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Structure Investigation of Polysaccharides Extracted from Spent Coffee Grounds Using an Eco-Friendly Technique

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Abstract: Coffee is one of the most marketable products worldwide but throughout the production chain, many by-products and waste are generated, e.g., spent coffee grounds (SCG). SCG are considered a promising source of polysaccharides. In the present study, the polysaccharides from SCG were recovered through microwave-assisted extraction. Detailed structural analysis showed that SCG were mainly composed of low-branched galactomannan, followed by various lengths and branches of galactan or arabinogalactan chains whose side chains are randomly ended by glucuronic acid. These findings indicate that glucuronic acid remains attached to the arabinogalactan chain even after coffee roasting and brewing. The investigation confirmed that microwave-assisted extraction, as a green extraction technique, is a promising method for preparing polysaccharides with lower molecular weight. This extraction method ensures the recovery of SCG polysaccharides with potential biological activity without destroying the glucuronic acid at the arabinogalactan chains.

Keywords: coffee by-products; microwave-assisted extraction; galactomannans; acid arabinogalactan; NMR

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1. Introduction

The latest food trends show that coffee is one of the most consumed beverages worldwide due to its unique taste. To meet the demands, coffee trees are cultivated in enormous fields in about 80 countries worldwide and the coffee market is comparable to that of petroleum [1]. Since 1963 and the establishment of the International Coffee Organization, the economic importance of coffee has been constantly rising. Due to this massive production, several by-products are generated during coffee processing, including spent coffee grounds, husks, silverskin, and defective coffee beans. Spent coffee grounds (SCG), the organic compounds-rich residue obtained during the brewing process, accounts for approximately 45–50% of the total by-products [1]. It is estimated that almost 6 million tons of SCG are generated by the production of instant coffee and coffee brewing [2]. To date, SCG have been disposed of in landfills leading to environmental pollution, but the valorization of this material for the recovery of bioactive compounds and the development of value-added products is an effective way of minimizing their disposal in landfills [3].

SCG are composed of polysaccharides and oligosaccharides, lipids, and proteins. They are a source of essential amino acids, minerals, alkaloids (e.g., caffeine), melanoidins, and phenolic compounds [2]. Due to their composition, especially regarding phenolic content, they can be used as additives, possessing antioxidant, and anti-inflammatory properties, metal scavengers, dermatological anti-melanogenesis agents as well as animal feed supplement [2]. For example, SCG have been investigated as additives in food

products. Martinez-Saez et al. [4] added SCG directly to cookie dough, while Murthy and Madhava Naidu [5] have used it in flakes, bread, cookies, and snacks to enhance the nutritional value of the products.

As a matrix, SCG is an important source of polysaccharides [6]. The polysaccharide fraction is comprised of galactomannans, arabinogalactans, hemicelluloses, and cellulose with galactomannans accounting for 50% of it [7]. The extraction of valuable polysaccharides from SCG could be accomplished by various methods that exhibit different efficiency. While water-extractable polysaccharides are eluted into the coffee beverage, the release of polysaccharides from SCG requires more rigorous techniques. Mussatto et al. [7] extracted polysaccharides from SCG by acid hydrolysis, while alkaline pre-treatment was used by Ballesteros et al. [8]. However, the disadvantage of these methods is environmental pollution due to the use of chemical agents. As an alternative, a «green» way of extracting polysaccharides has been recently studied. More specifically, subcritical water extraction [9], and microwave irradiation [10,11] using only hot compressed water are becoming a feasible extraction method.

In addition, it is worth noting that the polysaccharides extracted from SCG have demonstrated biological activity. This finding highlights the potential value of these compounds in various applications. Ballesteros et al. [8] confirmed their antioxidant potential and reported significant antimicrobial inhibition against *Phoma violacea* and *Cladosporium cladosporioides*. In addition, Getachew et al. [12] reported the hypoglycaemic activity of SCG polysaccharides through the inhibition of α-glucosidase and α-amylase activity, revealing their potential as a promising alternative therapy for diabetes. Gu et al. [13] isolated SCG galactomannan, which exhibits in vitro immunostimulatory activities and prebiotic effects that promote bifidobacteria growth in the intestines. Moreover, the prebiotic activity of SCG oligosaccharides (3–6 units) on *Lactobacilli* strains has been reported by Sarghini et al. [14], highlighting the potential for these compounds to promote gut health. This article also points out the importance of the structure of oligosaccharides concerning biological effects.

Articles dealing with SCG polysaccharides only described neutral polysaccharides such as galactomannan and arabinogalactan, although Redgwell et al. [15] and Nunes et al. [16] confirmed that glucuronic acid is a part of arabinogalactan in green coffee beans. It is unclear whether uronic acid is present in SCG after roasting and brewing, and, for example, influences biological activities.

The aim of the present study was to fully characterize the polysaccharide extract isolated with a «green» extraction technique, namely microwave-assisted extraction, using NMR spectroscopy. This method provides additional valuable information about the structure of polysaccharides from SCG.

2. Materials and Methods

2.1. Materials and Reagents

SCG, remaining after the extraction of ground coffee (*Arabica* variety) with water at 95 °C, were provided by a local coffee shop in Thessaloniki, Greece. Immediately after collection, the material was placed in a drying oven (MOD type, 2100 High-Performance Oven, Fratelli Galli, Milano, Italy) at 60 °C for 10 h until constant weight and then stored in sealed containers in the freezer (−18 °C) until use.

Diethyl ether, methanol, Folin–Ciocalteau phenol reagent, potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$), sodium carbonate and sodium hydroxide (NaOH) were obtained from CHEM-LAB NV (Zedelgem, Belgium). Phenol solution (99.5%) was purchased from Panreac (Barcelona, Spain), while the gallic acid standard compound was obtained from Alfa Aesar (Karlsruhe, Germany). The sulfuric acid solution, trifluoroacetic acid, all monosaccharides' standards, and deuterium oxide were purchased from Merck (Darmstadt, Germany) or Duchefa (Haarlem, The Netherlands), and 3,5-dinitrosalicylic acid (DNS) was from TCI (Tokyo, Japan), respectively.

2.2. Soxhlet Extraction

For the removal of a lipid fraction from SCG, Soxhlet extraction was performed using diethyl ether as a solvent for 2 h. The crude fat content was calculated based on the following Equation (1):

$$TFC\ (\%) = \left(\frac{F - C}{S}\right) \times 100 \quad (1)$$

where F is the weight of the cartridge containing the lipid fraction (g), C is the weight of the empty cartridge (g), and S is the dry sample weight (g) placed in the cartridge. The TFC % refers to the dry weight of SCG. The extraction process was performed in triplicate and the results were expressed as mean of three replicates ± standard deviation.

2.3. Recovery and Determination of Total Phenolic Content

The removal of the phenolic fraction from SCG was carried out by organic solvent extraction according to the methodology of Mussatto et al. [17] with a few modifications. In a glass laboratory bottle, dry SCG and 60% v/v of aqueous methanol solution were added to obtain a solvent/solid (L/S) ratio of 40 mL/g. The bottle (covered with a cap) was placed in a magnetic stirrer for 90 min at 60 °C. Afterwards, the obtained crude extract was filtered through filter paper and the obtained clear extract (SCG extract) was stored in the freezer (−18 °C) until use. The total phenolic content was determined based on the Folin–Ciocalteau method as described by Arnous et al. [18]. The absorbance was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan) at 750 nm, and results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight of SCG. The experiments were performed in triplicate.

2.4. Polysaccharides Extraction

The protocols of Passos and Coimbra [10] and Passos et al. [19] were used for the extraction of polysaccharides from SCG, after the removal of lipid and phenolic fractions. Microwave irradiation of the material was performed using a Microwave Accelerated Reaction System of 1.2 kW power (CEM MARS 5, New York, NY, USA) using a 300-psi high-pressure reactor. Operating conditions were as described in Passos and Coimbra [10] with a SCG/water ratio of 1/30 g/mL. Nine vessels were used, and an expansion valve was attached to each. A thermocouple and a pressure sensor were placed in the control vessel to monitor the temperature and the pressure, respectively. The temperature was set at 200 °C and was preserved for 2 min. After the first extraction cycle (MAE_1), the unextracted insoluble material was re-dissolved in water and subjected to a second microwave-assisted extraction (MAE_2) under the same operating conditions. A total of three consecutive cycles were performed to ensure the maximum recovery of polysaccharides from SCG. At each new extraction, the reactors were cooled at room temperature. All samples were filtered using 90 mm glass microfiber grade GF/A filters (Whatman, 1.6 μm pore size). The total collected filtrate (MAE_t), which was the sum of the extracts from the three-consecutive microwave-assisted extractions, was stored at −18 °C until further analysis.

2.5. Sugars and Nitrogen Content Analysis of SCG

For the determination of total sugars (mono-, di-, oligo-, polysaccharides) in the MAE_t extract, the concentrated sulfuric acid-phenol method (Dubois method) was applied [20]. Briefly, 1 mL of the extract, suitably diluted, was placed in test tubes, followed by the addition of 1 mL of 5% w/v phenol solution. Then, 5 mL of concentrated sulfuric acid solution (37%) was added, and the tubes were vortexed. The samples were left to rest for 10 min and then vortexed again. The tubes were then cooled, and the absorbance was measured using a UV-Vis spectrophotometer at 490 nm. As blank, the sample containing water instead of extract was used. The results were expressed as g glucose equivalents/L of MAE_t extract. The experiments were performed in triplicate.

For the determination of reducing sugars in MAE_t extract, the DNS method [21] was used with some modifications. In large test tubes, 0.5 mL of appropriately diluted sample

and 0.5 mL of DNS solution were added. The mixture was vortexed and transferred to a water bath (Doubnoff Waterbath Mod. 750, Asal srl, Milan, Italy) at 100 °C for 5 min. The tubes were then cooled, and 5 mL H_2O was added to each. The absorbance was then measured at 540 nm. As a blank, the sample containing water instead of extract was used. The results were expressed as g glucose equivalents/L of MAE_t extract. The experiments were performed in triplicate. For the preparation of 100 mL DNS solution, 80 mL of distilled water was gently heated, and 1.6 g of NaOH was added. Then, 1 g of DNS powder ($C_7H_4N_2O_7$) and 30 g of $KNaC_4H_4O_6{\cdot}4H_2O$ were dissolved in the mixture. The solution was placed in a bottle and stored in the dark at room temperature.

For determination of neutral sugar composition, the sample was hydrolysed with 2 M trifluoroacetic acid (TFA) for 2 h at 120 °C, the monosaccharides were reduced with $NaBH_4$ and acetylated with pyridine-acetic anhydride to their alditol acetates and analyzed on a TRACE Ultra Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Restek RT-2330-NB column (0.32 mm × 105 m). A mixture composed of rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, and glucose was used as a standard [22].

The total nitrogen content was measured by FLASH 2000 Organic elemental analyser (Thermo Fisher Scientific, Dreieich, Germany). The protein factor (6.25) was used to calculate the protein content (% N × 6.25) [23].

2.6. Size Exclusion Chromatography of Polysaccharide Extract

The weight-average molecular weight (M_w) was estimated by HPLC, with two HEMA-BIO 40 and 100 columns (Tesssek, 8 × 250 mm) connected in series. The analyses were conducted on an Agilent LC 1260 GPC/SEC system equipped with RI detectors. Samples were prepared by dissolving them in 0.1 M $NaNO_3$ to achieve a concentration of 1 mg/mL, and 20 µL of each sample was injected into the column. The mobile phase used was also 0.1 M $NaNO_3$, with a flow rate set at 0.4 mL/min. For calibration purposes, a set of dextran standards with molecular weights of 0.5, 5.2, 25.5, 72.7, and 158.1 kg mol^{-1} (American Polymer Standard Corporation, Mentor, OH, USA) was utilized. The Cirrus GPC/SEC 3.4.1 software was employed to calculate the polydispersity index (PDI).

2.7. Purification of Polysaccharide Extract in Gel Chromatography

The 20 mg of the sample was fully solubilized in 0.5 mL of distilled water, applied on a column (145 cm × 2.5 cm) of Bio-Gel P4 fine monitored by a RI detector (RI2000, Ricoh Deutschland GmbH, Hannover, Germany), and eluted with deionized water. The fraction collector was set to 5 mL tubes. Eight distinct fractions (F1–F8) were separated (Figure 1). Tubes of each peak were pooled, concentrated and lyophilized.

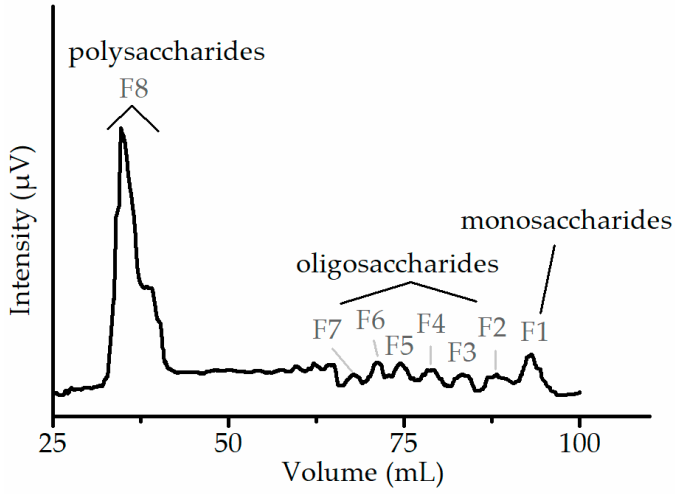

Figure 1. Purification of the SCG in Biogel-P4. F1–F8 represent the separated fractions.

2.8. FTIR Spectroscopy of Polysaccharide Fraction

The Fourier transform infrared spectroscopy-Attenuated Total Reflectance (FTIR-ATR) spectra were measured using a Nicolet iS50 FTIR (Thermo Scientific, USA) spectrometer equipped with a DTGS detector and controlled by Omnic 9.0 software. The spectra were collected within the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. 128 scans were performed. A diamond Smart Orbit ATR accessory was used for measurements in the solid state.

2.9. NMR Spectroscopy of Polysaccharide Fraction

The NMR spectra of coffee by-product samples were recorded by a Bruker AVANCE III HDX 600 MHz spectrometer equipped with a triple inverse TCI H-C/N-D-05-Z liquid He cooled cryoprobe (Bruker BioSpin, Karlsruhe, Germany). The spectra were measured in 99.9% D_2O at 313 K. The chemical shifts were referenced to the internal standard 3-methyl-silyl-propionic acid sodium salt-TSP-d_4 with 0.00 and −1.9 ppm for ^{1}H and ^{13}C, respectively.

3. Results and Discussion

3.1. Total Fat Content of SCG

SCG are a good source of lipids, such as tocopherols, cafestol, caffeol, linoleic and palmitic acids, with a high economic value [1,2]. In the present study, the percentage of total fat content was calculated at 15.5 ± 0.2% on dry matter. Loyao et al. [24] employed Soxhlet extraction to recover the total fat from SCG with a maximum extraction of 20 mg/100 g of dry SCG using ethyl acetate and n-propanol as solvents. Other studies reported extraction yields of 6.5–45.0 mg/100 g dry SCG [25]. In a study by Martinez-Saez et al. [4], total fat content was recovered by Soxhlet extraction using petroleum ether after acid hydrolysis. The percentage of fat was found to be 24.3%, similar to the results of the present study. However, compared to the aforementioned studies, the total fat content in this case appears quite high and it can be probably attributed to the variety of coffee selected as well as the combination of extraction method and solvent.

3.2. Total Phenolic Content of SCG

The total phenolic content, as determined by the Folin–Ciocalteu method, was 15.26 ± 0.5 mg GAE/g of SCG. In similar studies, the phenolic compounds from SCG were extracted by conventional solid–liquid extraction methods. Extraction with 60% methanol (40 mL/g SCG, 90 min) produced an extract with a high phenolic content (16 mg GAE/g SCG) [17]. In another study, the phenolic compounds from SCG were extracted by an environmentally friendly and cost-effective process, using aqueous ethanol solution at low temperatures to preserve the volatile phenolic compounds. The total SCG-1 and SCG-2 phenolic contents were 17.75 and 21.56 mg GAE/g, respectively, in the two different types of coffee used [26]. Also, Kovalcik et al. [2] have reported that the phenolic content in SCG ranged from 9.9 mg/g SCG to 17.3 mg GAE/g SCG. Finally, it is worth noting that the phenolic content in the coffee fruit is significantly higher compared to SCG. Coffee grounds are roasted before brewing and this process results in significant degradation of phenolic compounds [27].

3.3. Polysaccharides Content of SCG

The extraction conditions were chosen based on the knowledge that the lower molecular weight of polysaccharides positively affects their biological activity [14]. One of the suitable methods is microwave treatment in an aqueous environment. This technique should be gentle enough to avoid degrading individual sugars while still strong enough to depolymerize the backbone. As reported by Campos-Vega et al. [1], microwave-assisted extraction allows the recovery of arabinogalactans, while re-extraction of the residual material allows the recovery of galactomannans with a higher yield. Through this method, 74% and 66% of total galactose and mannose can be extracted from SCG [10]. Therefore, sequential extraction plays an important role.

In this study, the extraction of polysaccharides from SCG was performed with the help of microwaves. After the first microwave-assisted extraction (MAE_1) in which 9 g of SCG was used, the unextracted insoluble material was weighed and redissolved in water and subjected to a second microwave-assisted extraction (MAE_2). After 3 cycles, the process was complete, extracts were combined, and the amount of total and reducing sugars were determined, respectively. The total sugar content was determined at 8.12 ± 0.5 g/L, while the total reducing sugars were found equal to 1.5 ± 0.3 g/L extract (or 17.07 g reducing sugars/100 g SCG). In the study of Getachew and Chun [9], the reducing sugar content of the extracts after supercritical fluid extraction ranged from 8.88 to 37.91 g/100 g of dried and defatted SCG, results that are in line with the present study. However, it should be noted, that each extraction process may result in different content of total and reducing sugars since different processes taking place during extraction may cause degradation of polysaccharides.

Mannose (51.2 rel. wt%) and galactose (38.0 rel. wt%) were two dominant neutral monosaccharides found in the sugar analysis, while the amount of other sugars was minimal (arabinose 5.5, glucose 3.0, and other sugar 3 rel. wt%). It could be assumed that the coffee by-product contains mannan, galactomannan or galactan, or a combination of polysaccharides [28]. In this case, the NMR analysis allows a closer look at the structure and helps determine more precisely to which polysaccharide the sugar units belong.

3.4. Purification of Polysaccharide Extract

The molecular weight analysis (Figure 2) revealed one wide peak with Mw 2173 g·mol^{-1} with PDI 2.5 and one sharp peak of monosaccharides with Mw 180 g mol^{-1} and PDI 1.1. Similar low-molecular-weight polysaccharides (1.7–17 kDa) were isolated using microwaves by Passos et al. [19]. Compared to galactomannan isolated from SCG by autohydrolysis (44.5 kDa) and enzymatic hydrolysis (28.0 kDa) [13], the microwave treatment produced a sample with lower molecular weight and higher solubility. The NMR data of the non-purified sample supported the GPC/SEC results and affirmed that a portion of the material was converted during processing into simple monosaccharide units. This degradation of polysaccharides was already indicated by the higher content of reducing sugars determined in the isolated material (Section 3.3). For enhanced readability of NMR signals, the sample was homogenized by fractionation according to molecular weight on a Biogel P4 column (Figure 1). Eight different factions were separated, i.e., monomers (F1), oligomers (F2–F7), and polymers residues (F8). Only polysaccharides fraction F8 was employed to determine the characterization of polysaccharides structure for the coffee by-product.

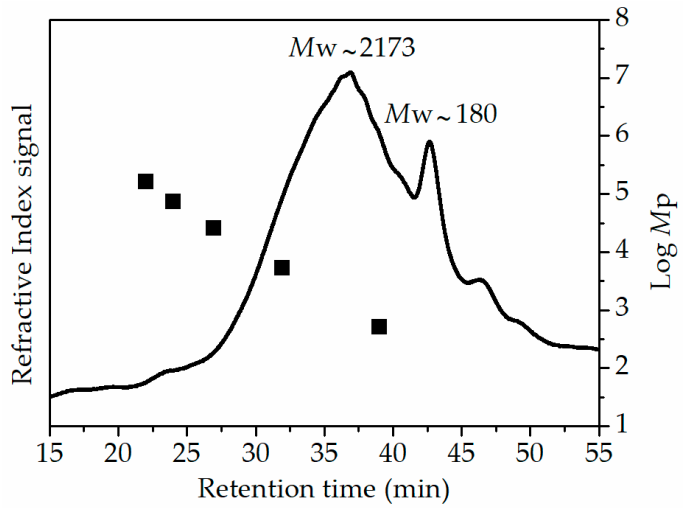

Figure 2. Molecular weight distribution patterns of the whole spent coffee grounds extract. The squares represent Log *M*p of Dextran standards (*M*w 505, 5220, 25,500, 72,700, 158,100 Da).

3.5. FTIR Spectroscopy of Polysaccharide Fraction

The FTIR–ATR spectrum of polymer fraction F8 (Figure 3) revealed the presence of various components, with their signals overlapping. The band at 1645 cm^{-1} indicates the presence of proteins, corresponding to the Amide I. Amide II, observed at 1560 cm^{-1}, is likely overlapping with other broad bands in the region 1500 to 1700 cm^{-1} [29]. Additionally, the band at 1596 cm^{-1} represents the antisymmetric stretching of COO^{-}, coupled with the band at 1407 cm^{-1}, which corresponds to the symmetric stretching of COO^{-}, suggesting the presence of carboxylic acids [30]. The band at 1444 cm^{-1}, associated with C-C vibrations, indicates the presence of phenolic substances. However, the aromatic bands C-C at 1520 cm^{-1} and C=C at 1610 cm^{-1} are overlapped with the COO^{-} and Amide I bands [31].

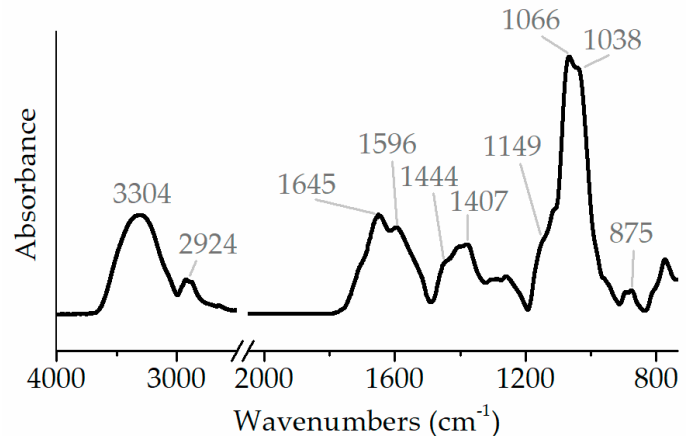

Figure 3. FTIR–ATR spectrum of polysaccharide fraction F8.

The region from 1300 to 800 cm^{-1}, known as the fingerprint region for polysaccharides, is also complex. The bands at 1149 cm^{-1} (O-C-O), 1066 cm^{-1} (C-O, C-C), and 875 cm^{-1} correspond to stretching vibrations of mannose-containing polysaccharides. However, the band at 1038 cm^{-1} is characteristic of the C-C stretching ring in galactan [30]. Therefore, identifying the polysaccharides using only this method is challenging.

3.6. NMR Spectroscopy of Polysaccharide Fraction

To gain a deeper structural insight, NMR is a powerful analytical approach for more precise determination of the carbohydrates, including α-, β-configuration, branching or linkage sequence of the residues and glycosidic bond.

According to Ripper et al. [32], the ^{1}H spectrum (Figure 4a) contains, besides the carbohydrate signals, signals between 0.5 and 2.5 ppm, which can be attributed to the aliphatic hydrogens of proteins and some not removed fatty acids, as well as aromatic signals between 6.6 and 7.5 ppm. The protein content was established from elemental analysis to 6.42 wt%. While the 1D-^{1}H spectrum was not well readable due to the overlapping signals in the region 4.5–4.8 ppm, the 2D ^{1}H-^{13}C HSQC, HMBC, and HSQC-TOCSY techniques provided nicely resolved peaks, allowing the further composition analysis of higher molecular weight fraction F8.

An expansion of 2D HSQC and HMBC spectra, in the area of carbohydrate bands, is shown in Figure 4b. The anomeric region of HSQC showed at least 15 different cross-peaks. One of them with the signal at 4.73/101.0 ppm was assigned to β-D-Man*p* (unit A) based on the literature [33,34] and confirmed by the remaining chemical shifts from HSQC-TOCSY (Table 1).

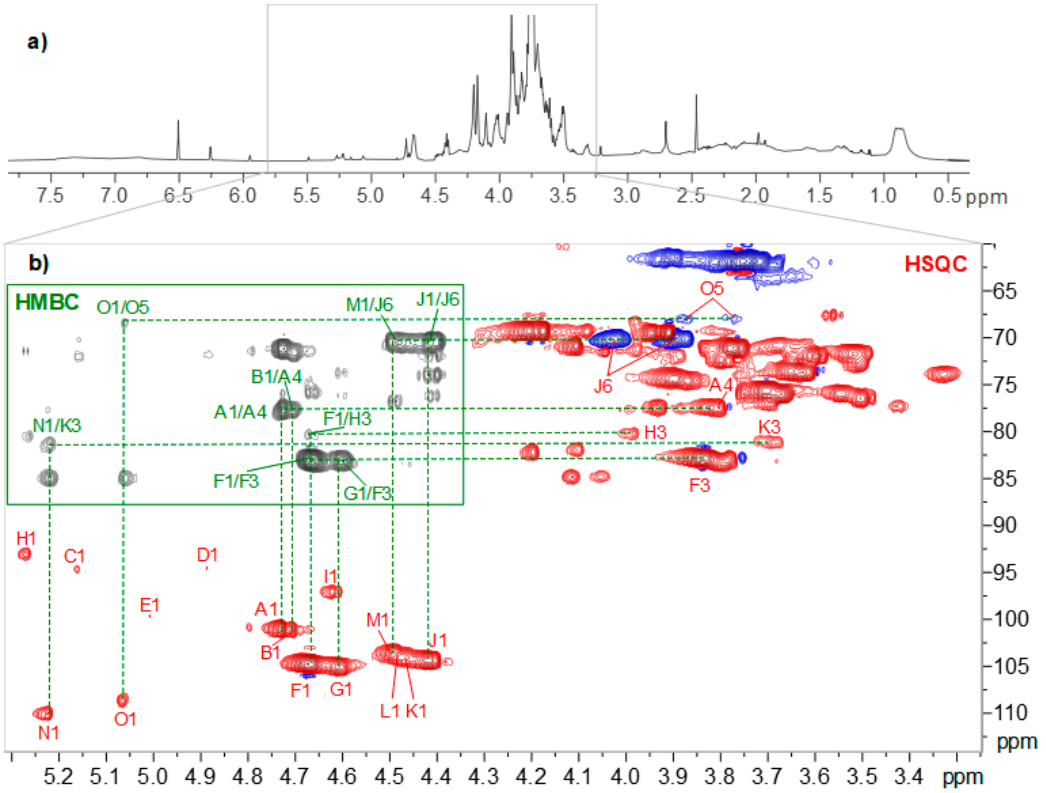

Figure 4. The selected region of 2D NMR spectra of fraction F8 (**a**) ^{1}H NMR spectra (**b**) ^{1}H-^{13}C HSQC as a red/blue spectrum, and ^{1}H-^{13}C HMBC as a grey cross-peaks. The green lines and labels indicate HMBC intercorrelations.

Table 1. ^{1}H and ^{13}C NMR data of polysaccharides fraction H from spent coffee grounds extract.

Unit	Residue	Chemical Shifts (ppm) 1	2	3	4	5	6	Interconnectivity H and C (ppm) from HMBC
A	→ 4)-*β*-D-Man*p*-(1 →	4.73 101.0	4.10 70.8	3.7972.3	3.8177.3	3.5475.8	61.5	**A**H1/**A**C4, BH1/**A**C4
B	*β*-D-Man*p*-(1 →	4.71 101.0	4.04 71.3	3.6373.7	3.5667.6	3.4177.3	61.9	**B**H1/AC4
C	→ 4)-*α*-D-Man*p*	5.16 94.7	3.97 71.2					
D	→ 4)-*β*-D-Man*p*	4.88 94.5	71.5					
E	*α*-D-Gal*p*-(1 →	5.00 99.7						
F	→ 3)-*β*-D-Gal*p*-(1 →	4.67 104.8	3.78 71.1	3.8482.8	4.2069.3	3.7075.6	61.7	**F**H1/**F**C3, **F**H1/HC3, GH1/**F**C3, IH1/**F**C3
G	*β*-D-Gal*p*-(1 → 3	4.61 105.1	3.59 71.9	3.6773.5	3.9169.4	3.6576.0		**G**H1/FC3
H	→ 3)-*α*-D-Gal*p*	5.27 93.0	3.98 68.2	3.9880.2	4.2370.0			FH1/**H**C3
I	→ 6)-*β*-D-Gal*p*	4.62 97.1		83.2	4.1769.3			**I**H1/FC3
J	→ 6)-*β*-D-Gal*p*-(1 →	4.41 104.4	3.52 71.7	3.6273.7	3.9469.5	3.8974.3	3.89–4.0370.1	**J**H1/**J**C6, MH1/**J**C6
K	→ 3, 6)-*β*-D-Gal*p*-(1 → subs. *α*-Ara*f*-(1 →	4.47 104.1		3.6981.1				NH1/**K**C3
L	→ 3, 6)-*β*-D-Gal*p*-(1 → subs. *β*-D-Gal*p*-(1 →	4.48 103.7		3.7883.0				
M	*β*-D-GlcA*p*-(1 →	4.50 103.6	3.33 73.8	3.5076.4	3.5172.7	3.6976.6		**M**H1/JC6
N	*α*-L-Ara*f*-(1 →	5.22 110.0	4.19 82.2	3.9377.5	4.1184.78	62.1		**N**H1/KC3

Table 1. *Cont.*

Unit	Residue	Chemical Shifts (ppm) 1	2	3	4	5	6	Interconnectivity H and C (ppm) from HMBC
O	α-L-Ara*f*-(1 → 5	5.06 108.7	4.10 81.9	3.9377.4	4.0584.8	62.1		**O**H1/PC5
P	→ 5)-α-L-Ara*f*-(1 →	5.04 108.7				3.76– 3.8768.1		OH1/**P**C5
R	β-D-Gal*p*-(1 → 6	4.42 104.4			75.9			

Abbreviations: D-Man*p* = D-Mannopyranose, D-Gal*p* = D-Galactopyranose, L-Ara*f* = L-Arabinofuranose, D-GlcA*p* = D-Glucopyranuronic acid.

In the HMBC spectrum, a long-range correlation of the H1 proton with the C4 atom confirmed the 1 → 4 bond of the mannose unit. The HMBC spectrum showed more detail about this mannan chain (Figure 4b). The long-range connection between C4 of unit A and H1 (4.71 ppm) of unit B, which was established as terminal β-D-Man*p* unit (Table 1) confirmed the chain's termination from one side. The opposite side of the mannan chain was presented by two small H1/C1 cross-peaks of α- and β- mannose reducing ends (5.00/99.7 and 4.88/94.5 ppm) in the HSQC spectrum. The tinny cross-peak at 5.00/99.7 ppm belongs to the α-D-Gal*p* unit (unit E) and it originates from the branching of the mannose residue at position O-6 [33]. The signal from the 6-branched mannose unit, which C6 should be down-filed shifted, could not be found because of low abundance. The low branching of galactomannan may be caused by the degradation of the polysaccharide during sample preparation, which is also confirmed by the lower molecular weight. A similar effect of reducing galactose content with reducing molecular weight was observed by Muschin and Yoshida [34]. The expected structure of the galactomannan component is shown in Figure 5a.

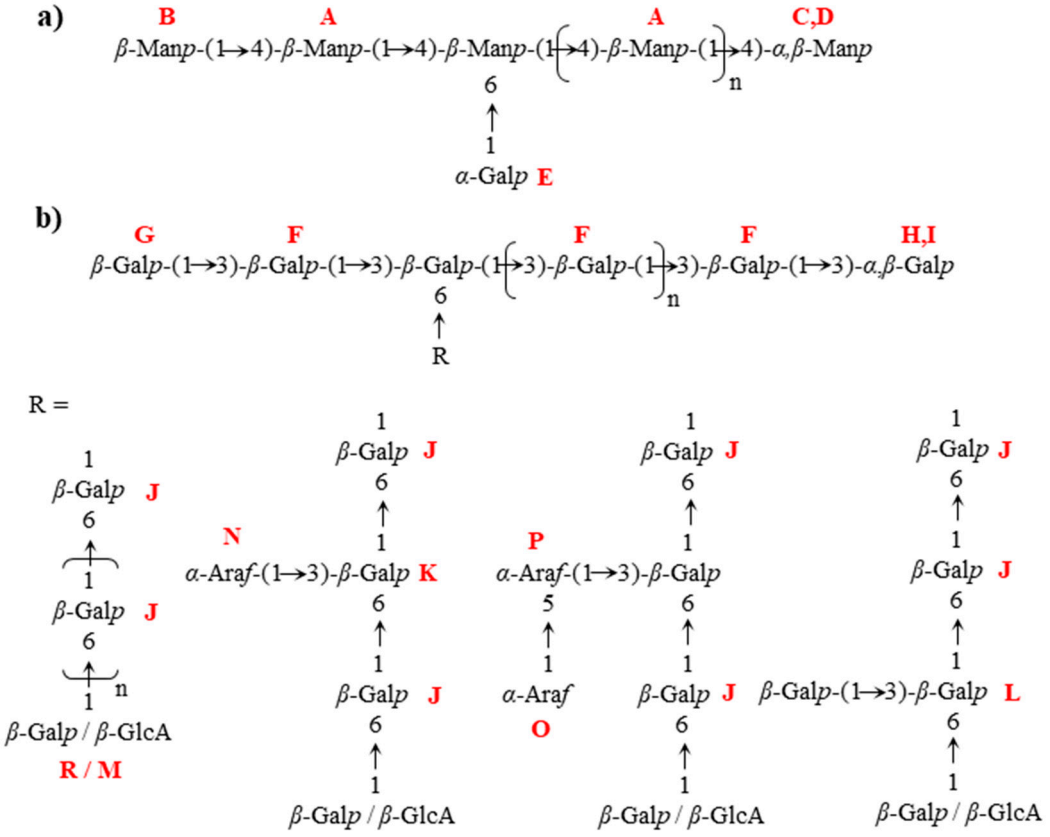

Figure 5. Presumed structural fragments in spent coffee grounds extract: (**a**) galactomannan, (**b**) arabinogalactan. The labels A–R correspond to sugar unit attributions in Table 1.

Except for the α-D-Gal*p* from the galactomannan chain, the other eight galactose H1/C1 cross-peaks were visible in the HSQC spectrum. The assignments for the proton and carbon resonances presented in Table 1 were derived from HSQC, HSQC-TOCSY, and HMBC and they agreed with Nunes et al. [16] and Capek et al. [35].

For anomeric signal at 4.67/104.8 ppm, the HSQC-TOCSY revealed H-2/C-2 (3.78/71.1 ppm), H-3/C-3 (3.84/82.8 ppm), H-4/C-4 (4.20/69.3 ppm), and H-5/C-5 (3.70/75.6 ppm), respectively. The residue was assigned as (1 → 3)-linked β-D-Gal*p* (Unit F). The linkage between H1 4.67 ppm and C3 82.8 ppm was directly assigned from the heteronuclear HMBC spectrum (Figure 4b). However, the H1 4.67 ppm of unit F correlated further with 80.2 ppm which is C3 of reducing α-D-Gal*p* residue (Unit H). The backbone, as shown in Figure 5b, is terminated on the other side by T-β-D-Gal*p* (unit G), which has an anomeric peak at 4.61/105.1 ppm and its binding to the chain is confirmed in HMBC by a cross-peak between H1 (4.61 ppm) of unit G and C3 (82.8 ppm) of unit F. The unit J (H1/C1 4.413/104.4 ppm) was, due to the downfield shift in C6 atom values (C6 70.1 ppm, Table 1), attributed to (1 → 6)-linked β-D-Gal*p*. As was described previously [28,35], the (1 → 6)-linked galactan chain should represent the side chain of the main 1 → 3 linked backbone. The presence of unit K [(1 → 6)-linked β-D-Gal*p* with attached T-α-L-Araf], or unit L [(1 → 6)-linked β-D-Gal*p* with attached T-β-D-Gal*p*], or the presence of (1 → 5)-α-L-Araf unit (Unit P) indicated the branching of the galactan side chain as shown at Figure 5b. The (1 → 6)-linked galactose side chain was randomly ended by glucuronic acid (D-GlcA*p*, unit M, Figure 5b), which is confirmed by observed correlation between the signal at 4.50 ppm (H1 of unit M) and the signal at 70.1 ppm (C6 of unit J).

The presence of glucuronic acid at the arabinogalactan chain was observed by Redgwell et al. [15] and Nunes et al. [16] in green coffee beans. However, another study [36] confirmed that during the roasting process, 70% of the glucuronic acid was destroyed. According to the provided information, it is expected that glucuronic acid will be absent from SCG. However, the presenting NMR study revealed the presence and binding of glucuronic acid to the arabinogalactan chain even after the roasting and brewing process. This assumption has not yet been confirmed in SCG. It could be caused by using inappropriate isolation techniques [7,8] or the limitations of the analytical techniques used to determine the acid polysaccharide structure (glycosidic linkages, sugar analyses used by Mussatto et al. [7], Passos and Coimbra [10] and Passos et al. [19]. The presence of a carboxylic group in the polysaccharide chain could offer opportunities for modification and application.

4. Conclusions

As a conclusion, SCG are rich in bioactive and functional compounds and the recovery of the latter is an important means of utilization. In the framework of the study, the extraction of polysaccharides from SCG was carried out employing a «green» extraction technique, namely accelerated microwave-assisted extraction. From the carbohydrate point of view, this extraction provided a good soluble product, which consists of mono-, oligo- and a very short chain of polysaccharides. Low-branched galactomannan is the predominant polysaccharide. The rest are represented by the galactan or arabinogalactan chains of various lengths and branches, whose sidechains are randomly ended by glucuronic acid. These data show that some glucuronic acid remains attached to the arabinogalactan chain even after coffee roasting and brewing. Additionally, microwave extraction is an effective technique for extracting a water-soluble acidic polysaccharides complex. This characterization of polysaccharide profile is of great importance regarding potential future applications of such extracts, including their integration into food products, thus providing the possibility of its exploitation by the food industry in innovative/functional products.

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