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Chemical Characterization of Potentially Prebiotic Oligosaccharides in Brewed Coffee and Spent Coffee Grounds

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Supporting Information

ABSTRACT: Oligosaccharides are indigestible carbohydrates widely present in mammalian milk and in some plants. Milk oligosaccharides are associated with positive health outcomes; however, oligosaccharides in coffee have not been extensively studied. We investigated the oligosaccharides and their monomeric composition in dark roasted coffee beans, brewed coffee, and spent coffee grounds. Oligosaccharides with a degree of polymerization ranging from 3 to 15, and their constituent monosaccharides, were characterized and quantified. The oligosaccharides identified were mainly hexoses (potentially galacto-oligosaccharides and manno-oligosaccharides) containing a heterogeneous mixture of glucose, arabinose, xylose, and rhamnose. The diversity of oligosaccharides composition found in these coffee samples suggests that they could have selective prebiotic activity toward specific bacterial strains able to deconstruct the glycosidic bonds and utilize them as a carbon source.

KEYWORDS: *coffee, coffee industrial residues, mass spectrometry, oligosaccharides, prebiotics*

INTRODUCTION

Coffee is one of the most popular beverages worldwide and is usually prepared from two commercially grown varieties—*Coffea arabica* and *Coffea canephora* (also known as *robusta*) or their blends. Because of the great demand for coffee, large amounts of industrial waste products, such as spent coffee grounds (from instant coffee production and the direct preparation of beverages in cafeterias, restaurants, or homes), silver skin (from roasting), and other byproducts of the fruit and bean processing, are generated annually.[1,2] Industrial coffee residues are sometimes disposed of inappropriately and, therefore, represent an environmental concern, especially in developing countries. New publications have shown that spent coffee grounds represent potent sources of functional compounds and exhibit beneficial activities such as prebiotic, antimicrobial, and antioxidant properties.[2] Therefore, there is a pressing need to identify such compounds and utilize the industrial residues for the extraction of functional molecules, thus generating added value.[3]

Several components, including caffeine, polyphenols, melanoidins, diterpenes, etc., have been investigated in brewed coffee and its spent coffee grounds for several functional properties.[4] Even though carbohydrates make up 50−54% of green coffee beans and 38−42% of roasted coffee beans by weight (dry basis),[5] they have not been as extensively studied as a result of their high structural complexity. Green *C. arabica* beans have a content of polysaccharides as high as 50% (on dry basis), which include galactomannans, arabinogalactans, and cellulose.[3]

Unlike coffee polysaccharides, coffee oligosaccharides are small in size and have not been fully isolated and characterized. Oligosaccharides are carbohydrates generally consisting of 2−20 monomers linked by a variety of *O*-glycosidic bonds.[6] Other than sucrose, there is no evidence for the presence of naturally occurring oligosaccharides in green coffee beans. However, a few oligosaccharides have been found in roasted coffee beans, and they seem to derive from two polysaccharides, arabinogalactans and galactomannans, through several chemical reactions during the roasting process.[7−9]

The amount of oligosaccharides that are transferred into brewed coffee depends on factors such as the variety and origin of green beans, the degree of roasting, and the brewing conditions used. Structural elucidation and quantification of oligosaccharide profile in coffee will aid the understanding of their functional properties. Oligosaccharides are targets of new investigations because they exhibit highly specific functions such as acting as prebiotics by feeding beneficial bacteria, blocking attachment of pathogens in the gut, and interacting directly with intestinal cells. Prebiotics are dietary ingredients that cannot be digested by human-produced digestive enzymes, yet they provide a health benefit to the host mediated by selectively stimulating the growth and/or activity of one or a limited number of host gut microbiota.[10] Some abundant food oligosaccharides, including galactooligosaccharides and inulin, have prebiotic activity.[11]

The prebiotic properties of oligosaccharides highly depend on structural conformation, including their degree of polymerization, monosaccharide composition, and their glycosidic linkages. Identification of novel oligosaccharide structures can be challenging because of a range of molecular diversity and complexity of molecular structure, e.g., the heterogeneous

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compositions and stereochemistry of each monosaccharide unit, the possibility of branching, and different linkages. As a matter of fact, arabinogalactooligosaccharides and galactooligosaccharides—which may be composed of the same group of constituent monosaccharides and linked by the same glycosidic linkages as coffee oligosaccharides—possess biological activity on several bacteria known as probiotic in in vitro studies.[12−17]

Especially, mannooligosaccharides derived from mannan in spent coffee grounds have been isolated, and their beneficial effect on large bowel function was documented through a series of studies. Mannooligosaccharides were shown to promote growth of bifidobacteria as well as increase the production of short-chain fatty acids by in vitro studies,[18,19] in animal models,[20] and in human studies.[21,22] In addition, previous publications have also discussed their beneficial effect on reduction of fat absorption by animal and humans.[23−25] However, no results have been published yet to discuss the structure and potential prebiotic effect of intact oligosaccharides in brewed coffee or spent coffee grounds.

High-resolution mass spectrometry and tandem mass spectrometry offer the ability to comprehensively characterize oligosaccharide structures; these spectral technologies have already been successfully used to provide detailed information about the consumption of human milk oligosaccharides by probiotic bacteria in in vitro studies.[26] Here, we present the isolation, identification, and characterization of oligosaccharides from roasted coffee beans, brewed coffee, and its spent coffee grounds. Multiple chromatographic and spectroscopic analytical tools were used to identify and quantify the purified oligosaccharides.

■ MATERIALS AND METHODS

Samples, Reagents, and Instruments. Dark-roasted ground *C. arabica* and liquid coffee brew were provided by Keurig Green Mountain. A blend of coffee beans sourced from throughout the world was used. The blend contained green coffee components from Southeast Asia, East Africa, and South America. Roasted beans were ground using no. 5 grinder (see Table S1 for particle size information). All the samples were stored at −20 °C until ready to use.

To study the effect of brewing conditions, coffee samples were brewed using four brewing appliances, including a French press, a Bunn brewer, a K-cup coffee maker, and an espresso machine (hereafter referred to as French press, Bunn, K-cup, and espresso). All the beans were roasted to espresso roast (25 Agtron units color) and ground with Ditting Grinder (Ditting Maschinen AG, Bachenbülach, Switzerland) to different sizes (described in Table S1). French press method used beans ground with no. 9.0 grinder. Coffee was left to steep for 4 min in a bodum Chambord model French press, before depressing plunger and decanting coffee extract. Multiple extractions were performed and decanted extracts were pooled together to ensure the maximum extraction. The Bunn extract was made by using beans ground with no. 3.5 grinder, Bunn Axiom Brewer, with brewing temperature set at 93.3 °C. K-Cup brew was made by using beans ground with no. 3.5 grinder, on a B70 Keurig brewer at an 8 oz setting (∼236 mL). Espresso was prepared with beans ground with no. 3.0 grinder, in a La Marzocco espresso machine (Florence, Italy) with water pressurized to 130 psi. The spent coffee grounds were also analyzed. The values of total dissolved solid of the industrial samples are summarized in Table 1. The detailed parameters for all brewing techniques are summarized in Table S2.

D-Galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-rhamnose, and L-allose were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The LC-MS system used for analysis was Agilent 6520 Nano LC Chip-QToF (Agilent Technology, Santa Clara, CA, U.S.A.). The gas chromatograph with a flame ionization detector (GC-FID) was from Hewlett-Packard HP-6890 (Wilmington, DE, U.S.A.).

Table 1. Summary of Industrial Samples Received and Tested and Their Corresponding Total Dissolved Solids

sample type	sample phase	sample	total dissolved solids[a]
liquid coffee	liquid (brewed coffee)	French press	1044
		Bunn	1322
		Kcup	1443
		espresso	7518
spent coffee grounds[b]	solid (residues after brewing liquid coffee)	French press	160
		Bunn	177
		Kcup	235
		espresso	347

[a]Total dissolved solids (ppm) is measured at 18 °C. [b]Spent coffee grounds are brewed using the same methods as for the roasted beans.

A dryer was used for evaporation of solvent with nitrogen (Reacti-Vap III, Pierce, Rockford, IL, U.S.A.).

Oligosaccharides Extraction and Purification. Oligosaccharides from ground *C. arabica* beans were extracted with hot water according to a published method[27] with some modifications. A 5 g sample of ground coffee beans was extracted with 100 mL of water at 100 °C for 20 min under constant stirring. The extract was centrifuged at 4225*g* for 5 min to obtain the supernatant. Total dissolved solids (ppm) were measured at 18−23 °C depending on the room temperature (Ultramete II 4P, Myron L Company, Carlsbad, CA, U.S.A.). For liquid coffee samples, 5 mL of each coffee sample was used for further purification. The liquid coffee was defatted using the Folch method.[28] Briefly, 1 volume of liquid coffee was mixed with 4 volumes of 2:1 chloroform/methanol, mixed with a vortex mixer, and centrifuged to obtain the aqueous layer that contained the hydrophilic oligosaccharides. The aqueous layer was vacuum-dried and resuspended in water prior to purification by solid-phase extraction using reverse-phase C8 cartridges, followed by extraction on porous graphitized carbon cartridges.[29] Residual hydrophobic lipids, small peptide, and polysaccharides were bound to the C8 bonded silica, and the hydrophilic oligosaccharides were captured by a subsequent purification with porous graphitized carbon cartridges and stepwise elution with 20% and 40% acetonitrile/0.05% trifluoroacetic acid. The acetonitrile was removed with vacuum, and the dry oligosaccharides were resuspended in water for further analysis by LC-MS or GC-FID.

Characterization of Oligosaccharides by Chip-Quadrupole Time-of-Flight Mass Spectrometry. An Agilent 6520 NanoChip-LC-QToF was used for compositional analysis of intact coffee oligosaccharides. Dried samples were suspended in 200 μL of nanopure water and analyzed using nanoLC-Chip-QToF according a published method.[30] Oligosaccharide separation was achieved with the microfluidic high-performance liquid chromatograph (HPLC)-Chip consisting of an enrichment column (4 mm, 40 nL) and an analytical column (75 μm × 43 mm) with a nanoelectrospray tip. Both columns were packed with 5 μm of 250 Å porous graphitized carbon material. Binary solvent gradients were applied for oligosaccharides separation. Elution solvent A contained 97% nanopure water, 3% acetonitrile, and 0.1% formic acid; solvent B contained 90% acetonitrile, 10% nanopure water, and 0.1% formic acid. The columns were equilibrated with 100% solvent A. The 65 min gradient was programmed as follows: from 0 to 16% B from 2.5 to 20 min; from 16 to 40% B from 20 to 30 min; from 40% to 100% B from 30 to 40 min, followed by 100% B for 10 min; from 100 to 0% B from 50 to 55.01 min, and re-equilibrium at 0% B for another 10 min.

Mass spectrometry was set in the positive mode. Mass calibration was performed using internal reference masses (m/z 922.009798, 1221.990637). Automated precursor selection was applied for selecting peaks for tandem fragmentation. The threshold for peak selection was set at 200 ion counts for MS and 5 ion counts for MS/MS. The acquisition rate was 0.63 spectra/s. The isolation width for tandem MS was medium (∼4 m/z). The collision energy was set at 1.8 V/100 Da with an offset of −3.6 V.

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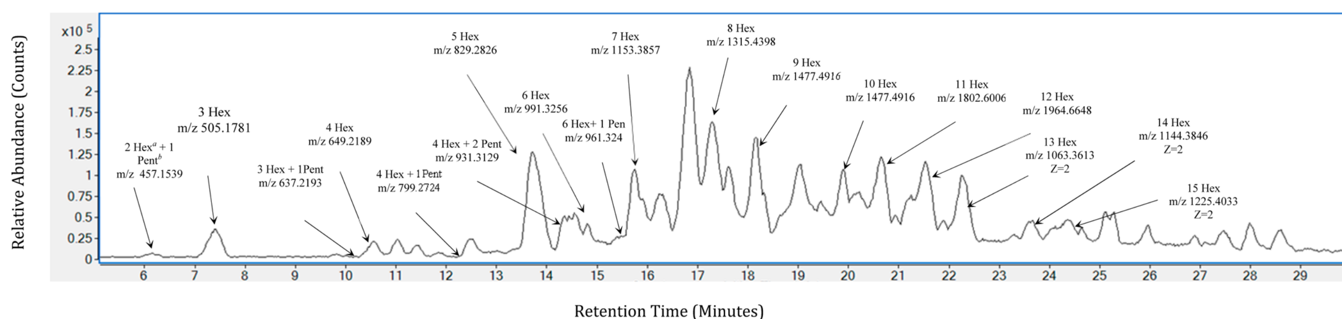

Figure 1. NanoLC-Chip-QToF base peak chromatogram of oligosaccharides in dark roasted coffee. Peaks were annotated with their compositional information obtained through MS and tandem MS.

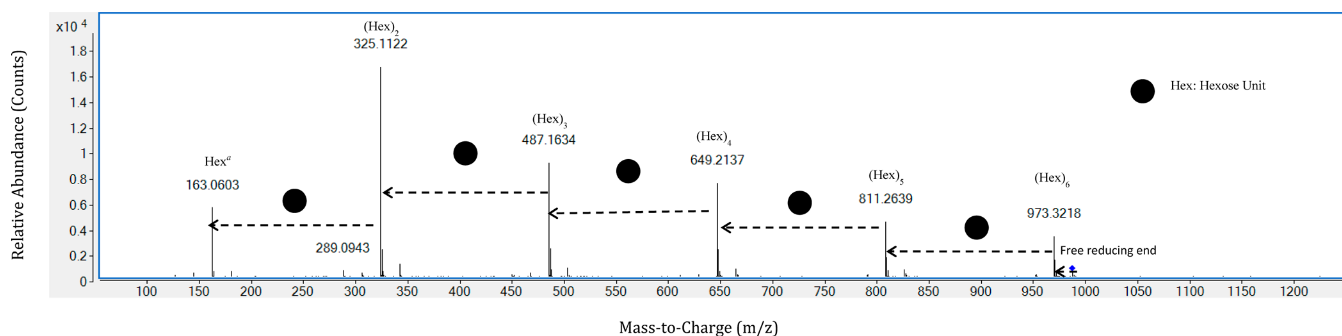

Figure 2. NanoLC-Chip-QToF MS/MS spectra of oligosaccharides with a DP of 6 (m/z 991.3328) in dark-roasted coffee. Fragment ions correspond to glycosidic bond cleavages. A difference of mass of 162.05 indicates loss of one hexose residue.

Mass spectra were analyzed using the molecular feature extraction in Mass Hunter Qualitative Analysis software version B.06.00. Oligosaccharide molecular formulas were determined for masses with an error as low as 10 ppm.

Characterization and Quantification of Monosaccharides Fingerprint by GC-FID. A gas chromatograph with an FID (GC-FID) was used to characterize the composition of monosaccharides and their absolute amounts. Carbohydrates have low volatility; therefore, preparation of derivatives was necessary for GC analysis. Methanolysis and trimethylsilylation (TMS) of purified coffee oligosaccharides was performed following a published procedure.[31,32] Methanol containing 0.5 M HCl was prepared by adding 2.8 mL of acetyl chloride to 20 mL of anhydrous methanol. Purified coffee oligosaccharides (0.5 mL) were dried, suspended in 0.5 mL of MeOH/HCl, and incubated at 80 °C for 16 h. The solutions were cooled to room temperature and dried under a stream of nitrogen; 250 μL of methanol was added and then dried under a stream of nitrogen. TriSil reagent (300 μL) was added to each sample, and the vials were incubated at 80 °C for 1 h. The residues were cooled at room temperature, and excess solvent was removed under a stream of nitrogen. The TMS derivatives were extracted by adding 1 mL of hexane and centrifuging at 10 000 rpm for 10 min. The solution was dried and suspended in 150 μL of hexane prior to injection (1 μL) into the GC-FID. The GC-FID was equipped with a capillary split/splitless inlet, coupled to an FID controlled by an HP ChemStation. A DB-1 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, U.S.A.) was used for separation of monosaccharides. Hydrogen was used as carrier gas with a flow rate of 2.5 mL/min. Samples were injected in the pulsed split mode with the split ratio of 5:1. The injector and the FID temperature was 280 °C. The GC temperature program was as follows: 120 to 200 °C by 1.5 °C/min, 200 °C for 5 min, and a post run of 2 min at 250 °C. Calibration curves were built for the following monosaccharide standards: glucose, galactose, mannose, arabinose, xylose, and rhamnose. Allose was used as internal standard. The detector response factors for individual monosaccharides were calculated to quantify each monosaccharide in oligosaccharide chains.

Statistical Analysis. The differences in constituent monosaccharides and total amount of oligosaccharides among different samples were analyzed using ANOVA followed by Tukey's posthoc test. All statistical analyses were conducted in R software, version 3.1.2. The threshold of significant difference was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Structural elucidation of coffee oligosaccharides is the first step to understanding their biological activity; at the same time, achieving high sample purity is critical for successful oligosaccharide analysis. Additionally, the method for extracting oligosaccharides from the cell wall matrix must be chosen with careful consideration, based on the particular properties of the compounds (such as polarity and solubility) and keeping in mind the goal of minimizing degradation or structural alterations of the oligosaccharides. Hot water extraction, a well-known method for plant oligosaccharide extraction, is often followed by protein precipitation with ethanol, dialysis, and ultrafiltration.[27,33,34] However, in our work, solid-phase extraction was a more efficient way to obtain high-purity oligosaccharides, which was a crucial requirement to accomplish characterization by mass spectrometry.

MS Analysis of Roasted Coffee Beans. Oligosaccharides were analyzed by nanoLC-Chip-QToF to obtain compositional information, including the size of individual oligosaccharides (degree of polymerization) and type of constituent monosaccharides. A typical chromatogram for oligosaccharides in dark-roast coffee is shown in Figure 1. The oligosaccharides were eluted with retention times between 5 and 30 min of the solvent gradient. Because single-stage MS only reveals information about size of molecules, to determine the individual monosaccharides and their size (degree of polymerization), the purified coffee oligosaccharides were also analyzed by tandem MS, which provided high resolution and accuracy

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for compositional identification. An example of a tandem spectrum for one oligosaccharide in dark-roasted coffee (m/z = 991.3382) and its fragments is shown in Figure 2. The loss of 162.05 Da corresponded to the loss of a hexose residue due to the cleavage of glyosidic bonds. Overall, the fragment ions allowed identification of the composition as six hexose residues and one free reducing end.

Table 2 presents the details of oligosaccharide composition obtained by tandem MS and Mass Hunter Quantitative analysis

Table 2. Details of Oligosaccharides and Constituent Monosaccharides[a] in Dark-Roasted Coffee

index	m/z	z	mass	hexose[b] Gal, Glc, Man	pentose[b] Ara, Xyl
1	505.1759	1	504.1687	3	
2	667.2235	1	666.2221	4	
3	829.2826	1	828.276	5	
4	991.3356	1	990.3282	6	
5	1153.3857	1	1152.3785	7	
6	1315.4398	1	1314.4355	8	
7	1477.4916	1	1476.4882	9	
8	820.2776	2	1638.5428	10	
9	901.3053	2	1800.5964	11	
10	982.3324	2	1962.6509	12	
11	1063.3613	2	2124.7079	13	
12	1144.3846	2	2286.7545	14	
13	1225.4033	2	2448.7855	15	
14	457.1563	1	474.1599	2	1
15	637.2193	1	636.2126	3	1
16	799.2724	1	798.2585	4	1
17	961.324	1	960.3071	5	1

[a]Gal, galactose; Glc, glucose; Man, mannose; Ara, arabinose; Xyl, xylose. [b]Numbers in these two columns indicate the number of monosaccharide units in the specific oligosaccharide structure.

(using a mass error as low as 10 ppm). Oligosaccharides identified in the roasted coffee samples were mainly of the hexose type, with different degrees of polymerization. Several ions corresponded to oligosaccharides made of pentose residues linked to hexose. Overall, the oligosaccharides in roasted coffee comprised from 3 to 15 hexose or hexose–pentose combinations.

Understanding the precursor polysaccharide structures will enable prediction of some coffee oligosaccharide structural information. Structurally, type II arabinogalactans in coffee beans have a backbone composed of β-(1 → 3)-linked galactopyranosyl residues frequently substituted at O-6 position by side chains formed by 1 → 6- and 1 → 3-linked β-galactosyl units and α-arabinosyl residues.[33] The 1 → 6 galactosyl side chains can be terminated by glucuronic acid residues.[35] The occurrence of rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains was also reported as a structural feature of coffee arabinogalactans.[36] As far as galactomannans are concerned, the structure has been described as linear polysaccharides with a main backbone of β-(1 → 4)-linked D-mannopyranose residues, 4–5% of which were substituted at O-6 with side chains of single α-(1 → 6)-linked D-galactopyranose residues.[9] The mannan backbones were sometimes interspersed with β-(1 → 4)-linked D-glucopyranose, and a side chain of single arabinose was also reported.[37] Previous research also reported the presence of singly, doubly, and consecutively acetylated mannose residues in coffee galactomannan.[37]

As degradation products from the two polysaccharides described above, we hypothesize that the hexose oligosaccharides would also be mainly β-(1 → 3)-linked galactopyranosyl residues or β-(1 → 4)-linked D-mannose residues with β-(1 → 6)-galactosyl/glucosyl units. The hexose–pentose oligosaccharides in coffee were possibly β-(1 → 3)-linked galactopyranosyl residues or β-(1 → 4)-linked D-mannose residues with an α-(1 → 3)-arabinosyl/xylosyl residue. The oligosaccharide profiles described in the present work are in partial agreement with the structural characterization of galactomannan derivatives published by Nunes, Coimbra, and co-workers.[7] These authors performed electrospray ionization (ESI)-MS analysis of galactomannan-derived oligosaccharide in roasted coffee and also found the major DP3 fractions were hexose oligosacchar-

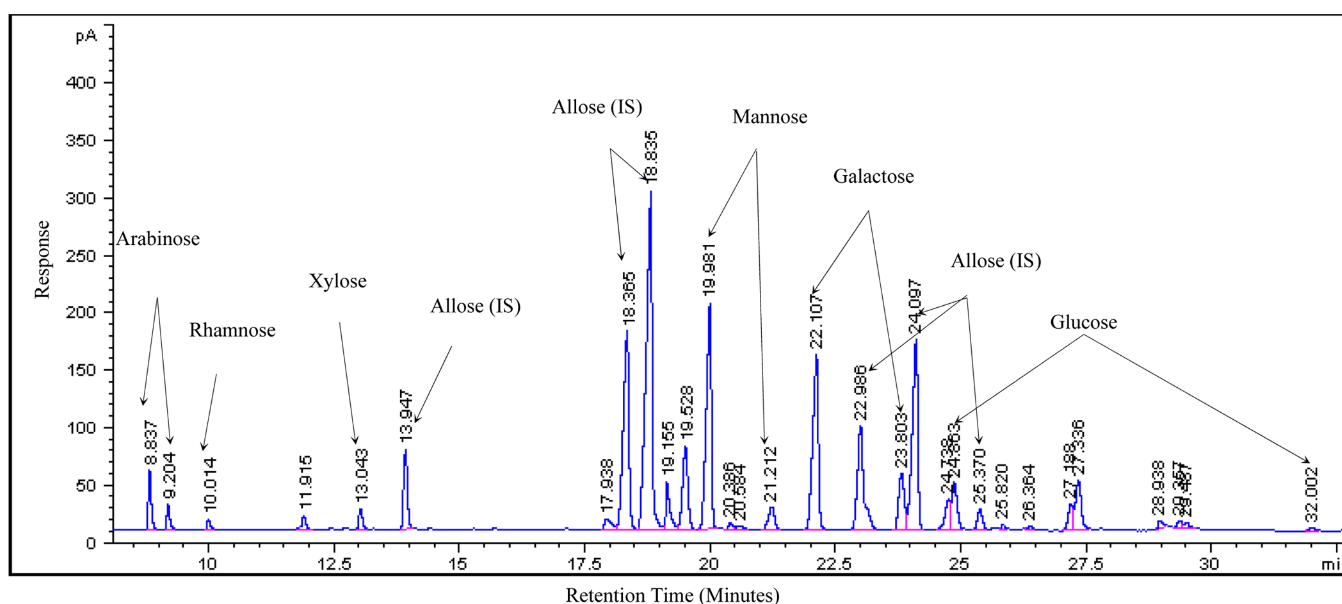

Figure 3. Gas chromatography profile of the TMS methyl glycoside derivatives generated after methanolic HCl treatment of coffee oligosaccharides. Allose was used as internal standard.

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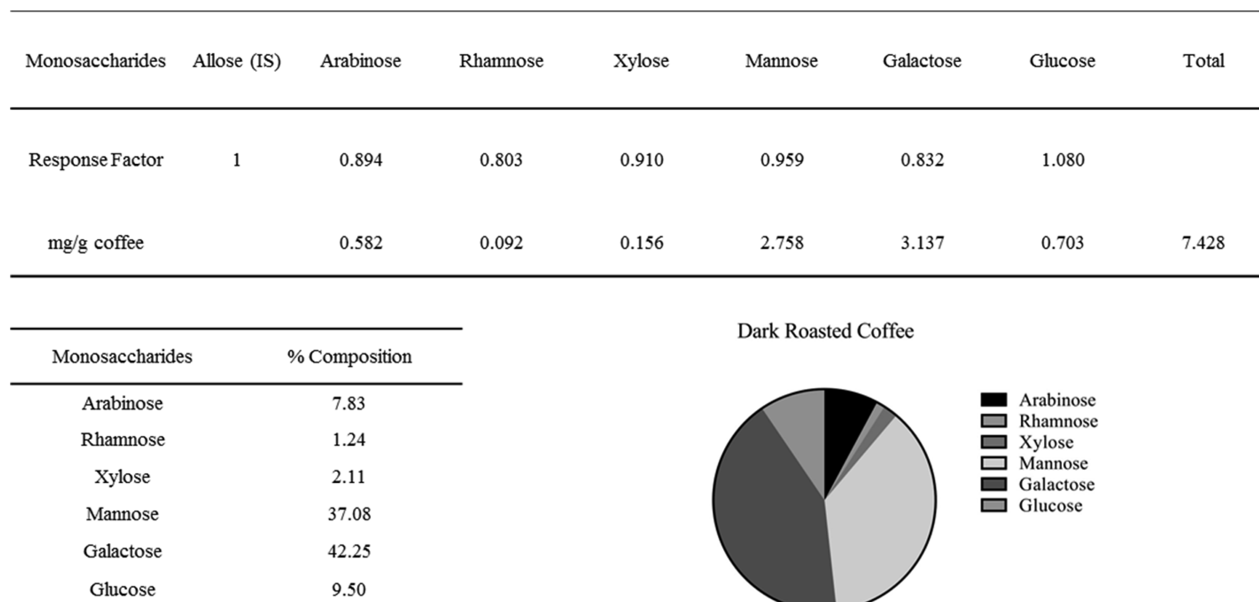

Monosaccharides	Allose (IS)	Arabinose	Rhamnose	Xylose	Mannose	Galactose	Glucose	Total
Response Factor	1	0.894	0.803	0.910	0.959	0.832	1.080	
mg/g coffee		0.582	0.092	0.156	2.758	3.137	0.703	7.428

Monosaccharides	% Composition
Arabinose	7.83
Rhamnose	1.24
Xylose	2.11
Mannose	37.08
Galactose	42.25
Glucose	9.50

Figure 4. Typical composition of dark-roasted coffee obtained by gas chromatography analysis; allose was used as the reference standard.

ides and pentosyl-containing trisaccharide Pent-Hex_2. Besides, they demonstrated the occurrence of oligosaccharide with acetylated species, oligosaccharide derivatives containing acid residues, modified residues of caramelization reactions, and Amadori compounds. The prediction of monosaccharide composition based on both mass spectrum and precursor information enabled the selection of appropriate monosaccharide standards for quantification (see GC analysis of constituent monosaccharides of coffee oligosaccharides). However, analysis and enzymatic assays beyond the scope of this work would be required to fully illustrate the oligosaccharide structures, including all linkages.

Limitations of MS. The nanoLC-Chip-QToF provided the necessary high resolution and high mass accuracy for the identification of oligosaccharide compositions. However, it must be noted that all hexose residues have the same molecular formula ($C_6H_{10}O_5$) and thus the same mass (162.0528 Da); therefore, MS, by virtue of measuring solely accurate mass, cannot discriminate among hexose isomers nor pentose isomers. Moreover, MS can only provide oligosaccharide relative abundance in part due to changes in ionization. The accurate quantification of coffee oligosaccharides could not be achieved without appropriate standards to obtain the unique response factors for each oligosaccharide, yet no commercial standards are available for coffee oligosaccharides. Therefore, the total amount of oligosaccharides was extrapolated via quantification of the constituent monosaccharides after methanolytic cleavage and derivatization.

GC Analysis of Constituent Monosaccharides of Coffee Oligosaccharides. The GC profile of the TMS derivatives of coffee oligosaccharides after methanolic HCl treatment is shown in Figure 3. Pentose (arabinose and xylose) and a deoxyhexose (rhamnose) were in lower abundance in general and eluted earlier than hexose (galactose, mannose, and glucose) molecules. Figure 4 shows an example of a typical monosaccharide composition of oligosaccharides in dark-roasted coffee, including the response factors of each monosaccharide standard. Mannose and galactose, respectively, at 37.1% and 42.2%, were the predominant constituents of oligosaccharides. Arabinose (7.8%) and glucose (9.5%) were also detected as major monosaccharide constituents, whereas xylose and rhamnose were found only in trace amounts (2.1% and 1.2%, respectively).

Partial structural information on coffee oligosaccharides was inferred by combining the results from MS and GC analysis. The oligosaccharides in brewed coffee mainly belonged to galactomannans and type II arabinogalactan, with galactose and mannose being the most represented in the backbone and other lower abundant monosaccharides (arabinose, xylose, and rhamnose) either mainly located in the side chain or interspersed in the main backbones. Mannose and galactose were found in approximately similar amounts. This fact is in agreement with the previously published results that soluble coffee fiber contained almost equal proportions of arabinogalactans and galactomannans.[17] Also, ∼9.5% glucose was detected in our oligosaccharide extracts. Similar results were reported by Redgwell et al., who found >10% of glucose residues as nonreducing terminal units on β-(1 → 6)-galactosyl side chains of type II arabinogalactan.[35] Arabinose was found to constitute about 7.8% (weight %) or 9% (mol %) of the total oligosaccharides, which is similar to the results (8 mol %) reported by Gniechwitz et al.[17] Trace amounts of xylose were also reported in previous research,[35] possibly deriving from the side chain type II arabinogalactan, too. The presence of rhamnose residues indicated the possibilities that rhamnoarbinosyl and rhamnoarabinoarabinosyl side chains of type II arabinogalactan were cleaved off during the roasting process, hence generating smaller oligosaccharides.

The structure−function properties of oligosaccharides in many foods have been studied, and one of the well-demonstrated activities is their prebiotic function. However, the efficacy of prebiotics may vary due to the specificity of their interactions with target commensal bacteria. The oligosaccharides in coffee are, in principle, nondigestible, thus making them possible candidates for prebiotic activity. Unlike commercially available prebiotics, which consist of repetition of the same monomer (fructose for inulin and galactose for galactooligosaccharides), the identified coffee oligosaccharides exhibited

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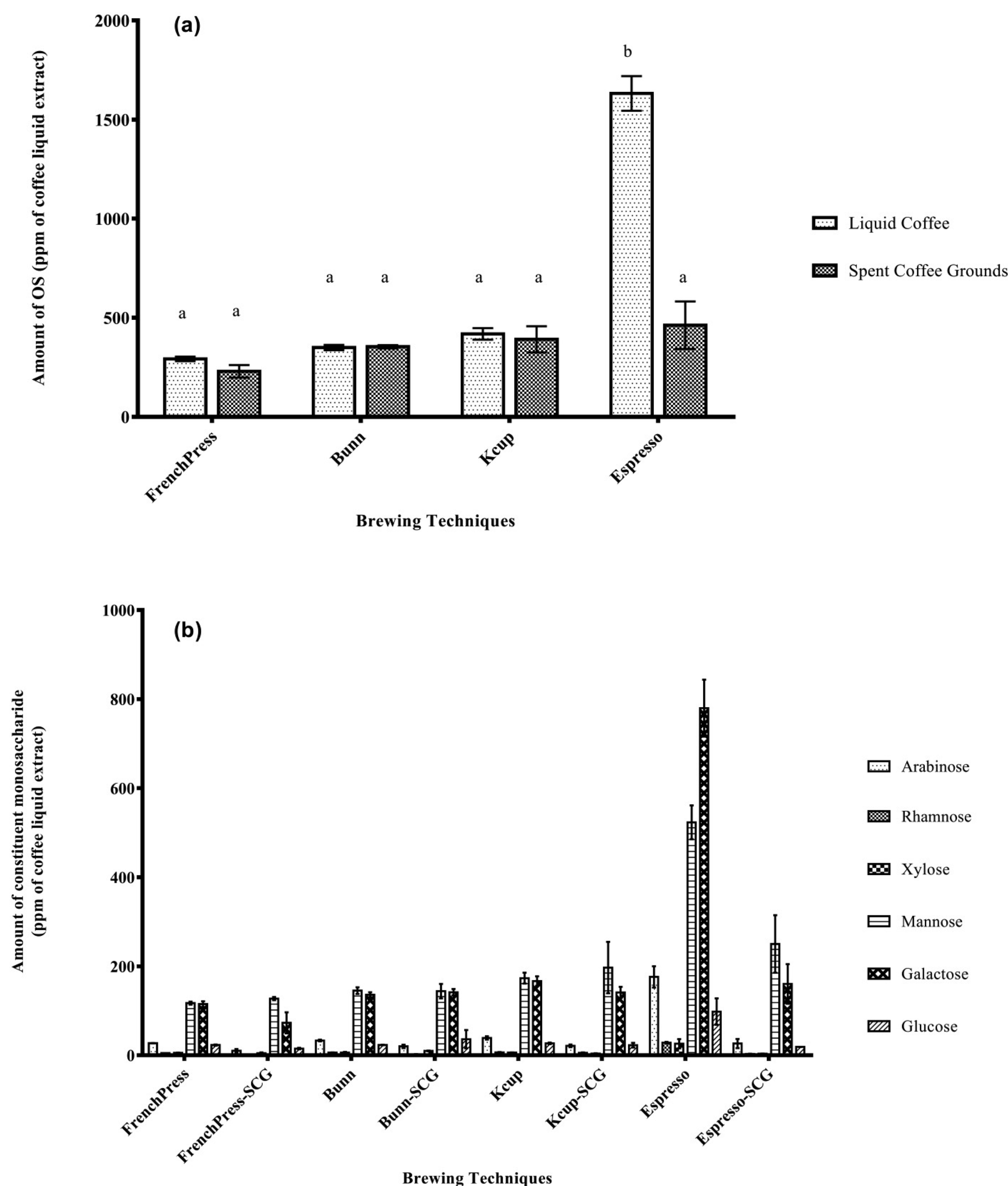

Figure 5. (a) Oligosaccharides and (b) their constituent monosaccharides in liquid coffee and corresponding spent coffee grounds (SCGs) from four brewing techniques. Different letters above the bar chart indicate the significant difference between various liquid coffees and their spent coffee grounds.

higher diversity in both size and composition. Their sizes ranged from those of 3 hexoses to 15 hexoses or hexose−pentose combination, and their constituent monosaccharides included 6 different monosaccharide types. The diversity in both the size and the monosaccharide composition provides the basis for future matching of coffee oligosaccharides with select probiotic strains.

Effects of Brewing on Oligosaccharide Distribution in Liquid Coffee and Spent Coffee Grounds. The effects of brewing techniques on the presence and abundance of oligosaccharides were investigated by analyzing coffee brewed using the following methods: French press, Bunn, K-cup, and espresso machine. The spent coffee grounds were subjected to hot water extraction and also analyzed for oligosaccharides.

Oligosaccharide compositions reported in Table 2 were found in all four liquid coffee samples. The chromatograms of French press, Bunn, and K-cup were similar, whereas espresso coffee had a rather distinct oligosaccharide profile with more abundant molecules larger than DP 10. Analysis by MS of the spent coffee grounds indicated the presence of oligosaccharides similar to what was observed in the corresponding liquid coffee samples, albeit in lower relative abundance. The only exception was that the espresso coffee extract contains more abundant oligosaccharides with DP above 10, while oligosaccharides with DP below 10 are in lower relative abundance in the espresso coffee extract than in the extract of the spent grounds. The oligosaccharide profile of extract and spent coffee grounds of espresso appears to correlate well and provide complementary oligosaccharides profiles. This could be explained by the finer

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bean size (no. 3.0 grinder) and the pressure applied during espresso brewing, which might have accelerated the release of oligosaccharides with higher DP in the liquid phase compared to the other brewing methods.

Figure 5 and Table 3 present the total amount of oligosaccharides (Figure 5a) and amount of constituent monosaccharides (Figure 5b) in liquid coffee and spent grounds obtained by different brewing techniques. ANOVA was conducted to compare the amount of oligosaccharides in liquid coffee and in hot water extract of spent coffee grounds. The result indicated a significant difference with p value equal to 2.06×10^{-7}. Tukey's method for post hoc test indicated a significantly higher level of oligosaccharides in espresso coffee than the rest of three brewing techniques and all spent coffee grounds.

Comparing the amount of oligosaccharides between liquid coffee and their corresponding spent coffee grounds in each technique shows that a comparable amount of oligosaccharides were found in the residues compared to their corresponding liquid coffee, but espresso coffee contained a significantly higher amount of oligosaccharides than its spent coffee grounds. The presence of oligosaccharides in spent coffee grounds provided another possible source of bioactive oligosaccharides. Moreover, the presence of significant amounts of DP < 10 oligosaccharides in spent coffee grounds obtained as byproduct of espresso suggests that a potential recovery strategy could be implemented.

As shown in Figure 5b, all six constituent monosaccharides were present in all four types of liquid coffee, and galactose and mannose were the major constituent monosaccharides. Looking at the difference among constituent monosaccharides in each brewing technique, per volume of liquid coffee, espresso contained a significantly higher level of all the constituent monosaccharides. Previous research has shown that extraction volume and temperature can influence the amount of carbohydrates released into brewed coffee.[38−41] The preparation of espresso includes grinding of roasted beans, powder dosing and tamping, and percolation, all of which may affect the yield of total dissolved solids, including oligosaccharides. The espresso coffee is characterized with higher bean-to-water ration and large amounts of bean fines of high specific surface, both of which allow the extraction of a large quantity of soluble material.[41] The pressurization used in percolation also increases the extraction of oligosaccharides. This was confirmed by comparing the total dissolved solids in liquid coffee samples. French press, Bunn, and K-cup had a similar range of ∼1000 ppm, whereas the espresso sample had up to 7500 ppm total dissolved solids. The high yield ensured extraction of oligosaccharides from coffee bean matrix throughout. However, considering the regular serving size of those liquids coffee, −60 mL (2 shots) for expresso and 470 mL (size of one mug) for the other liquid coffees, the amount of oligosaccharides consumed in each serving size is comparable.

Spent coffee grounds are a rich source of galactomannans and arabinogalactans. However, achieving complete extraction of polysaccharides/oligosaccharides with high yield remains a challenge. Several attempts have been made to extract beneficial polysaccharides from matrixes such as spent coffee grounds, including alkali extraction,[42,43] diluted acid hydrolysis,[44] and combination of dilute alkali treatment with addition of the enzyme cellulose.[45] Most recently, microwave superheated water extraction was reported by Passos and Coimbra as a feasible way to extract polysaccharides from spent coffee

Table 3. Constituent Monosaccharides and Total Oligosaccharides (OS) in Brewed Liquid Coffee and Spent Coffee Grounds from Different Brewing Techniques as Analyzed by Gas Chromatograph[a,b]

sample	technique	arabinose	rhamnose	xylose	mannose	galactose	glucose	total OS
liquid coffee	French press	27.03 ± 0.32 a	4.81 ± 0.01 bc	5.44 ± 0.54 a	117.23 ± 3.42 a	114.80 ± 6.64 a	23.02 ± 1.31 a	292.33 ± 11.15 a
	Bunn	33.13 ± 1.96 a	6.03 ± 0.51 b	6.18 ± 1.37 a	145.36 ± 7.42 a	135.89 ± 5.50 a	23.19 ± 0.54 a	349.80 ± 13.48 a
	Kcup	39.10 ± 3.71 a	6.64 ± 0.55 b	6.02 ± 0.36 a	173.31 ± 12.00 a	166.60 ± 10.59 a	26.72 ± 1.90 a	418.40 ± 29.11 a
	espresso	176.16 ± 23.83 b	28.36 ± 1.74 a	26.02 ± 10.19 b	523.43 ± 38.02 b	779.85 ± 63.90 b	98.10 ± 29.69 b	1631.94 ± 87.61 b
spent coffee grounds	French press	9.98 ± 4.19 a	ND	4.50 ± 1.91 a	127.31 ± 3.41 a	72.97 ± 23.73 a	15.12 ± 1.67 a	229.87 ± 31.58 a
	Bunn	19.95 ± 3.96 a	2.11 ± 0.82 c	9.81 ± 0.78 a	144.14 ± 16.14 a	141.69 ± 7.20 a	36.09 ± 20.53 a	353.79 ± 8.37 a
	Kcup	21.19 ± 2.91 a	5.33 ± 1.31 bc	4.06 ± 0.43 a	196.97 ± 57.73 a	141.17 ± 12.50 a	22.59 ± 5.12 a	391.32 ± 66.29 a
	espresso	26.47 ± 10.42 a	3.19 ± 0.01 bc	3.49 ± 0.42 a	250.00 ± 64.56 a	160.26 ± 44.40 a	18.86 ± 0.19 a	462.28 ± 119.88 a

[a]Monosaccharides are expressed as mean ppm ± SD ($n = 3$); ND, not detected. [b]Numbers followed by the same letter, within a column, are not significantly different ($p > 0.05$). Different letters in the same column indicate statistical difference.

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grounds.[46] These same authors further explored the feasibility of using sequential microwave superheated water extraction and achieved higher recovery of coffee mannans.[47] Roasting of spent coffee grounds was reported to improves the extractability of galactomannans without their apparent degradation.[48] Hot water extraction was chosen for this initial study; however, the newly developed technologies can be adapted to improve the yield in the future studies.

This study revealed the presence of many types of oligosaccharides in brewed coffee and spent grounds. The composition, structure, and abundance of oligosaccharides varied among different brewing conditions. Espresso liquid extract was found to contain a significantly higher amount of oligosaccharides, but the amount of oligosaccharides consumed in each serving size is comparable with other types of brewing techniques. Despite the fact that spent coffee grounds contained smaller amounts of oligosaccharides compared with beans and brewed coffee, they are still a promising source for bioactive oligosaccharides that may be a source of high value-added products. The release of oligosaccharides from industrial coffee residues may not be as easy as direct extraction from roasted coffee beans, but this could be partially solved by using enzymes to carry on a partial hydrolysis from the matrix, diluted alkali extraction, or with the aid of microwave superheated water extraction.

The characterization of coffee oligosaccharides in this study suggests their possible role as prebiotics. The diversity in monosaccharide compositions, linkages, and isomers and the range of sizes provides the basis for a selective support of probiotic growth, which could lead to the development of novel prebiotics and synbiotics (combination of probiotics and prebiotics). The demonstrated diversity of monosaccharide composition suggests that only bacteria possessing specific enzymatic (glycosidases) capabilities would be able to cleave the various monosaccharides in the side chains prior to their utilization as a carbon source. The combination of probiotics with synergistically acting prebiotic substrates would improve bacterial survival and potentiate the efficacy of beneficial microorganisms. This could provide the basis for developing the next generation of selective synbiotics by matching coffee oligosaccharides to specific probiotic bacteria that possess the necessary enzymes for utilization. The present work demonstrates that coffee and coffee byproducts represent a promising source of a novel functional food rich in bioactive oligosaccharides. The data on brewing techniques may lead to more effective and targeted extraction of bioactive oligosaccharides from coffee through careful manipulation of the key processing parameters.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b04716.

Roast and ground particle size characterization; detailed parameters for four brewing techniques in this study (PDF)

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Notes

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■ ABBREVIATIONS USED

DP, degree of polymerization; LC, liquid chromatography; QToF, quadrupole time-of-fight; TMS, trimethylsilylation

■ REFERENCES

(1) Mussatto, S. I.; Carneiro, L. M.; Silva, J. P.; Roberto, I. C.; Teixeira, J. A. A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydr. Polym.* **2011**, *83*, 368−374.

(2) Jiménez-Zamora, A.; Pastoriza, S.; Rufián-Henares, J. A. Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. *LWT–Food Sci. Technol.* **2015**, *61*, 12−18.

(3) Mussatto, S. I.; Machado, E. M.; Martins, S.; Teixeira, J. A. Production, composition, and application of coffee and its industrial residues. *Food Bioprocess Technol.* **2011**, *4*, 661−672.

(4) Clifford, M., Chemical and physical aspects of green coffee and coffee products. In *Coffee*; Springer: 1985; pp 305−374.

(5) Esquivel, P.; Jiménez, V. M. Functional properties of coffee and coffee by-products. *Food Res. Int.* **2012**, *46*, 488−495.

(6) Eggleston, G.; Cote, G. L. Oligosaccharides in Food and Agriculture. In *Oligosaccharides in food and agriculture*; Eggleston, G., Cote, G. L., Eds.; American Chemical Society: Washington, DC, 2003; p 2.

(7) Nunes, F. M.; Reis, A.; Domingues, M. R. M.; Coimbra, M. A. Characterization of galactomannan derivatives in roasted coffee beverages. *J. Agric. Food Chem.* **2006**, *54*, 3428−3439.

(8) Arya, M.; Rao, L. J. M. An impression of coffee carbohydrates. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 51−67.

(9) Moreira, A. S.; Coimbra, M. A.; Nunes, F. M.; Simoes, J.; Domingues, M. R. r. M. Evaluation of the effect of roasting on the structure of coffee galactomannans using model oligosaccharides. *J. Agric. Food Chem.* **2011**, *59*, 10078−10087.

(10) Glenn, G.; Roberfroid, M. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401−1412.

(11) Gibson, G. R.; Probert, H. M.; Van Loo, J.; Rastall, R. A.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.* **2004**, *17*, 259−275.

(12) Barreteau, H.; Delattre, C.; Michaud, P. Production of oligosaccharides as promising new food additive generation. *Food Technol. Biotechnol.* **2006**, *44*, 323−333.

(13) Van Laere, K. M.; Hartemink, R.; Bosveld, M.; Schols, H. A.; Voragen, A. G. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. *J. Agric. Food Chem.* **2000**, *48*, 1644−1652.

(14) Olano-Martin, E.; Mountzouris, K.; Gibson, G.; Rastall, R. Continuous production of pectic oligosaccharides in an enzyme membrane reactor. *J. Food Sci.* **2001**, *66*, 966−971.

(15) Olano-Martin, E.; Gibson, G.; Rastall, R. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. *J. Appl. Microbiol.* **2002**, *93*, 505−511.

(16) Oosterveld, A.; Beldman, G.; Voragen, A. G. Enzymatic modification of pectic polysaccharides obtained from sugar beet pulp. *Carbohydr. Polym.* **2002**, *48*, 73−81.
(17) Gniechwitz, D.; Reichardt, N.; Blaut, M.; Steinhart, H.; Bunzel, M. Dietary fiber from coffee beverage: degradation by human fecal microbiota. *J. Agric. Food Chem.* **2007**, *55*, 6989−6996.
(18) Asano, I.; Hamaguchi, K.; Fujii, S.; IINO, H. In vitro digestibility and fermentation of mannooligosaccharides from coffee mannan. *Food Sci. Technol. Res.* **2003**, *9*, 62−66.
(19) Asano, I.; Nakamura, Y.; Hoshino, H.; Aoki, K.; Fujii, S.; Imura, N.; Iino, H. Use of mannooligosaccharides from coffee mannan by intestinal bacteria. *Nippon Nogei Kagaku Kaishi* **2001**, *75*, 1077−1083.
(20) Asano, I.; Ikeda, Y.; Fujii, S.; Iino, H. Effects of mannooligosaccharides from coffee on microbiota and short chain fatty acids in rat cecum. *Food Sci. Technol. Res.* **2004**, *10*, 273−277.
(21) Asano, I.; Umemura, M.; Fujii, S.; Hoshino, H.; Iino, H. Effects of mannooligosaccharides from coffee mannan on fecal microflora and defecation in healthy volunteers. *Food Sci. Technol. Res.* **2004**, *10*, 93−97.
(22) Walton, G. E.; Rastall, R. A.; Martini, M.; Williams, C.; Jeffries, R.; Gibson, G. R. A double-blind, placebo controlled human study investigating the effects of coffee derived manno-oligosaccharides on the faecal microbiota of a healthy adult population. *Int. J. Probiotics Prebiotics* **2010**, *5*, 75−84.
(23) Asano, I.; Fujii, S.; Kaneko, M.; Takehara, I.; Fukuhara, I. Investigation of mannooligosaccharides blended coffee beverage on abdominal fat reduction in humans. *Jpn. J. Med. Pharm. Sci.* **2006**, *55*, 93−103.
(24) Hoshino-Takao, I.; Fujii, S.; Ishii, A.; Han, L.-K.; Okuda, H.; Kumao, T. Effects of mannooligosaccharides from coffee mannan on blood pressure in Dahl salt-sensitive rats. *J. Nutr. Sci. Vitaminol.* **2008**, *54*, 181−184.
(25) Kumao, T.; Fujii, S.; Ozaki, K.; Takao, I. Effect of diets with mannooligosaccharides from coffee mannan on fat in blood serum in healthy volunteers. *Jpn. J. Food Eng.* **2005**.
(26) Barile, D.; Rastall, R. A. Human milk and related oligosaccharides as prebiotics. *Curr. Opin. Biotechnol.* **2013**, *24*, 214−9.
(27) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted arabica coffee. *J. Agric. Food Chem.* **2001**, *49*, 1773−1782.
(28) Folch, J.; Lees, M.; Sloane-Stanley, G. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.
(29) Ninonuevo, M. R.; Park, Y.; Yin, H.; Zhang, J.; Ward, R. E.; Clowers, B. H.; German, J. B.; Freeman, S. L.; Killeen, K.; Grimm, R.; Lebrilla, C. B. A strategy for annotating the human milk glycome. *J. Agric. Food Chem.* **2006**, *54*, 7471−7480.
(30) Barile, D.; Marotta, M.; Chu, C.; Mehra, R.; Grimm, R.; Lebrilla, C.; German, J. Neutral and acidic oligosaccharides in Holstein-Friesian colostrum during the first 3 days of lactation measured by high performance liquid chromatography on a microfluidic chip and time-of-flight mass spectrometry. *J. Dairy Sci.* **2010**, *93*, 3940−3949.
(31) Ayestarán, B.; Guadalupe, Z.; León, D. Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Anal. Chim. Acta* **2004**, *513*, 29−39.
(32) Bordiga, M.; Travaglia, F.; Meyrand, M.; German, J. B.; Lebrilla, C. B.; Coïsson, J. D.; Arlorio, M.; Barile, D. Identification and characterization of complex bioactive oligosaccharides in white and red wine by a combination of mass spectrometry and gas chromatography. *J. Agric. Food Chem.* **2012**, *60*, 3700−3707.
(33) Bradbury, A. G. W. Chemistry I: Non-volatile Compounds. In *Coffee: Recent developments*; Clarke, R., Vitzthum, O., Eds.; John Wiley & Sons: Oxford, 2008; pp 1−17.
(34) Nunes, F. M.; Coimbra, M. A.; Duarte, A. C.; Delgadillo, I. Foamability, foam stability, and chemical composition of espresso coffee as affected by the degree of roast. *J. Agric. Food Chem.* **1997**, *45*, 3238−3243.
(35) Redgwell, R. J.; Curti, D.; Fischer, M.; Nicolas, P.; Fay, L. B. Coffee bean arabinogalactans: acidic polymers covalently linked to protein. *Carbohydr. Res.* **2002**, *337*, 239−253.
(36) Nunes, F. M.; Reis, A.; Silva, A. M.; Domingues, M. R. M.; Coimbra, M. A. Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains as structural features of coffee arabinogalactans. *Phytochemistry* **2008**, *69*, 1573−1585.
(37) Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. *Carbohydr. Res.* **2005**, *340*, 1689−1698.
(38) Petracco, M. In *Physico-chemical and structural characterization of espresso coffee brew*; Proceedings of the 13th International Colloquium on the Chemistry of Coffee, 1989; ASIC: Paris, France, 1989.
(39) Thaler, H. The chemistry of coffee extraction in relation to polysaccharides. *Food Chem.* **1979**, *4*, 13−22.
(40) Leloup, V.; Liardon, R. In *Analytical characterization of coffee carbohydrates*; Colloque Scientifique International sur le Cafe, 1993; ASIC Association Scientifique Internationale: 1993; pp 863−863.
(41) Illy, E.; Navarini, L. Neglected food bubbles: the espresso coffee foam. *Food biophysics* **2011**, *6*, 335−348.
(42) Fischer, M.; Reimann, S.; Trovato, V.; Redgwell, R. J. Polysaccharides of green Arabica and Robusta coffee beans. *Carbohydr. Res.* **2001**, *330*, 93−101.
(43) Simões, J.; Madureira, P.; Nunes, F. M.; do Rosário Domingues, M.; Vilanova, M.; Coimbra, M. A. Immunostimulatory properties of coffee mannans. *Mol. Nutr. Food Res.* **2009**, *53*, 1036−1043.
(44) Mussatto, S. I.; Machado, E. M.; Martins, S.; Teixeira, J. A. Production, composition, and application of coffee and its industrial residues. *Food Bioprocess Technol.* **2011**, *4*, 661.
(45) Iwai, K.; Kasai, N. Method of producing polysaccharides from coffee beans or/and coffee extraction residue. US Patent 20100227366 A1, 2010.
(46) Passos, C. P.; Coimbra, M. A. Microwave superheated water extraction of polysaccharides from spent coffee grounds. *Carbohydr. Polym.* **2013**, *94*, 626−633.
(47) Passos, C. P.; Moreira, A. S.; Domingues, M. R. M.; Evtuguin, D. V.; Coimbra, M. A. Sequential microwave superheated water extraction of mannans from spent coffee grounds. *Carbohydr. Polym.* **2014**, *103*, 333−338.
(48) Simões, J.; Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Extractability and structure of spent coffee ground polysaccharides by roasting pre-treatments. *Carbohydr. Polym.* **2013**, *97*, 81−89.

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