and ultrafiltration for the removal of lipids and proteins, respectively. The mobile phase composition, injection volume and column temperature were optimized to improve resolution. Limits of detection were 0.1 mg/mL for 2-FL and 0.2 mg/mL for 3-FL in whole milk, while LOD values of 0.6 mg/mL were observed for both 2-FL and 3-FL in infant formula and a cereal bar. The method was successfully applied for stability studies due to the high sensitivity, excellent linearity (R^2 > 0.9995) and acceptable recovery for 2-FL (88-105%) and 3-FL (94-112%). Furthermore, it can also be utilized for shelf-life studies as well as quality control of 2-FL and 3-FL in other food products and is a less expensive alternative for LC-MS.

2.4 Xylooligosaccharides

Xylooligosaccharides (XOS) are derived from the hydrolysis of xylan by xylanase enzyme and are composed of xylose units linked via β-1,4 glycosidic bonds. Depending on the source of xylan and mode of production, the structure of XOS can vary greatly in terms of degree of polymerization (2-10), side groups (i.e. acetyl groups, arabinofuranosyl residues, 4-O-methyl derivative, and uronic acids.) and their substitution pattern in the xylose chain (Amorim, Silvério, Prather, et al., 2019; de Freitas et al., 2019; Samanta et al., 2015). Apart from their prebiotic activity, XOSs were reported to inhibit colon carcinogenesis (Aachary et al., 2015), and exhibit anti-inflammatory, antiallergic (Nieto-domínguez et al., 2017), and antioxidant properties (de Freitas et al., 2019). In addition, XOS presents a potential as food ingredients due to their stability over a wide range of pH (2.5 – 8.0) and temperature (up to 100°C) as well as their acceptable organoleptic properties and price competitiveness due to higher bifidogenic activity compared to other prebiotics (Amorim, Silvério, Cardoso, et al., 2019; Antov & Dordevic, 2017; de Freitas et al., 2019). Currently, much of the studies are focused on developing a sustainable production process for XOS using lignocellulosic residues (Amorim, Silvério, Prather, et al., 2019) such as corncobs (Boonchuay et al., 2018; H. Zhang et al., 2017), sugarcane biomass (Avila et al., 2019; X. Zhou & Xu, 2019; X. Zhou et al., 2019), hazelnut shells (Surek & Buyukkileci, 2017), almond shells (R. D. Singh et al., 2019), others.

Production efficiency and xylanase activity are commonly monitored by XOS determination using HPAEC-PAD. In a study conducted by Cürten et al. (2017), a rapid automated detection method for XOS in enzymatic hydrolyzates was developed. Optimization of column temperature, and flow rate along with gradient elution of 200 mM sodium hydroxide solution and 100 mM sodium hydroxide with 500 mM sodium acetate enabled sufficient elution and separation of XOS of interest (i.e. xylobiose, xylotriose, xylotetraose, and xylopentose) within a chromatographic run time of 10 mins. The method also demonstrated excellent linearity (R^2 > 0.999) and sensitivity (LOD: 0.35 – 1.83 mg/L). Subsequently, HPAEC-PAD was applied for stability studies of XOS in orange juice after high-intensity ultrasound processing (Silva et al., 2020). Juice samples were diluted with ultra-pure water and filtered through a 0.22µm PTFE syringe filter before column injection. XOSs were separated using gradient elution on a Carbopac PA100 column, and their corresponding peaks were identified by comparing their retention times with commercially available standards.

The application of HILIC-ELSD for the determination of XOS in enzymatic hydrolyzates was recently proposed by Li et al. (2016). One advantage of HILIC is that highly polar samples are separated with high selectivity using a polar organic-aqueous mobile phase and a polar stationary phase. The authors extracted and purified XOS standards (DP 2-6) from a mixture of XOSs using solid phase extraction, followed by semi-preparative liquid chromatography using an HILIC column; hence demonstrating the feasibility of XOS purification using HILIC. Increasing the column temperature to 60°C, and passing a mobile phase consisting of H_2O and acetonitrile over a diol column with weaker retention capacity, enabled complete separation of XOS (DP 2-6) within a 20 min run time. Furthermore, method validation showed that the technique is precise (%RSD < 4.8%), accurate (%recovery: 90.0% - 110.8%). However, this

method provided lower sensitivity (LOD: 0.1585 – 0.5259 µg/µL) compared to the HPAEC-PAD method that was previously discussed. A similar method was developed by (Pu et al., 2017). A linear gradient elution of 75% - 50% acetonitrile (v/v) was applied to improve peak resolution of short chain XOS (DP 2-4) and accelerate the elution of long chain XOS (DP > 4). The column temperature and flow rate were also modified to improve baseline separation, and peak shape and resolution. The method was successfully applied for the simultaneous separation and detection of non-substituted and acetylated XOS (DP 2-8) with comparable sensitivity (LOD: 9.6 – 11.8 µg/mL) with previous studies over a 30 min run time.

Capillary electrophoresis with a photodiode array detector (CE-PDA) was also used for the analysis of xylooligosaccharides and other wood-based oligosaccharides such as manno-oligosaccharides and cellooligosaccharides (Hiltunen et al., 2016). Wood-based OSs including diastereomers were successfully separated and analyzed using the method without prior derivatization with the exception of cellohexose, xylotriose and xylotetraose. The electrophoretic mobility of the analytes was adjusted using a highly concentrated background electrolyte solution to improve peak resolution between the target analytes. However, this increases analysis time hence presenting a drawback for this method.

Table 1
Overview of analytical methods for determination of functional oligosaccharides in different food and biological matrices.

Technique	Analytes of Interest	Samples Analyzed	Sample preparation	LOD	Recoveries (%)	RSD (%)	Remarks	References
RP-HPLC-RID	FOS (DP 2-3)	Wine, and grape juices	Dilution and filtration	0.074 – 0.090 g/L	90.3 – 108.8	1.79 – 2.14	RP-C18 column with polar endcapping	(dos Santos Lima et al., 2019)
HPLC-RID	FOS (DP 2-4)	Milk and milk related products	Protein precipitation by zinc and tungsten salt in acidic media and centrifugation	60 µg/mL	~ 100	2.0 – 2.5	Luna 5u NH_2 column	(Rodríguez-Gómez et al., 2015)
HPLC-ELSD	FOS (DP 3-15)	*Atractylodis sp.* rhizome powder	20-fold reflux extraction with 60% (v/v) ethanol, centrifugation, and filtration	0.01 – 0.04 mg/mL	83.16 – 99.66	< 2.66	Amide column in gradient elution mode	(Zhuang et al., 2019)
UHPLC-ELSD	FOS (DP 2-11)	*Morinda officinalis*	Ultrasonic extraction with 50% (v/v) methanol, and filtration	12.87 – 37.44 µg/mL	98.59 – 102.72	0.41 – 2.85	UPLC BEH amide C18 column	(Hao et al., 2019)
UHPLC-ELSD	FOS (DP < 10)	Onions	Crude onion juice extraction, centrifugation, dilution, and filtration	7.87 – 9.07 ng on column	89.5 – 102.7	1.1 – 3.9 (CV)	UPLC BEH amide column; baseline separation of isomers was not achieved	(Pöhnl et al., 2017)
HPAEC-PAD	FOS (DP up to 18)	Onions	Crude onion juice extraction, centrifugation, dilution, and filtration	0.80 – 2.29 ng on column	95.3 – 107.3	1.3 – 7.4 (CV)	CarboPac PA-200 column; complete separation of isomers	(Pöhnl et al., 2017)
HPAEC-PAD	GOS	GOS syrup and powder	Enzymatic hydrolysis of GOS into glucose and galactose	0.3 µg/mL	95.5 - 107	< 3.65	CarboPac PA-20 column; AOAC 2001.02 method calculation was simplified	(Lin et al., 2018)
GC-FID	GOS/FOS (DP up to 7)	Infant formula	Protein and fat precipitation with Carrez reagents;	-	-	< 12.3	Results are within the range indicated in package labels	(Sabater et al., 2016)

			derivatization to TMSO					
GC-FID	GOS	Reaction hydrolyzate	Derivatization to TMSO	-	-	-	Commercial fused silica capillary column	(Sabater et al., 2019)
GCLC-MS	Neutral and acidic HMO	Human milk	Centrifugation, dilution, homogenization, ultrafiltration, and reduction with $NaBH_4$	0.039 – 0.156 µg/mL (LOQ)	89 - 110	< 13%	PGC column and electrospray ionization	(Tonon et al., 2019)
UPLC-QqQMS	Total HMO	Human milk	Dilution, centrifugation, protein precipitation with 60% ethanol	0.6 – 6.8 fmol	-	-	UPLC BEH amide column and electrospray ionization in MRM mode	(Xu et al., 2016)
UPLC-QqQMS	HMO	Human milk	Dilution, centrifugation, and ultrasonic protein precipitation with acetonitrile	-	89.3 – 110.29	< 8.64	UPLC amide column and electrospray ionization in MRM mode	(W. Zhang et al., 2019)
SPE-HILIC-MS	Sialylated milk OS	Mammalian milk	Centrifugation, freeze drying, and extraction ethanol: water (2:1)	-	90 – 106	-	On-line zwitterionic SPE, and amide column	(Yan et al., 2018)
UHPLC-FLD	Neutral and acidic HMOs	Human milk	10 to 100-fold dilution with ultrapure water, centrifugation, and fluorescent labelling with 2-AB	0.8 – 7.6 pg	88 - 107	1.2 - 3.6	UPLC BEH amide column	(Huang et al., 2019)
MEKC-CE	Sialylated OS	Mammalian milk	Centrifugation, extraction with 60% ethanol, and drying with N_2	-	-	-	Uncoated capillary; detection at 205 nm	(Monti et al., 2015)
CE-LIF	Neutral and acidic OS	Cow milk	Protein and fat precipitation, lactose removal by SPE, and derivatization with APTS	-	-	-	Rapid characterization of 33 milk OS	(Difilippo et al., 2016; Vicaretti et al., 2018)
HPLC-RID	Commercial HMO	Infant formula, whole milk, and cereal bar	Homogenization, centrifugation, and ultrafiltration	0.1 – 0.7 mg/mL	88 - 112	< 5	UPLC BEH amide column	(Christensen et al., 2020)
HPAEC-PAD	XOS (DP 2 – 5)	Reaction hydrolyzate	Direct injection	0.35 – 1.83 mg/mL	-	-	CarboPac PA-100 column	(Cürten et al., 2017)
HILIC-ELSD	XOS (DP 2 – 6)	Reaction hydrolyzate	Clean-up by SPE	91.6 – 315 mg/L	95.0 -110.8	< 4.8	Lab-purified standards by SPE and semi-preparative HILIC system	(Li et al., 2016)
HPLC-ELSD	XOS (DP 2 – 8)	Commercial XOS	Dilution with ultrapure water	9.6 – 11.8 µg/mL	-	< 2.15	Zwitterionic HILIC column	(Pu et al., 2017)
CE-PDA	XOS (DP 2 – 6), cello-OS	Birch kraft pulp	Hot water extraction (100 – 160 °C)	3.8 – 6.0 mg/mL	-	< 5	Fused silica capillary; detection at 270 nm	(Hiltunen et al., 2016)

3 Conclusion

Given the structural complexity and diversity of functional oligosaccharides depending on the source and production process, the development of quantitative analysis for OS has been challenging. Furthermore, the lack of available standards, especially for GOS, limits the quantification of OS at an individual level; hence, OS contents are typically expressed as relative abundance. However, recent studies demonstrated the feasibility of extracting and purifying OS standards in the laboratory by SPE, preparative HPLC, or a combination of both in place of commercially available standards.

Common drawbacks of conventional techniques used to analyze OS are long analysis time, and complicated sample pretreatment such as SPE and derivatization. Hence, recent research efforts have directed to simplifying sample pre-treatment through. The application of an on-line SPE system has simplified sample pre-treatment and automation, and enhanced recovery rates and sensitivity compared with conventional off-line analysis. Furthermore, several studies have demonstrated the suitability of extraction with organic solvents (i.e. acetonitrile, methanol, and ethanol) coupled with centrifugation and ultrafiltration in the analysis of OS in various food matrices.

Subsequently, several methods have been proposed and evaluated for the analysis of OS in food and reaction hydrolyzates with each method having their own set of advantages and disadvantages. Due the lack of chromophores in OS molecules, they are commonly detected using refractive index (RID), evaporative light scattering detection (ELSD), pulsed amperometric detection (PAD), and mass detection (MS). Liquid chromatographic methods coupled with RID are commonly used owing to its convenience, and they are relatively less expensive than other instrumental techniques. However, the incompatibility of RID with gradient elution greatly limits LC-RID techniques in separating higher molecular weight OS (DP > 4). Hence, they are commonly used for the analysis of low molecular weight OS while higher molecular weight OS are detected by either ELSD or PAD. Detection by ELSD and MS are commonly used for HILIC-based liquid chromatographic techniques. However, HILIC-based techniques are limited by the differences in solubility of OS molecules. Hence, among these techniques, quantification by HPAEC-PAD was the most promising due to its higher sensitivity and specificity compared to other methods discussed in this review. Moreover, HPAEC-PAD was not limited by solubility differences of target OS and was able to present better separation between isomers and lower limits of detection and quantification without the need for elaborate sample preparation. However, the relatively longer analysis time was observed for this method; hence other methods discussed such as HPLC-RID and HPLC-ELSD could still be more applicable for routine analysis of OS in food samples.

Abbreviations

OS – Oligosaccharides
DP – Degree of polymerization
FOS – Fructooligosaccharides
GOS – Galactooligosaccharides
XOS – Xylooligosaccharides
HMO – Human milk oligosaccharide
MOS – Milk oligosaccharide
BMO – Bovine milk oligosaccharide
HPLC – High performance liquid chromatography
UPLC – Ultra performance liquid chromatography
RID – Refractive index detector
ELSD – Evaporative light scattering detector
FLD – Flourescent detector
BEH – ethylene bridged hybrid
HPAEC-PAD – High performance anion exchange chromatography – pulsed amperometric detection
TMSO – Trimethyl silated oximes
PGC-SPE – Porous graphitized carbon – solid phase extraction
MRM – Multiple reaction monitoring
HILIC – Hydrophilic interaction liquid chromatography
QqQMS – Triple quadrupole mass spectrometry
GC-FID – Gas chromatography fluorescence induced detection
CE-LIF – Capillary electrophoresis lased induced fluorescence
MEKC-CE – Micellar electrokinetic chromatography – capillary electrophoresis
CE-PDA – Capillary electrophoresis photodiode array detector
LOD – Limits of detection
LOQ – Limits of quantitation

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5 References

Aachary, A. A., Gobinath, D., Srinivasan, K., & Prapulla, S. G. (2015). Protective effect of xylooligosaccharides from corncob on 1 , 2-dimethylhydrazine induced colon cancer in rats. *Bioactive Carbohydrates and Dietary Fibre*, *5*(2), 146–152.

Aburto, C., Guerrero, C., Vera, C., Wilson, L., & Illanes, A. (2018). Enzyme and Microbial Technology Co-immobilized β -galactosidase and Saccharomyces cerevisiae cells for the simultaneous synthesis

and puri fi cation of galacto-oligosaccharides. *Enzyme and Microbial Technology*, *118*, 102–108.

Amorim, C., Silvério, S. C., Cardoso, B. B., Alves, J. I., Pereira, M. A., & Rodrigues, L. R. (2019). In vitro assessment of prebiotic properties of xylooligosaccharides produced by Bacillus subtilis 3610. *Carbohydrate Polymers*, *229*, 115460.

Amorim, C., Silvério, S. C., Prather, K. L. J., & Rodrigues, L. R. (2019). From lignocellulosic residues to market : Production and commercial potential of xylooligosaccharides. *Biotechnology Advances*, *37*(7), 107397.

Antov, M. G., & Dordevic, T. R. (2017). Environmental-friendly technologies for the production of antioxidant xylooligosaccharides from wheat chaff. *Food Chemistry*, *235*, 175–180.

Avila, P., Cairo, J. P. L. F., Damasio, A., Forte, M. B. S., & Goldbeck, R. (2019). Xylooligosaccharides production from a sugarcane biomass mixture: effects of commercial enzyme combinations on bagasse/straw hydrolysis pretreated using different strategies. *Food Research International*, *128*, 108702.

Azcarate-peril, M. A., Ritter, A. J., Savaiano, D., Monteagudo-mera, A., & Anderson, C. (2016). Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *PNAS Early Edition*, *114*(3), 1–9.

Babbar, N., Dejonghe, W., Gatti, M., Sforza, S., & Kathy, E. (2015). Pectic oligosaccharides from agricultural by-products : production , characterization and health benefits. *Critical Reviews in Biotechnology*, *36*(4), 594-606.

Bali, V., Panesar, P. S., Bera, M. B., & Panesar, R. (2015). Fructo-oligosaccharides: Production, Purification and Potential Applications. *Critical Reviews in Food Science and Nutrition*, *55*(11), 1475–1490.

Balthazar, C. F., Silva, H. L. A., Cavalcanti, R. N., Esmerino, E. A., Cappato, L. P., Abud, Y. K. D., Moraes, J., Andrade, M. M., Freitas, M. Q., Anna, C. S., Raices, R. S. L., Silva, M. C., & Cruz, A. G. (2017). Prebiotics addition in sheep milk ice cream : A rheological , microstructural and sensory study. *Journal of Functional Foods*, *35*, 564–573.

Balthazar, C. F., Silva, H. L. A., Celeguini, R. M. S., Santos, R., Pastore, G. M., & Junior, C. A. C. (2015). Effect of galactooligosaccharide addition on the physical, optical, and sensory acceptance of vanilla ice cream. *Journal of Dairy Science*, *98*(7), 4266–4272.

Bersaneti, G. T., Pan, N. C., & Baldo, C. (2017). Co-production of Fructooligosaccharides and Levan by Levansucrase from Bacillus subtilis natto with Potential Application in the Food Industry. *Applied Biochemistry and Biotechnology*, *184*, 838–851.

Blanco-morales, V., López-garcía, G., & Cilla, A. (2018). The impact of galactooligosaccharides on the bioaccessibility of sterols in a plant sterol-enriched beverage : adaptation of the harmonized INFOGEST digestion method. *Food and Function*, *9*, 2080–2089.

Boonchuay, P., Techapun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., & Takenaka, S. (2018). An Integrated Process for Xylooligosaccharide and Bioethanol Production from Interdisciplinary Program in Biotechnology , Graduate School , Chiang Mai University , Bioprocess Research Cluster (BRC), Faculty of Agro-Industry , Chiang Mai Department of Fo. *Bioresource Technology*, *256*, 399–407.

Caetano, B. F. R., de Moura, N. A., Almeida, A. P. S., Dias, M. C., Sivieri, K., & Barbisan, L. F. (2016). Yacon (Smallanthus sonchifolius) as a food supplement: Health-promoting benefits of fructooligosaccharides. *Nutrients*, *8*(436).

Cezar, M., Mano, R., Neri-numa, I. A., Bueno, J., Paulino, B. N., Pessoa, M. G., & Pastore, G. M. (2017). Oligosaccharide biotechnology : an approach of prebiotic revolution on the industry. *Applied Microbiology and Biotechnology*, *102*, 17–37.

Chanalia, P., Gandhi, D., Attri, P., & Dhanda, S. (2018). Purification and characterization of β-galactosidase from probiotic Pediococcus acidilactici and its use in milk lactose hydrolysis and galactooligosaccharide synthesis. *Bioorganic Chemistry*, *77*, 176–189.

Charoenwongpaiboon, T., Sitthiyotha, T., Pramoj, P., & Ayutthaya, N. (2019). Modulation of fructooligosaccharide chain length and insight into the product binding motif of Lactobacillus reuteri 121 inulosucrase. *Carbohydrate Polymers*, *209*, 111–121.

Chen, B., Du, L., He, H., Kim, J. J., Zhao, Y., Zhang, Y., Luo, L., Dai, N., Chen, B., Du, L., He, H., & Zhang, Y. (2017). Fructo-oligosaccharide intensifies visceral hypersensitivity and intestinal inflammation in a stress-induced irritable bowel syndrome mouse model. *World Journal of Gastroenterology*, *23*(47), 8321–8333.

Chen, X. Y., & Michael, G. G. (2017). Lactose and lactose-derived oligosaccharides : More than prebiotics? *International Dairy Journal*, *67*, 61–72.

Christensen, A. S., Skov, S. H., Lendal, S. E., & Hornshøj, B. H. (2020). Quantifying the human milk oligosaccharides 2 ’ -fucosyllactose and 3-fucosyllactose in different food applications by high-performance liquid chromatography with refractive index detection. *Journal of Food Science*, *85*(2), 332–339.

Costa, M. P., & Conte-Junior, C. A. (2018). Analytical Applications of Evaporative Light Scattering Detection for Determination of Carbohydrates and Organic Acids in Food. *Reference Module in Food Science*, 1–4.

Cürten, C., Anders, N., Juchem, N., Ihling, N., Volkenborn, K., Knapp, A., Jaeger, K., Büchs, J., Spiess, A. C., & Anders, N. (2017). Fast automated online xylanase activity assay using HPAEC-PAD. *Analytical and Bioanalytical Chemistry*, *410*, 57–69.

de Freitas, C., Carmona, E., & Brienzo, M. (2019). Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. *Bioactive Carbohydrates and Dietary Fibre*, *18*, 100184.

Difilippo, E., Pan, F., Logtenberg, M., Willems, R., Braber, S., Schols, H. A., & Gruppen, H. (2016). Milk oligosaccharide variation in sow milk and their fermentation in piglet intestine. *Journal of Agricultural and Food Chemistry*, *64*(10), 2087–2093.

dos Santos Lima, M., Nunes, P. C., de Lourdes de Araujo Silva, B., & da Silva Padilha, C. (2019). Determining 1-kestose , nystose and raffinose oligosaccharides in grape juices and wines using HPLC : method validation and characterization of products from Northeast Brazil. *Journal of Food Science and Technology*.

Duan, W., Ji, W., Wei, Y., Zhao, R., Chen, Z., & Geng, Y. (2018). Separation and Purification of Fructo-Oligosaccharide by High-Speed Counter-Current Chromatography Coupled with Precolumn Derivatization. *Molecules*, *23*, 381.

Farias, D. D. P., Araújo, F. F. De, & Neri-numa, I. A. (2019). Prebiotics : Trends in food , health and technological applications. *Trends in Food Science & Technology*, *93*, 23–35.

Figueroa-Lozano, S., Ren, C., Yin, H., Pham, H., van Leeuwen, S., Dijkhuizen, L., & de Vos, P. (2020). The impact of oligosaccharide content, glycosidic linkages and lactose content of galcto-oligosaccharides (GOS) on the expression of mucus-related genes in goblet cells. *Food & Function*,

11, 3506–3515.

Flores-Maltos, D. A., Mussatto, S. I., Contreras-Esquivel, J. C., Rodríguez-Herrera, R., Teixeira, J. A., & Aguilar, C. N. (2016). Biotechnological production and application of fructooligosaccharides. *Critical Reviews in Biotechnology*, *36*(2), 259–267.

Gill, B., Indyk, H., & Woollard, D. (2016). Current Methods for the Analysis of Selected Novel Nutrients in Infant Formulas and Adult Nutritionals. *Journal of AOAC International*, *99*(1), 30–41.

Gómez, B., Gullón, B., Yáñez, R., & Schols, H. (2016). Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp : A comparative. *Journal of Functional Foods*, *20*, 108–121.

Grabarics, M., Csernák, O., Balogh, R., & Béni, S. (2017). Analytical characterization of human milk oligosaccharides – potential applications in pharmaceutical analysis. *Journal of Pharmaceutical and Biomedical Analysis*, *146*, 168–178.

Guo, M., Chen, G., & Chen, K. (2016). Chapter 5 Fructooligosaccharides: Effects, Mechanisms, and Applications. In H. Yin & Y. Du (Eds.), *Research Progress in Oligosaccharins* (pp. 51–62). Springer Science+Business Media New York.

Hao, Q., Nan, T., Zhou, L., Kang, L., Guo, L., & Yu, Y. (2019). Rapid simultaneous quantification of fructooligosaccharides in Morinda officianalis by ultra-high performance liquid chromatography. *Journal of Separation Science*, *42*(13), 2222–2230.

Hiltunen, S., Siren, H., Heiskanen, I., & Backfolk, K. (2016). Capillary electrophoretic profiling of wood-based oligosaccharides. *Cellulose*, *23*, 3331–3340.

Huang, X., Zhu, B., Jiang, T., Yang, C., Qiao, W., Hou, J., Han, Y., Xiao, H., & Chen, L. (2019). Improved Simple Sample Pretreatment Method for Quantitation of Major Human Milk Oligosaccharides Using Ultrahigh Pressure Liquid Chromatography with Fluorescence Detection. *Journal of Agricultural and Food Chemistry*, *67*(44), 12237–12244.

Huazano-garcía, A., & López, M. G. (2017). Enzymatic Hydrolysis of Agavins to Generate Branched Fructooligosaccharides (a-FOS). *Applied Biochemistry and Biotechnology*, *184*, 25–34.

Ibrahim, O. (2018). Functional Oligosaccharide: Chemicals Structure, Manufacturing, Health Benefits, Applications and Regulations. *Journal of Food Chemistry & Nanotechnology*, *4*(4), 65–76.

Jain, M., Gote, M., Dubey, A. K., Narayanan, S., Krishnappa, H., Kumar, D. P. S., & Ravi, G. S. (2019). Safety evaluation of fructooligosaccharide subchronic oral toxicity study in Wistar rats. *Toxicology Research and Application*, *2*(1), 1–20.

Kaczynski, L., Cais-Sokolinska, D., & Szwengiel, A. (2019). Kinetics of lactose hydrolysis and galactooligosaccharides formation in beverages based on goat ' s milk and its permeate. *Food Science and Biotechnology*, *28*(5), 1529–1534.

Ko, H., Bae, J. H., Sung, B. H., Kim, M. J., Park, H. J., & Sohn, J. H. (2019). Microbial production of medium chain fructooligosaccharides by recombinant yeast secreting bacterial inulosucrase. *Enzyme and Microbial Technology*, *130*, 109364.

Kumar, C. G., Sripada, S., & Poornachandra, Y. (2018). Status and Future Prospects of Fructooligosaccharides as Nutraceuticals. In *Role of Materials Science in Food Bioengineering*. Elsevier Inc.

Lans, A. M., Frelka, J. C., Paluri, S., & Vodovotz, Y. (2018). Physical properties and sensory analysis of

galacto-oligosaccharide glassy confections. *LWT - Food Science and Technology*, *96*, 499–506.

Li, F., Wang, H., Xin, H., Cai, J., Fu, Q., & Jin, Y. (2016). Development, validation and application of a hydrophilic interaction liquid chromatography-evaporative light scattering detection based method for process control of hydrolysis of xylans obtained from different agricultural wastes. *Food Chemistry*, *212*, 155–161.

Licitra, R., Li, J., Liang, X., Altomonte, I., Salari, F., Yan, J., & Martini, M. (2019). Profile and content of sialylated oligosaccharides in donkey milk at early lactation. *LWT - Food Science and Technology*, *115*, 108437.

Lin, H., Li, S., Xu, C., Pang, M., & Wang, S. (2018). Simultaneous determination of galactose, glucose, lactose and galactooligosaccharides in galactooligosaccharides raw materials by high-performance anion-exchange chromatography with pulsed amperometric detection. *Food Chemistry*, *263*(639), 29–36.

Ma, L., Mcjarrow, P., & Fong, B. Y. (2019). Quantification of major milk oligosaccharides in a range of formulated milk powder products using high performance liquid chromatography-multi reaction monitoring-mass spectrometry. *International Dairy Journal*, *94*, 1–6.

Ma, L., Mcjarrow, P., Jan, H., Mohamed, B. J., Liu, X., Welman, A., & Fong, B. Y. (2018). Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *International Dairy Journal*, *87*, 1–10.

Mellado-Mojica, E., Gonzalez de la Vara, L., & Lopez, M. (2016). Fructan active enzymes (FAZY) activities and biosynthesis of fructooligosaccharides in the vacuoles of Agave tequilana Weber Blue variety plants of different age. *Planta*, *245*, 265–281.

Menéndez, C., Martínez, D., Pérez, E. R., Musacchio, A., Ramírez, R., López-Munguía, A., & Hernández, L. (2019). Engineered thermostable β–fructosidase from Thermotoga maritima with enhanced fructooligosaccharides synthesis. *Enzyme and Microbial Technology*, *125*, 53–62.

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

Moreno, F. J., Corzo, N., Montilla, A., & Villamiel, M. (2017). Current state and latest advances in the concept , production and functionality of prebiotic oligosaccharides. *Current Opinion in Food Science*, *13*, 50–55.

Nieto-domínguez, M., Eugenio, L. I. De, York-durán, M. J., Rodríguez-colinas, B., Plou, F. J., Chenoll, E., Pardo, E., Codoñer, F., & Jesús, M. (2017). Prebiotic effect of xylooligosaccharides produced from birchwood xylan by a novel fungal GH11 xylanase. *Food Chemistry*, *232*, 105–113.

Nobre, C., Cerqueira, M. Â., Rodrigues, L. R., Vicente, A., & Teixeira, J. A. (2015). Production and Extraction of Polysaccharides and Oligosaccharides and Their Use as New Food Additives. In *Industrial Biorefineries and White Biotechnology*. Elsevier B.V.

Pöhnl, T., Böttcher, C., Schulz, H., Stürtz, M., Widder, S., Carle, R., & Schweiggert, R. M. (2017). Comparison of high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and ultra-high performance liquid chromatography with evaporative light scattering (UHPLC-ELSD) for the analyses of fructooligosaccharides in onion. *Journal of Food Composition and Analysis*, *63*, 148–156.

Porras-domínguez, J. R., Rodríguez-alegría, M. E., & Ávila-fernández, Á. (2017). Levan-type fructooligosaccharides synthesis by a levansucrase-endolevanase fusion enzyme (LevB 1 SacB).

Carbohydrate Polymers, *177*, 40–48.

Prokopov, T., Slavov, A., Petkova, N., Yanakieva, V., Bozadzhiev, B., & Taneva, D. (2018). Study of Onion Processing Waste Powder for Potential Use in Food Sector. *Acta Alimentaria*, *47*(2), 181–188.

Pu, J., Zhao, X., Xiao, L., & Zhao, H. (2017). Development and validation of a HILIC-ELSD method for simultaneous analysis of non-substituted and acetylated xylo-oligosaccharides. *Journal of Pharmaceutical and Biomedical Analysis*, *139*, 232–237.

Rad, A. H., Azizi, A., Dargahi, R., Bakhtiari, O., Javadi, M., & Jafarzadeh, M. (2018). Development of Synbiotic Milk Chocolate Enriched with Lactobacillus. *Applied Food Biotechnology*, *5*(2), 59–68.

Robinson, R. C., Colet, E., Tian, T., Poulsen, N. A., & Barile, D. (2018). An improved method for the purification of milk oligosaccharides by graphitised carbon-solid phase extraction. *International Dairy Journal*, *80*, 62–68.

Rodríguez-Gómez, R., Jiménez-Díaz, I., Zafra-Gómez, A., & Morales, J. C. (2015). Improved sample treatment for the determination of fructooligosaccharides in milk related products by liquid chromatography with electrochemical and refractive index detection. *Talanta*, *144*, 883–889.

Sabater, C., Fara, A., Palacios, J., Corzo, N., Requena, T., Montilla, A., & Zárate, G. (2019). Synthesis of prebiotic galactooligosaccharides from lactose and lactulose by dairy propionibacteria. *Food Microbiology*, *77*, 93–105.

Sabater, C., Prodanov, M., Olano, A., Corzo, N., & Montilla, A. (2016). Quantification of prebiotics in commercial infant formulas. *Food Chemistry*, *194*, 6–11.

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., & Sridhar, M. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrates and Dietary Fibre*, *5*(1), 62–71.

Sánchez-Martínez, M. J., Soto-Jover, S., Antolinos, V., Martínez-Hernández, G. B., & López-Gómez, A. (2020). Manufacturing of Short-Chain Fructooligosaccharides: from Laboratory to Industrial Scale. *Food Engineering Reviews*, *12*(2), 149–172.

Silva, E. K., Arruda, H. S., Pastore, G. M., Meireles, M. A. A., & Saldaña, M. D. A. (2020). Xylooligosaccharides chemical stability after high-intensity ultrasound processing of prebiotic orange juice. *Ultrasonics Sonochemistry*, *63*, 104942. https://doi.org/10.1016/j.ultsonch.2019.104942

Singh, R. D., Banerjee, J., & Arora, A. (2015). Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. *Bioactive Carbohydrates and Dietary Fibre*, *5*(1), 19–30.

Singh, R. D., Gracy, C., Muir, J., & Arora, A. (2019). Green and clean process to obtain low degree of polymerisation xylooligosaccharides from almond shell. *Journal of Cleaner Production*, *241*, 118237.

Singh, S. P., Jadaun, J. S., Narnoliya, L. K., & Pandey, A. (2017). Prebiotic Oligosaccharides: Special Focus on Fructooligosaccharides, Its Biosynthesis and Bioactivity. *Applied Biochemistry and Biotechnology*, *183*(2), 613–635.

Solaiman, D. K. Y., Ashby, R. D., & Crocker, N. V. (2020). Bioprocess for hydrolysis of galacto-oligosaccharides in soy molasses and tofu whey by recombinant Pseudomonas chlororaphis. *Biocatalysis and Agricultural Biotechnology*, *24*, 101529.

Surek, E., & Buyukkileci, A. O. (2017). Production of xylooligosaccharides by autohydrolysis of hazelnut (Corylus avellana L.) shell. *Carbohydrate Polymers*, *174*, 565–571.

Tonon, K. M., Miranda, A., Cristina, A., Abrão, F. V, Morais, M. B. De, & Morais, T. B. (2019). Validation and application of a method for the simultaneous absolute quantification of 16 neutral and acidic human milk oligosaccharides by graphitized carbon liquid chromatography – electrospray ionization – mass spectrometry. *Food Chemistry*, *274*, 691–697.

Trollope, K. M., Volschenk, H., Görgens, J. F., Bro, R., & Nieuwoudt, H. H. (2015). Direct, simultaneous quantification of fructooligosaccharides by FT-MIR ATR spectroscopy and chemometrics for rapid identification of superior, engineered β-fructofuranosidases. *Analytical and Bioanalytical Chemistry*, *407*(6), 1661–1671.

Vandenplas, Y., Berger, B., Carnielli, V. P., Id, J. K., Id, H. L., Sanchez, M., Id, L., Id, N. M., & Mosselmans, J. (2018). Human Milk Oligosaccharides: 2'-Fucosyllactose (2'-FL) and Lacto-N-Neotetraose (LNnT) in Infant Formula. *Nutrients*, *10*(1161).

Vandenplas, Y., Zakharova, I., & Dmitrieva, Y. (2015). Oligosaccharides in infant formula: More evidence to validate the role of prebiotics. *British Journal of Nutrition*, *113*(9), 1339–1344.

Vega, R., & Zuniga-Hansen, M. E. (2015). The effect of processing conditions on the stability of fructooligosaccharides in acidic food products. *Food Chemistry*, *173*, 784–789.

Vera, C., Cordova, A., Aburto, C., Guerrero, C., Suarez, S., & Illanes, A. (2016). Synthesis and purification of galacto-oligosaccharides : state of the art. *World Journal of Microbiology and Biotechnology*, *32*(197).

Vicaretti, S. D., Mohtarudin, N. A., Garner, A. M., & Zandberg, W. F. (2018). Capillary Electrophoresis Analysis of Bovine Milk Oligosaccharides Permits an Assessment of the Influence of Diet and the Discovery of Nine Abundant Sulfated Analogues [Research-article]. *Journal of Agricultural and Food Chemistry*, *66*(32), 8574–8583.

Walsh, C., Lane, J. A., Sinderen, D. Van, & Hickey, R. M. (2020). From lab bench to formulated ingredient : Characterization, production, and commercialization of human milk oligosaccharides. *Journal of Functional Foods*, *72*, 104052.

Wang, G., Zhu, J., Liu, L., Yaqoob, M. U., Pei, X., Tao, W., Xiao, Z., Sun, W., & Wang, M. (2020). Optimization for galactooligosaccharides synthesis: A potential alternative for gut health and immunity. *Life Sciences*, *245*, 117353.

Wang, X., & Rastall, R. A. (2018). Prebiotic Ingredients in Probiotic Dairy Products. In *Probiotic Dairy Products* (pp. 253–292).

Wei, J., Wang, Z. A., Wang, B., Jahan, M., Wang, Z., Wynn, P. C., & Du, Y. (2018). Characterization of porcine milk oligosaccharides over lactation between primiparous and multiparous female pigs. *Scientific Reports*, *8*, 1–16.

Weiß, K., & Alt, M. (2017). Determination of Single Sugars , Including Inulin , in Plants and Feed Materials by High-Performance Liquid Chromatography and Refraction Index Detection. *Fermentation*, *3*(3), 36.

Xu, G., Davis, J. C. C., Goonatilleke, E., Smilowitz, J. T., German, J. B., & Lebrilla, C. B. (2016). Absolute Quantitation of Human Milk Oligosaccharides Reveals Phenotypic Variations during Lactation 1 – 3. *The Journal of Nutrition*, *147*(1), 117–124.

Yan, J., Ding, J., Jin, G., Yu, D., Yu, L., Long, Z., Guo, Z., Chai, W., & Liang, X. (2018). Profiling of

Sialylated Oligosaccharides in Mammalian Milk Using Online Solid Phase Extraction-Hydrophilic Interaction Chromatography Coupled with Negative-Ion Electrospray Mass Spectrometry [Research-article]. *Analytical Chemistry*, *90*(5), 3174–3182.

Yan, J., Ding, J., & Liang, X. (2017). Chromatographic methods for analysis of oligosaccharides in human milk. *Analytical Methods*, *9*, 1071–1077.

Yang, J., & Xu, Y. (2018). Functional Carbohydrate Polymers: Prebiotics. In T. Gutierrez (Ed.), *Polymers for Food Applications* (pp. 651–691). Springer, Cham.

Zhang, H., Xu, Y., & Yu, S. (2017). Co-production of functional xylooligosaccharides and fermentable sugars from corncob with effective acetic acid prehydrolysis Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest. *Bioresource Technology*, *234*, 343–349.

Zhang, W., Wang, T., Chen, X., Pang, X., Zhang, S., Joy, O., Shilong, J., Lu, J., & Lv, J. (2019). Absolute quantification of twelve oligosaccharides in human milk using a targeted mass spectrometry-based approach. *Carbohydrate Polymers*, *219*, 328–333.

Zhao, C., Wu, Y., Liu, X., Liu, B., Cao, H., Yu, H., Sarker, S. D., Nahar, L., & Xiao, J. (2017). Functional properties, structural studies and chemo-enzymatic synthesis of oligosaccharides. *Trends in Food Science & Technology*, *66*, 135–145.

Zhou, X., & Xu, Y. (2019). Integrative process for sugarcane bagasse biore fi nery to co-produce xylooligosaccharides and gluconic acid. *Bioresource Technology*, *282*(159), 81–87.

Zhou, X., Zhao, J., Zhang, X., & Xu, Y. (2019). An eco-friendly biorefinery strategy for xylooligosaccharides production from sugarcane bagasse using cellulosic derived gluconic acid as e ffi cient catalyst. *Bioresource Technology*, *289*(159), 121755.

Zhou, Y., Kruger, C., Ravi, G. S., Kumar, D. P. S., Vijayasarathi, S. K., Lavingia, M., Chen, X., & Ambriz, P. (2017). Safety evaluation of galacto- oligosaccharides : Subchronic oral toxicity study in Sprague-Dawley rats. *Toxicology Research and Application*, *1*, 1–12.

Zhuang, D., Qin, J., Wang, H., Zhang, Y., Liu, C., Ding, Q., & Lv, G. (2019). Oligosaccharide-based quality evaluation of Atractylodis rhizome and a strategy for simplifying its quality control. *BMC Chemistry*, *13*, 92.