Chemical Characterization of the High Molecular Weight Material Extracted with Hot Water from Green and Roasted Arabica Coffee

Fernando M. Nunes and Manuel A. Coimbra*

Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

The polysaccharides present in coffee infusions are known to contribute to the organoleptic characteristics of the drink, such as the creamy sensation perceived in the mouth known as "body", the release of aroma substances, and the stability of espresso coffee foam. To increase the knowledge about the origin, composition, and structure of the polysaccharide fraction, the high molecular weight material (HMWM) was extracted with hot water from two green and roasted ground arabica coffees: Costa Rica (wet processed) and Brazil (dry processed). The polysaccharides present in the green coffees HMWM were arabinogalactans (62%), galactomannans (24%), and glucans, and those found in roasted coffees were galactomannans (69%) and arabinogalactans (28%). The polysaccharides of the HMWM of the roasted coffees were less branched than those of the green coffees. The major green coffee proteins had molecular weights of 58 and 38 kDa, and the 58 kDa protein had two subunits, of 38 and 20 kDa, possibly linked by disulfide bonds. The protein fraction obtained from roasted coffees had only a defined band with ≤14 kDa and a diffuse band with >200 kDa. The majority of the galactomannans were precipitated with solutions of 50% ethanol, and the size-exclusion chromatography of the roasted fractions showed coelution of polysaccharides, proteins, phenolics, and brown compounds. The use of strong hydrogen and hydrophobic dissociation conditions allowed us to conclude that the phenolics and brown compounds were linked by covalent bonds to the polymeric material.

Keywords: *Coffee; arabica; polysaccharides; roasting; galactomannan; arabinogalactan; protein; phenolics; polymeric interactions*

INTRODUCTION

The water-soluble polysaccharides extracted from ground roasted coffee are a major component of the soluble material present in coffee brews (*1*). They play an important role in the retention of volatile substances (*2*), and contribute to the coffee brew viscosity (*3*) and thus to the creamy sensation perceived in the mouth, known as "body" (*4*). They are also related to the foam stability of espresso coffee, an important quality attribute of this coffee brew (*5*).

Polysaccharides comprise nearly 50% of the green coffee bean weight (*6*). Early works on coffee polysaccharides were devoted mainly to the characterization of the major green coffee fraction, the holocellulose residue (*7*−*11*). Cellulose, mannans, and arabinogalactans are its major components (*12*). The hot-water-soluble fraction of green coffee polysaccharides accounts only for 1.6−3.2% of the total green coffee polysaccharides (*11*,*13*−*16*). These polysaccharides, although they are important for explaining the polysaccharide composition of the roasted coffee brews (*16*), have always been overlooked.

The polysaccharide composition of roasted coffee infusions have been studied in instant coffee (*17*−*19*) and in traditional hot water extractions (*11*, *14*, *20*). The structures of the polysaccharides have been inferred from the constituent monosaccharides. Recently, the water-soluble polysaccharides of roasted coffees were characterized by ^{1}H and ^{13}C NMR, allowing the identification of a β-D-(1 → 4)-mannan containing small amounts of galactose and arabinose, and an arabinogalactan (*21*). At present, the nature of the roasted coffee polysaccharides and their modifications, such as degradation and/or copolymerization, caused by the roasting process are still uncertain (*6*, *19*, *22*−*25*). Although the relevance of the water-soluble polysaccharides to the overall quality of roasted coffee brews is evident, these polymers were never structurally characterized in detail, neither were the changes in the structure of the green coffee polysaccharides caused by the roasting process elucidated.

Proteins are another high-molecular-weight component of the coffee brews. In espresso coffee, the protein content was shown to be correlated with the foam volume (*5*). The composition of coffee proteins was also shown to be profoundly changed by roasting of the green coffee bean (*26*, *27*), however, little information is available regarding the structure of the water soluble proteins of roasted coffee, and the variation of their amount with the roasting process.

The aim of this work was to isolate and characterize structurally the hot-water-soluble polysaccharides obtained from green and roasted coffees, and identify the nature of their interactions with proteins and phenolics due to the roasting process. Two arabica coffees (*Coffea arabica*) of different geographical origins: Costa Rica (wet-processed coffee) and Brazil (dry-processed coffee) were used.

* To whom correspondence should be addressed (fax +351 234 370084; e-mail mac@dq.ua.pt).

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MATERIALS AND METHODS

Materials. The green coffee samples (*Coffea arabica*), Costa Rica (wet-processed), and Brazil (dry-processed) were provided by a local factory. All chemicals were analytical grade or the highest purity available.

Coffee Roasting. The green coffees were roasted in a laboratory roaster (Probat, Germany) in batches of 150 g at 200 ± 5 °C and were degassed over 2 days at room temperature. The degree of roast (*DR*) was quantified by the percentage of dry weight loss of green coffee beans, on a dry basis (*5*). The two coffees were roasted to a *DR* of 8.7%.

Percentage of Water Content. The percentage of water content was determined in duplicate, for the green coffees, according to the ISO 1447-1978, by the method of two-stage oven drying at 130 °C, and for the ground roasted coffees, according to the ISO/DIS 11294-1993, by the method of oven drying at 105 °C during 4 h (*4*).

Coffee Grinding and Deffating. Because of the hard texture of green coffee beans, and with the purpose of avoiding the heat development that occurs during the normal grinding of this material, the green coffee beans were frozen in liquid nitrogen before being ground. The roasted coffee beans were ground directly with no prior treatment. The coffees were ground to a particle size of 0.350 ± 0.050 mm and deffated by Soxhlet extraction with petroleum ether (*5*).

Preparation of High Molecular Weight Material (HMWM). With constant stirring, 50 g of each ground and deffated coffee was extracted with 1 L of water at 80 °C during 20 min. The extracts were filtered through a size 2 sintered glass filter, and the material retained was washed with an additional 500 mL of water at 80 °C. The filtrate was concentrated under reduced pressure at 40 °C, and dialyzed (MW cutoff 12−14 kDa, Visking size 8, Medicell International Ltd, London, UK) at 4 °C with 8 water renewals. The retentate obtained was frozen and freeze-dried, giving the HMWM. The HMWM recovered from all coffees had a fluffy aspect; with light green and brown colors, when the origin was the green or the roasted coffees, respectively.

Ethanol Precipitation. The HMWM (1.0 g) was dissolved in 100 mL of water; the solution was stirred for 1 h at 4 °C and centrifuged at 24400*g* for 20 min at 4 °C. The residue obtained (WIppt) was suspended in water, frozen, and freeze-dried. Absolute ethanol (Riedel, Seelze, Germany, 100 mL) was added and the solution (50% ethanol, assuming additive volumes) was stirred for 1 h at 4 °C. This solution was then centrifuged and the residue obtained (Et50) was removed. To the supernatant was added 200 mL of absolute ethanol; the solution (75% ethanol) was stirred 1 h at 4 °C and centrifuged, and the residue obtained (Et75) was removed from the supernatant solution (EtSN). To remove the ethanol completely, each precipitate was dissolved in water, rotary evaporated at 40 °C, and freeze-dried (*28*).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). Electrophoresis in polyacrylamide gel (12.5% and 4% of acrylamide for the running and stacking gels, respectively) and under denaturing conditions (SDS), and under nonreductive and reductive conditions was as described by Shewry et al. (*29*). To each lane, 20 μL of sample (20 mg/mL) was applied. The run was done at 200 V, the protein bands were colored using the Coomassie R-250 procedure, and the molecular weights were estimated with the use of a standard protein kit (BioRad-Broad Range, 200−6.5 kDa).

Protein Analysis. Protein was quantified by multiplying the obtained percentage of Kjeldhal nitrogen by the 6.25 factor. The Kjeldhal nitrogen was determined according to the procedure described by Willis et al. (*30*).

Sugars Analysis. Neutral sugars were released by Saeman hydrolysis (*31*) and analyzed as their alditol acetates by GLC (*32, 33*) using a Hewlett-Packard 5890 with a split injector (split ratio 1:60) and a FID detector. A 25-m column CP-Sil-43 CB (Chrompack, Holland) with 0.15 mm i.d. and 0.20-μm film thickness was used. With the injector and detector operating at 220 °C, the following temperature program was used: 180 °C for 5 min and 200 °C for 20 min, with a rate of 0.5 °C/min. Linear velocity of the carrier gas (H_2) was set at 50 cm/s at 200 °C. Hexuronic acids were determined colorimetrically by a modification (*34*) of the method of Blumenkrantz and Asboe-Hansen (*35*).

Methylation Analysis. Polysaccharides were activated with powdered NaOH and methylated with CH_3I (*36, 37*) as described by Coimbra et al. (*34*). The sample (2−3 mg) was dispersed in 2 mL of dried DMSO and sonicated occasionally until it was fully dispersed. NaOH pellets (100 mg) powdered under argon were added to the solution. The sample was sonicated for 90 min and allowed to stand for an additional 90 min. To the frozen solution, methyl iodide (1 mL) was added and allowed to react 30 min in a ultrasonic bath and left to stand for a further 30 min. $CHCl_3$/MeOH (1:1, v/v, 3 mL) was added, and the solution was dialyzed (MW cutoff 12−14 kDa, Visking size 8, Medicell International Ltd, London, UK) against 3 lots of 50% EtOH. The dialyzate was evaporated to dryness and the material was remethylated using the same procedure. The remethylated material was hydrolyzed with 2 M TFA (1 mL) at 121 °C for 1 h (*38*), cooled, and rotary evaporated at 35 °C. The partially methylated sugars were then suspended in 0.3 mL of 2 M NH_3 and 20 mg of $NaBD_4$ was added. $NaBD_4$ was used instead of $NaBH_4$ to avoid eventual ambiguities when interpreting the mass spectra (*39*). The mixture was allowed to react at 30 °C for 1 h, and the reaction was terminated by the addition of 0.1 mL of glacial acetic acid. The acetylation of the partially methylated alditols was performed by adding 1-methylimidazole (0.45 mL) and acetic anhydride (3 mL) and allowing reaction for 30 min at 30 °C. This solution was treated with water (3 mL) to decompose the excess of acetic anhydride, and the partially methylated alditol acetates (PMAA) were extracted with dichloromethane (3−5 mL). The dichloromethane phase was washed with water and evaporated to dryness at 35 °C under a stream of argon. The PMAA were dissolved in dichloromethane (70 μL) and analyzed by GC−FID on an OV-1 capillary column (30 m length, 0.32 mm i.d., and 0.25 μm of film thickness) and characterized by GC/MS. The samples were injected in splitless mode (time of splitless 0.75 min), with the injector and detector operating at 210 and 220 °C, respectively, using the following temperature program: 55 °C for 0.75 min with a linear increase of 45 °C/min until 140 °C, and standing 1 min at this temperature, followed by a linear increase of 2.5 °C/min until 218 °C, with further 37 min at 218 °C. For quantification, the molar response factors of Sweet et al. (*40*) were used. Linear velocity of the carrier gas (H_2) was set at 50 cm/s at 218 °C. GC−MS analysis was performed in a HP series 2 gas chromatograph and Trio-1S VG mass-lab with scans between 400 and 35 m/e/s with a 70 eV ionization energy. The chromatographic conditions used were as described. Linear velocity of the carrier gas (He) was set at 40 cm/s at 200 °C, with a solvent delay of 4 min.

Gel-Filtration Chromatography. Gel-filtration chromatography on Sephacryl S-400 HR (Pharmacia) was performed on a 100 × 1.6 cm (XK 100/16, Pharmacia) column at a flow rate of 2.5 mL/min (*41*). The samples were suspended in 1 mL of 0.1 M potassium phosphate buffer pH 6.5 with 3 M urea, heated during 10 min at 80 °C, and sonicated 5 min for complete dissolution. The same phosphate-urea buffer was used as eluent. Fractions (5 mL) were collected and aliquots (20, 500, and 100 μL) were assayed for carbohydrate (phenol−sulfuric acid method) (*42*), protein (Coomassie staining) (*43*), and phenolic compounds ($FeCl_3$ + $K_3Fe(CN)_6$) (*44*). The absorption at 400 nm for detection of brown compounds was also measured, and the eluent was continuously monitored at 280 nm. To calibrate the column, standard dextrans of 2000, 487, 266, and 72 kDa (Sigma) were used. The column internal volume was determined by elution of glucose.

Gel-filtration chromatography on Sephadex G-25 was performed on a 25 × 1.6 cm (XK 25/16 Pharmacia) column at a flow rate of 0.5 mL/min. The samples (1 mg) were suspended in 1 mL of a solution of 0.1M acetate buffer at pH 5 containing 1% SDS, 5 M trietanolamine, and 7 M urea; heated during 10 min at 80 °C; and sonicated 5 min for complete dissolution;

Table 1. Chemical Composition of HMWM of Green and Roasted Coffees

coffee		HMWM[a]	sugar[b,c]	protein[b,d]	phenolics[b,e]
Costa Rica	green	7.56	15.0	43.7	6.8
	roasted	7.62	31.4	9.7	32.7
Brazil	green	10.05	18.6	34.3	10.5
	roasted	8.51	29.3	8.9	42.7

[a] As % of dry and deffated coffee weight. [b] As % of HMWM weight. [c] Anhydrosugar. [d] %N × 6.25. [e] As 3-CQA equivalents by the prussian blue method.

and were eluted with the same solution. Fractions (2.5 mL) were collected and assayed for absorption at 280 and 400 nm and for carbohydrate. The column internal volume was determined by elution of 3-chlorogenic acid.

Spectral Analysis of Roasted Coffees Et50 Fractions. The absorption spectra of solutions of the Et50 fractions obtained from the roasted coffees were determined in the range 200−500 nm. The solutions of Et50 fractions (1.0 mg/mL) were prepared in 1% SDS and 7 M urea, and buffer solutions with pH 2.0 (HCl 0.1M in 1% SDS and 7 M urea) and pH 10.0 (phosphate 0.05 M in 1% SDS and 7 M urea). The sample solutions were diluted 1+9 (v/v) with these buffers, giving each one two solutions with identical Et50 concentrations but different pH values. Each solution was prepared just before measurement. The blank solutions, containing only 1% SDS and 7 M urea, were added to the buffers in the same described proportions.

RESULTS AND DISCUSSION

Characterization of the High Molecular Weight Material (HMWM). Table 1 shows the chemical composition of the coffees HMWM solubilized with hot water. The amount of polysaccharides extracted from the green coffees were 1.13% for Costa Rica (CR) and 1.87% for Brazil (BR), on a dry and deffated weight basis. These values are within the range between 0.8 and 2.1% reported in the literature (*11*, *15*, *45*). The polysaccharide content of roasted coffees was higher than that obtained for the correspondent green coffees. When recalculated for the equivalent dry weight of green deffated coffee, it was 93% higher for Costa Rica and 22% higher for Brazil than that obtained for the green coffees. The mean value for the amount of polysaccharides in roasted coffees HMWM was 2.4% of their dry and defatted weight, a value closer to that obtained by Navarini et al. (*21*) but lower than the 3.5% that was obtained by Thaler and Arneth (*11*). This difference may be due to the different extraction temperature used (100 °C) as the increase in temperature increases the amount of the coffee polysaccharides extracted (*14*).

For the two green coffees, the protein accounted, on average, for nearly 40% of the HMWM, being the most abundant polymeric component (Table 1). The amount of nitrogen, in relation to the dry and deffated weight of green coffee, was on average 0.54% for the two coffees. This value is in agreement with the previously reported values of 0.43−0.70% (*15*, *26*, *46*). The roasting process caused an average decrease of 76% in the amount of protein extracted. This fact may be due to an insolubilization of denatured proteins caused by the high temperatures (*47*) or the loss of protein by degradation to small products (*26*) that would be lost during the dialysis step.

Although the HMWM recovered from the infusions of the two green coffees were subjected to dialysis through a 12−14 kDa cutoff membrane, there was a significant amount of phenolic compounds retained. The presence of these compounds, in particular chlorogenic acids, in the nondialyzable fractions of green coffees was also previously observed (*48*). These compounds may be responsible for the light green color of the HMWM extracted from the green coffees (*48*, *49*). Phenolic compounds can establish strong noncovalent interactions (hydrogen bonds and/or hydrophobic interactions) with proteins (*49*−*51*). The amount of phenolic compounds present in the HMWM of roasted coffees was considerably higher than that present in green coffees. All fractions recovered from the roasted coffees showed a brown color, indicating that some components could have undergone reactions such as Maillard or caramelization (*24*) from the high temperatures to which the beans were subjected during the roasting process.

Table 2. Monosaccharide Composition of the Hot-Water Soluble HMWM for the Green and Roasted Coffees

coffee		mol %						
		Rha	Ara	Man	Gal	Glc	HexA	total[a]
Costa Rica	green	2	25	23	43	7	t	1.13
	roasted	1	4	69	22	4	t	2.39
Brazil	green	3	20	22	34	20	t	1.87
	roasted	1	6	65	25	2	t	2.49

[a] g anhydrosugar/100 g of dry and deffated coffee; t, traces.

Table 3. Glycosidic-Linkage Composition of the Polysaccharides Present in the HMWM of Green and Roasted Coffees

linkage	Costa Rica		Brazil	
	green	roasted	green	roasted
	mol %			
T-Rha*p*	2.7	0.5	1.1	0.9
T-Ara*f*	17.0	2.9	12.4	4.8
5-Ara*f*	10.4	1.3	11.1	2.0
T-Man*p*	0.5	3.9	0.6	3.9
4-Man*p*	20.5	60.3	21.4	58.5
4,6-Man*p*	1.4	2.3	1.2	2.8
T-Gal*p*	4.7	6.5	4.3	6.0
3-Gal*p*	17.5	8.6	14.0	10.3
6-Gal*p*	1.4	3.0	2.4	2.2
3,6-Gal*p*	17.4	5.3	13.5	7.1
T-Glc*p*	nd[a]	nd	nd	nd
4-Glc*p*	5.4	4.8	4.9	1.2
6-Glc*p*	1.4	0.6	13.1	0.4

[a] nd, Not determined by GC−FID but detected by GC−MS.

Characterization of Green Coffee HMWM Polysaccharides. Table 2 shows the monosaccharide composition of the HMWM polysaccharides obtained for CR and BR green coffees. These polysaccharides were mainly composed of galactose (Gal), arabinose (Ara), mannose (Man), and glucose (Glc). The analysis of the glycosidic linkage composition (Table 3) showed that Gal residues were mainly (1 → 3)- and (1 → 3,6)-linked, with an almost equal abundance. These linkages are indicative of the presence of type II arabinogalactans (*52*). T-Gal*p* and (1 → 6)-Gal*p* residues were also present in green coffees HMWM. Type II arabinogalactans had been previously isolated from the water-insoluble residue of green coffee beans by treatment with hypochlorite and proteases (*9*, *12*) giving rise to arabinogalactans with a ratio (1 → 3-Gal)/(1 → 3,6-Gal) of 3:1. This composition allows inferring that the arabinogalactans present in the hot-water-soluble fraction had more branching points than the arabinogalactans previously isolated

from the insoluble residue with a stronger extraction treatment. The arabinosyl residues present were terminally- and (1 → 5)-linked, all in furanosidic form. The molar percentage of terminal residues was higher than that of (1 → 5)-linked, suggesting that the arabinosyl residues may form short side chains of the arabinogalactans, some of them possibly as a single arabinosyl residue. This was in agreement with the ratio T-Ara*f*/(1 → 3,6)-Gal*p* of 1 for CR; the value of 0.9 for BR suggests that T-Gal*p* was also present in coffee arabinogalactans, or formed during the isolation procedure (*9, 12*).

The majority of the mannosyl residues (92%, on average) occurred as (1 → 4)-linked; (1 → 4,6)-linked (6%) and terminally linked (2%) residues were also present. The nature of the linkages and the relative abundance of the terminal and substituted mannosyl residues are characteristic of galactomannans (*53, 54*). Also, the large abundance of T-Gal*p* reinforces this idea. The presence of galactomannan in the hot water extract is in accordance with the solubility characteristics of this polysaccharide. Its solubility depends of the degree of substitution, with the parent mannan being hot water insoluble (*54*−*56*).

The glucosyl residues present in both green coffees HMWM were (1 → 4)- and (1 → 6)-linked. The amount of Glc found in BR was much higher than that found in CR, and this was due to the occurrence of (1 → 6)-Glc*p*. This type of linkage, in such a high abundance, is not common in the plant kingdom, but it is known to occur in microbial exo-polysaccharides (*57*). During the green coffee processing by the dry process method the microbial contamination is very common. Also, during the wet process, the presence of microorganisms is inevitable during the fermentation step (*4*). The (1 → 4)-Glc*p* may belong to the water soluble amylose, present in green coffee beans (*58*).

The glycosidic composition of the polysaccharides present in the green coffees HMWM showed that, for CR, arabinogalactans represented 67% of the polysaccharide fraction, galactomannans accounted for 24%, and glucans accounted for 7%; for BR, arabinogalactans represented 57% of the polysaccharide fraction, galactomannans accounted for 24%, and glucans accounted for 18%.

Characterization of Roasted Coffee HMWM Polysaccharides. Mannose was the major monosaccharide residue of roasted coffees (Table 2); also present were Gal and low amounts of Ara, Glc, and rhamnose (Rha). The polysaccharides present in the HMWM of the roasted coffees, when compared with those present in the green coffees, had higher amounts on Man and lower amounts of Gal, Ara, and Glc. The amount of Ara residues extracted were, on average, 66% lower when recalculated for the equivalent dry weight of defatted green coffee, and the amount of Gal residues extracted were 5 and 13% lower, respectively, for CR and BR coffees.

The majority of the mannosyl residues were (1 → 4)-Man*p* (90%, in average) T-Man*p* (6%), and (1 → 4,6)-Man*p* (4%) (Table 3).

These results showed that the Man-rich polymers present in the roasted coffees HMWM had relatively lower amounts of (1 → 4,6)-Man*p* when compared to green coffees, indicating that the HMWM galactomannans of roasted coffees were less branched than those present in the green coffee extracts. This suggests that the roasting process may cause changes in the structure of the Man-rich polysaccharides present in coffee beans, making them more easily extractable in hot water. It was also possible that the modification of the structure of coffee beans during the roasting process, reflected in the increase of its volume (*4, 6, 59*), allowed an increase of the accessibility of water to the polysaccharides, increasing their extraction.

The linkage composition of the galactosyl residues of roasted coffees HMWM, when compared with that of the green coffees, showed a higher ratio of (1 → 3)-Gal*p*/(1 → 3,6)-Gal*p* (Table 3). This result allows us to infer that the arabinogalactans present in the roasted coffees HMWM were less branched. This may be due to the extraction of less branched arabinogalactans from roasted coffees and/or to a loss of arabinosyl residues from the arabinogalactans. The Ara residues present in HMWM were terminally- (70%, on average) and (1 → 5)-linked. The decrease in the amount of arabinosyl residues with the roasting process was lower for T-Ara*f* residues than for the (1 → 5)-linked. However, the decrease in the relative amount of T-Ara*f* was slightly higher than the decrease in (1 → 3,6)-Gal*p*, the branched galactosyl residues, which allows us to infer that part of the T-Gal*p* residues belong to the arabinogalactan side-chains, as previously reported (*10, 12, 21*).

The glycosidic composition of the polysaccharides present in the roasted coffees HMWM showed that, for CR, galactomannans represented 69% of the polysaccharide fraction, arabinogalactans were 25%, and glucans accounted for 6%; for BR, galactomannans represented 68% of the polysaccharide fraction, arabinogalactans were 30%, and glucans accounted for only 2%.

Characterization of HMWM Protein. Figures 1 and 2 show the SDS−PAGE patterns of the HMWM major protein components of green and roasted coffees. Both green coffees, under nonreducing conditions, presented a major protein band at 58 kDa and a second one at 38 kDa. In addition, four more bands were detected but with much less abundance: 170 kDa, 150 kDa, 16 kDa, and ≤14 kDa. The use of reducing conditions allowed us to infer that the band with 58 kDa had two subunits, with 38 and 20 kDa, possibly linked by disulfide bonds. A more extensive extraction would result in the increase of the intensity of the high molecular bands (*60, 61*). In the roasted coffees, the only defined band had a molecular weight of ≤14 kDa. During the entire run, there was a brown band that migrated in the front of the buffer and, in the high molecular weight region, a diffuse band was observed (>200 kDa). These results show that the roasting caused random protein degradation, which is in accordance with the possible loss of protein through the dialysis bag. Also, the roasting process may promote the covalent linkage between polysaccharides and proteins (*62, 63*) that would contribute to this diffuse pattern. Under reducing conditions, together with the band at 14 kDa, a delimited band at 22 kDa appeared, which allowed us to infer the occurrence of disulfide or other bonds sensitive to the reducing conditions used.

Ethanol Fractionation of HMWM. The HMWM of each coffee was fractionated by a graded addition of absolute ethanol into four fractions: WIppt, material insoluble in water; Et50, material precipitated with 50% ethanol; Et75, material precipitated with 75% ethanol; and EtSN, material that remained soluble in 75% ethanol solutions. Table 4 shows the yield and sugar

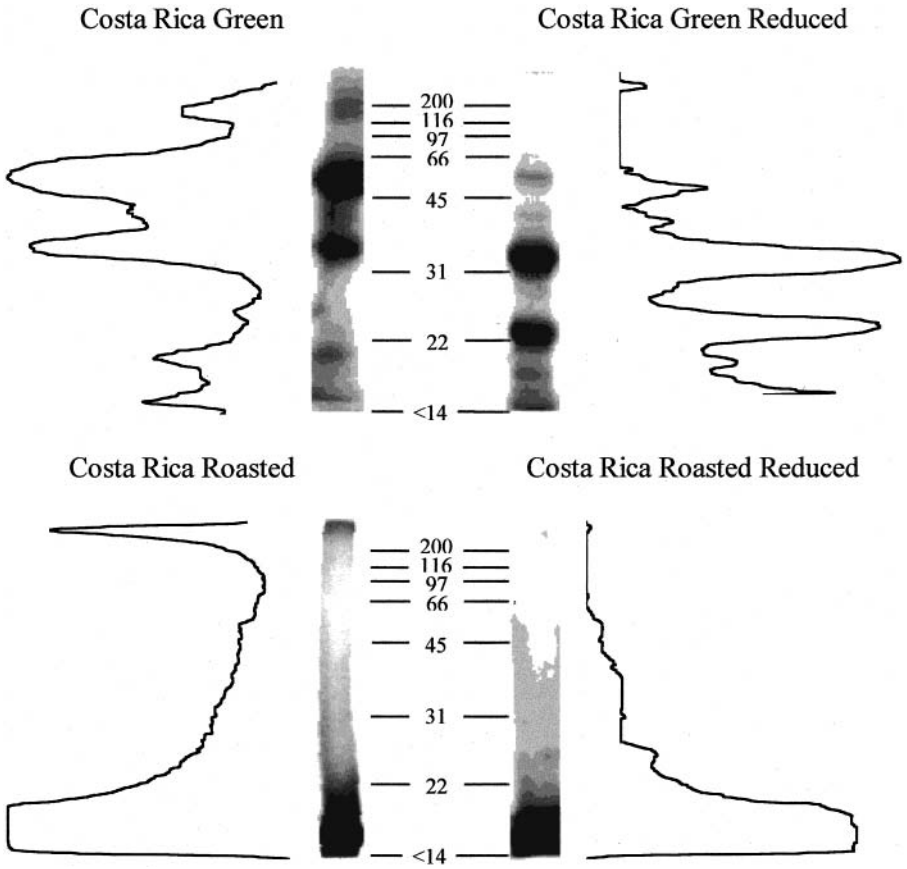

Figure 1. SDS−PAGE of HMWM proteins of Costa Rica coffee. Middle horizontal marks represent molecular weight markers.

composition of the fractions and the last column gives the amount of polysaccharides of each fraction in relation to the dry and deffated weight of coffee.

For green coffees, the majority of the material was collected in fraction WIppt. This material was very poor in sugars (10−11%). The majority of the polysaccharides solubilized occurred in fraction Et75 (34−35% of the HMWM polysaccharides). The major component sugars were Gal (53−55 mol %) and Ara (33−34 mol %), a glycosidic composition characteristic of arabinogalactans. Et50 contained a glycosidic composition characteristic of galactomannans mixed with arabinogalactans. For the roasted coffees, the fractions with higher amounts of material were Et50 and WIppt. Et50 was composed mainly of Man (86−90 mol %) and Gal (8−10 mol %); the relative amount of Ara and Rha was not significant. This sugar composition allowed us to infer that the majority of the polysaccharides present were galactomannans. These polysaccharides were also present in WIppt. Fractions Et75 and EtSN were mainly composed of Gal-rich polymers (52 mol %), containing in addition a relatively high amount of mannosyl and arabinosyl residues.

Polysaccharides, in general, are heterogeneous regarding their molecular weight and degree of branching. The solubility of galactomannans in water increases with the increase of the degree of branching and with the decrease of the molecular weight (*54*, *64*) due to the decreased tendency of chain association (*55*). The presence of relatively high amounts of arabinogalactans and galactomannans in the different fractions revealed a great heterogeneity in the structures of these polysaccharides. The results obtained for the ethanol fractionation of the HMWM were in accordance with the results previously described for espresso coffee extracts (*5*) concerning the nature and relative abundance of the sugar residues in the fractions and the amount of polysaccharides. As fractions Et50 contained the majority of the soluble galactomannans, which is the principal polysaccharide of the roasted coffee HMWM, they were further characterized.

Characterization of Fraction Et50. Table 5 shows the content of polysaccharides, protein, and phenolic compounds present in fractions Et50 obtained from CR and BR coffees. The UV−Vis spectra of the roasted samples at pH 2 showed a predominant peak at 208 nm, with one peak/shoulder at 287. Any distinct peak was observed in the visible region, even at higher concentrations. The spectra obtained at pH 10 had a maximum absorbance at 217 nm and one peak/shoulder at 302. These differences in the UV spectra in acid or alkali solutions were due to the presence of phenolic compounds (*43*, *65*), showing that they had an important contribution in the UV absorption spectra of the fractions.

Figures 3 and 4 show the elution profiles of the size-exclusion chromatography obtained for the Et50 fractions for the two coffee origins, monitored for polysaccharides (490 nm), proteins (595 nm), phenolics (720 nm), UV absorbing compounds (280 nm), and brown color (400 nm). Total solubilization of the samples was only achieved when the material was suspended in solutions of phosphate buffer pH 6.5 in urea 3M. The polysaccharide elution profiles for the green coffees showed to be more complex than those of the roasted samples. For the protein elution profile only a peak with 60 kDa was observed, which was in accordance with the occurrence of the band at 58 kDa showed by SDS−PAGE. During the elution, the phenolic compounds were not detected; and no variation in the absorbances at 280

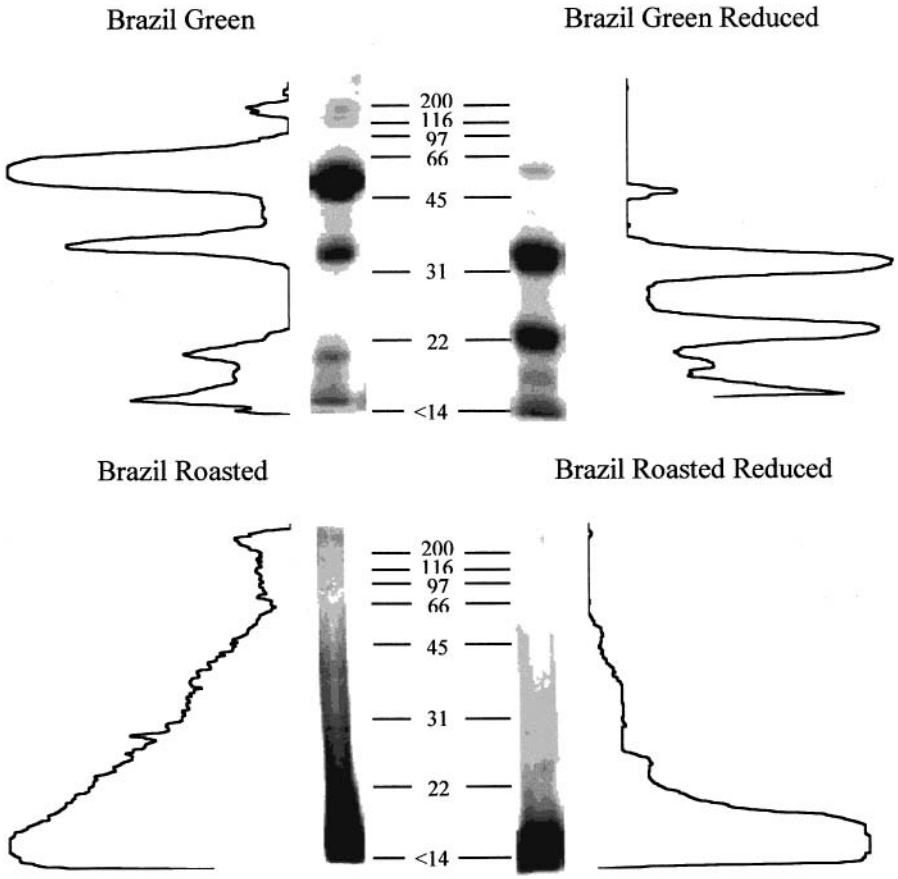

Figure 2. SDS−PAGE of HMWM proteins of Brazil coffee. Middle horizontal marks represent molecular weight markers.

Table 4. Sugar Composition of the Fractions Obtained by Ethanol Precipitation of the HMWM.

fraction	yield[a] (%)	mol % Rha	Ara	Man	Gal	Glc	total sugars (mg/g)	total sugars (mg/100 g coffee)[b]
Costa Rica green								
WIppt	53.8	0	15	43	31	11	104	420
Et50	12.2	0	17	28	41	14	332	306
Et75	25.6	3	34	8	53	2	243	465
EtSN	9.0	6	26	11	45	11	176	120
Costa Rica roasted								
WIppt	28.5	1	2	78	11	8	341	740
Et50	29.5	0	1	90	8	1	503	1130
Et75	16.8	1	7	37	52	2	317	405
EtSN	19.7	0	17	28	52	4	164	246
Brazil green								
WIppt	50.7	4	17	29	28	22	113	575
Et50	15.3	2	13	28	25	32	280	431
Et75	18.2	5	33	6	55	1	308	565
EtSN	13.1	5	11	12	35	37	83	109
Brazil roasted								
WIppt	25.5	0	2	89	6	3	433	939
Et50	24.0	1	2	86	10	1	440	899
Et75	17.3	1	10	35	52	2	346	508
EtSN	23.4	5	18	23	51	4	147	293

[a] As % of HMWM weight. [b] Dry and deffated coffee.

and 400 was observed with the detector attenuation used. For the roasted coffees, the elution of the polysaccharide material was closely followed by the peaks of absorbance at 280 and 400 nm, protein and phenolics. The band observed had a lower molecular weight than those found for the green coffee polysaccharides, which allowed us to infer the presence of shorter polymers. The coelution of the different types of molecules may indicate the probable occurrence of strong interactions between them, possibly by covalent linkages, and could also justify the heterogeneous SDS−PAGE patterns obtained for the roasted coffees. The brown material and phenolic compounds found in the nondialyzable fractions obtained from the roasted coffees might be loosely linked to the polysaccharide and/or protein material through weaker hydrogen bonds and/or hydrophobic interactions (*6, 22, 23*). To eliminate the hypothesis of the coelution of the colored and UV absorbing material through noncovalent bonds, the fractions were eluted in a Sephadex G-25 gel column using strong dissociating conditions in the eluent: urea 7 M, SDS 1% and 5 M triethanolamine (*49, 50, 66*). As only one band was

Table 5. Chemical Composition of the Et50 Fraction

coffee		yield[a]	polysaccharides[b,c]	protein[b,d]	phenolics[b,e]
Costa Rica	green	0.92	33.2	50.4	2.8
	roasted	2.25	50.3	15.0	15.5
Brazil	green	1.48	28.0	55.2	2.5
	roasted	2.04	44.0	14.6	19.8

[a] As a coffee % dry basis. [b] As % of Et50. [c] As anhydrosugar. [d] As BSA equivalents by the Coomassie staining procedure. [e] As 3-CQA equivalents by the Prussian blue method.

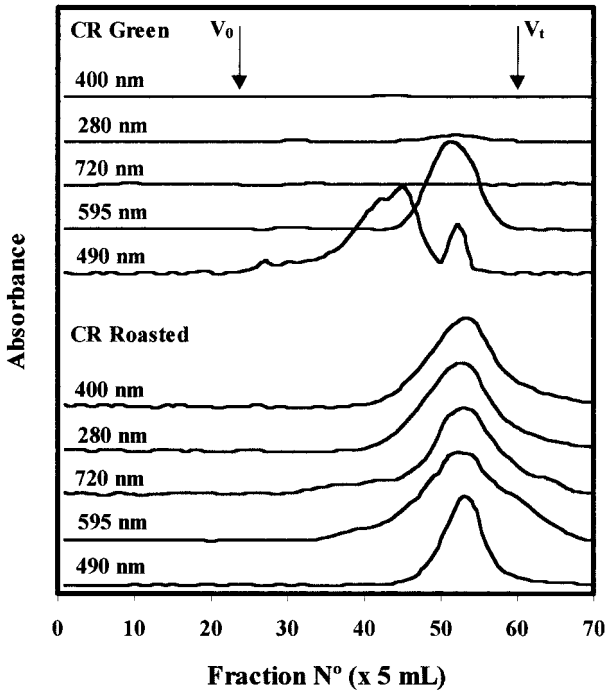

Figure 3. Chromatographic profile of Et50 fractions obtained for Costa Rica green and roasted coffee in Sephacryl S-400 HR.

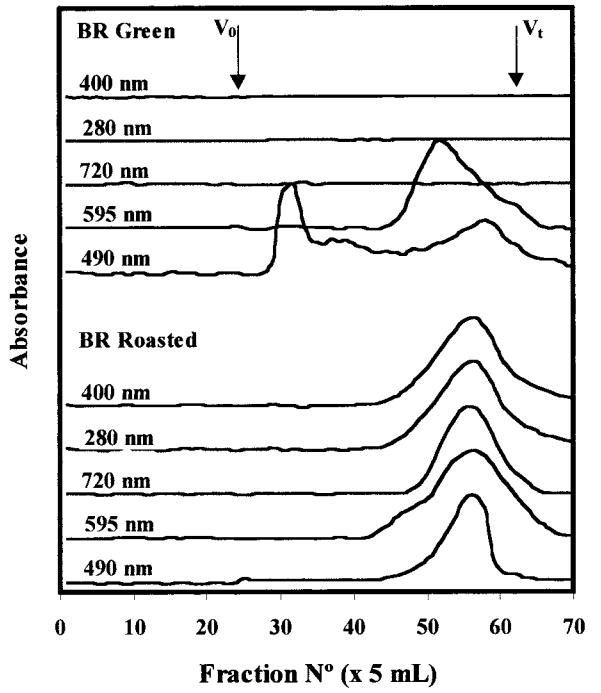

Figure 4. Chromatographic profile of Et50 fractions obtained for Brazil green and roasted coffee in Sephacryl S-400 HR.

observed in the exclusion limit of the gel, as determined by the Abs 280 nm, Abs 400 nm, and phenol-sulfuric acid, it was inferred that the phenolic compounds and brown material present in the roasted coffees Et50 fraction were linked by covalent bonds.

Table 6. Glycosidic-Linkage Composition of the Polysaccharides Present in Et50 Fractions of Green and Roasted Coffees

linkage	Costa Rica		Brazil	
	green	roasted	green	roasted
	mol %			
T-Rha*p*	1.5	0.2	2.6	0.6
T-Ara*f*	12.6	1.1	9.3	1.2
5-Ara*f*	4.8	0.3	4.8	0.4
T-Man*p*	1.0	4.4	0.9	4.2
4-Man*p*	25.7	81.5	24.6	79.7
4,6-Man*p*	2.8	3.8	2.5	3.1
T-Gal*p*	8.2	4.1	5.5	4.0
3-Gal*p*	16.1	2.2	9.0	3.1
6-Gal*p*	2.6	0.9	1.4	1.0
3,6-Gal*p*	14.1	1.3	9.5	1.9
T-Glc*p*	nd[a]	nd	nd	nd
4-Glc*p*	8.8	0.5	1.3	0.7
6-Glc*p*	1.8	-	28.7	0.2

[a] nd, Not determined by GC−FID but detected by GC−MS.

The mean molecular weight of the polymeric material in the Et50 fraction of roasted coffees was 90 kDa in CR and 60 kDa in BR. These values are in accordance with those found by Leloup and Liardon (*14*). The polysaccharide elution profiles were different from those obtained for the espresso coffee extracts (*25*) that showed two polysaccharide peaks, one with a comparable molecular weight (70 kDa), and another peak that eluted in the size exclusion volume of the column (2000 kDa). This difference was shown not to be due to the presence of urea in the eluting buffer (results not shown).

Methylation Analysis of Fraction Et50. Table 6 shows the glycosidic-linkage composition of the polysaccharides present in fraction Et50 for the two green and roasted coffees. For the roasted coffees, the major glycosidic linkages found were those characteristic of galactomannans: (1 → 4)-Man*p*, T-Man*p*, (1 → 4,6)-Man*p*, and T-Gal*p*. Also present were (1 → 3)- and (1 → 3,6)-Gal*p* residues and T- and (1 → 5)-Ara*f* residues characteristic of arabinogalactans, which accounted for nearly 6−8% of the Et50 polysaccharides.

The relative amount of (1 → 4,6)-Man*p* was lower in roasted coffees (4 and 9%, respectively, for the roasted and green coffees), which indicated the presence in roasted coffees of less branched galactomannans than those present in the green coffees.

The relative percentage of terminally linked mannosyl residues found for the green and roasted coffees was considerably high when a linear mannan backbone structure is considered (5 and 3% of total Man, respectively, for the Et50 fraction of roasted and green coffees). The use of these values for the determination of the *DP*

of the galactomannans, showed a *DP* of 30 for the green coffees and a *DP* of 20 for the roasted coffees. These values for the roasted coffee galactomannans are in accordance with the results obtained for the mannose-rich polymers isolated from instant coffees that were reported to have *DP*s between 13 and 45 (*17, 18*). However, although there is close agreement between the sugar composition obtained from the sugar analysis and the sugar composition obtained from the methylation analysis, these *DP* figures are considerably low, not in accordance with the results of molecular weights obtained by size exclusion chromatography. To be sure that the results of methylation were accurate, each sample was analyzed five times, with different length periods of solubilization (3 h to 2 days), different lengths of NaOH activation (3 to 6 h), and different lengths of methylation (2 to 12 h) and, in a last attempt, a re-methylation was done. Degradation during the methylation analysis may occur (*67*), however the methodology used has been implemented in our laboratory for determination of the glycosidic-linkages of cell wall polysaccharides without any problem regarding the estimation of the *DP* of polysaccharides.

CONCLUSIONS

The polysaccharides present in the green coffees HMWM were arabinogalactans (62%), galactomannans (24%), and glucans. The arabinogalactans were more branched than those previously isolated from the holocellulose residue. The major green coffee proteins had molecular weights of 58 and 38 kDa. The 58 kDa protein had two subunits, with 38 and 20 kDa, possibly linked by disulfide bonds.

Roasted coffees HMWM were composed mainly of galactomannans (69%) and arabinogalactans (28%). The galactomannans and arabinogalactans present in the roasted coffees extracts were less branched than those of green coffees.

The protein fraction obtained from roasted coffees had only a defined band with a molecular weight ≤14 kDa, and presented a diffuse band with a molecular weight >200 kDa, showing that their structure was changed by the roasting process.

The Et50 fraction obtained from the roasted coffees had a brown color, was composed mainly of polysaccharides, the majority of them galactomannan, and also contained protein and phenolic compounds. The use of strong hydrogen bond and hydrophobic dissociation conditions allowed us to conclude that the phenolics and brown compounds present were covalently linked to the polymeric material.

ABBREVIATIONS USED

Ara, arabinose; BR, Brazil; CR, Costa Rica; *DP*, degree of polymerization; *DR*, degree of roast; Et50, material precipitated with 50% ethanol; Et75, material precipitated with 75% ethanol; EtOH, ethanol; EtSN, material soluble in 75% ethanol; Gal, galactose; HMWM, high molecular weight material; Man, mannose; Rha, rhamnose; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; HexA, hexuronic acid; WIppt, water insoluble precipitate.

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