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Chemical Characterization of the High-Molecular-Weight Material Extracted with Hot Water from Green and Roasted Robusta Coffees As Affected by the Degree of Roast

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The hot-water-soluble polymeric material from green and roasted Uganda robusta coffees submitted to different degrees of roasting was isolated and characterized, and the changes in structure and amount of galactomannans and arabinogalactans were determined and discussed in relation to the data already available for arabica coffees, obtained under the same experimental conditions. The content of arabinogalactans extracted from robusta green coffee was higher than that extracted from arabica. For roasted coffees, the amount of galactomannans extracted ranged from 0.66% to 0.92% (w/w). These values were near 50% of those obtained from the arabica coffees using the same extraction procedure. However, the amount of arabinogalactans extracted from robusta coffees (0.56−0.72%) was in the range obtained from arabica. The structures of arabinogalactans and galactomannans extracted from green and roasted coffees were not sufficiently different between robusta and arabica coffees to explain the observed differences in extraction yields for the arabinogalactans from green coffees and for the galactomannans from roasted coffees. The total polysaccharide content and the structures of the galactomannans and arabinogalactans in the two green coffee varieties investigated were also very similar. These differences in the extraction of high-molecular-weight polysaccharides between arabica and robusta roasted coffees may be related to the different susceptibility of the cell walls during the roasting process, known to result in a different porosity between arabica and robusta roasted coffees.

KEYWORDS: Coffee; robusta; 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*). The viscosity of polysaccharide solutions increases with concentration and, for the same concentration, with increasing molecular weight (*2*, *3*). The high-molecular-weight polysaccharides are the main contributors to the viscosity of coffee solutions (*4*) and thus to the creamy sensation perceived in the mouth, known as "body" (*5*). They are also important in determining the physicochemical characteristics of coffee infusions, such as the foaming ability of espresso coffee (*6*), and play an important role in the retention of volatile substances (*7*).

The two main polysaccharides present in roasted arabica coffee infusions are galactomannans and type II arabinogalactans (*8*, *9*). The roasting process of the coffee beans was shown, in two arabica coffees, to change markedly the amount and structure of these hot-water-extracted polysaccharides (*9*, *10*). With roasting, the galactomannans present in the infusions became less branched and shorter, and the arabinogalactans had shorter and less arabinosyl side chains (*10*).

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 (*6*). The composition of coffee proteins was also shown to be profoundly changed by the roasting of the green coffee bean (*11*, *12*). The major proteins present in green arabica coffee infusions had molecular weights of 58 and 38 kDa (*9*). However, from roasted coffees, only a defined band with $\leq$14 kDa and a diffuse band with >200 kDa were observed (*9*, *10*). In these arabica roasted coffee infusions, strong associations were observed between phenolics and brown compounds and the polymeric material.

The roasting process of coffee changes the cell wall porosity (*13*, *14*). By scanning electron microscopy, it has been observed that, under the same roasting conditions, the cell walls of the arabica coffees are more degraded than the cell walls of robusta coffees. Also, more pores are observed in the cell walls of arabica coffees (*13*). This could be a key factor in the extraction process of ground roasted coffee, especially for the extraction of the polymeric material.

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Previous studies showed that the foam stability of espresso coffee depends on the degree of roasting of the coffee bean and is different for robusta and arabica coffees (*6*). This was related to the presence of polysaccharides, namely those that precipitated in 50% ethanol solutions. Following the detailed chemical analyses used to characterize the hot-water-soluble polymeric material from two origins of arabica coffees and their changes resulting from the roasting process (*9*, *10*), the aim of this work was to isolate and characterize the hot-water-soluble polymeric material for green and roasted Uganda robusta coffee submitted to different degrees of roasting, determine the changes in structure and amount of galactomannans and arabinogalactans extracted, and discuss them in relation to the data already available for arabica coffees.

MATERIALS AND METHODS

Materials. The green coffee sample (*Coffea canephora*), from Uganda (dry-processed), was provided by a local factory. All chemicals were analytical grade or of the highest purity available. The green robusta coffee was roasted, ground, and extracted for the same period of time as the two arabica coffee varieties studied in our previous reports (*9*, *10*).

Coffee Roasting, Grinding, and Defatting. 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 (*9*). The coffees were roasted to DRs of 5.3%, 8.2%, and 10.3%, ground to a particle size of 0.350 ± 0.050 mm, and defatted by Soxhlet extraction with petroleum ether (*9*). 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 beans were frozen in liquid nitrogen before being ground and were defatted as described for the roasted coffees.

Percentage of Water Content. The percentage of water content was determined in duplicate as previously described (*9*).

Preparation of High-Molecular-Weight Material (HMWM). With constant stirring, 50 g samples of each ground and defatted coffee were extracted with 1 L of water at 80 °C for 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 to approximately 200 mL and dialyzed (MW cutoff 12−14 kDa, Visking size 8, Medicell International Ltd., London, U.K.) at 4 °C with eight 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 or brown color 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 for 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, concentrated by rotary evaporation at 40 °C, and then freeze-dried (*9*).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). The electrophoresis in polyacrylamide gel (12.5% and 4% of acrylamide for the running and stacking gels, respectively) under denaturing conditions (SDS), and under nonreductive and reductive conditions, was done as described by Shewry et al. (*15*). To each lane, 20 μL of sample (20 mg/mL) was applied. The run was performed at 200 V, the protein bands were colored by using the Coomassie R-250 procedure (*15*), 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 Kjeldahl nitrogen by the factor 6.25. The Kjeldahl nitrogen was determined according to the procedure described by Willis et al. (*16*).

Phenolics Assay Procedure. The phenolic content of HMWM was determined by a modification of the Prussian blue method described by Price and Butler (*17*). To 100 μL of the sample were added 5 mL of water, 0.5 mL of 0.1 M $FeCl_3$ in 0.1 M HCl, and 0.5 mL of $K_3Fe(CN)_6$, with stirring between additions. The absorbance at 720 nm was read after 10 min, and the results were expressed in equivalents of 3-chlorogenic acid for a concentration range between 5 and 100 μg/mL. For the detection of phenolics in the chromatographic eluent of the Sephacryl column, the amount of water added was 2 mL.

Sugars Analysis. Neutral sugars were released by Saeman hydrolysis (*18*) and analyzed as their alditol acetates by gas chromatography (*19*, *20*). Hexuronic acids were determined colorimetrically by a modification (*21*) of the method of Blumenkrantz and Asboe-Hansen (*22*). The hydrolysis of all samples was performed in duplicate. The results obtained showed less than 5% variability in the major component sugars.

Methylation Analysis. Polysaccharides were activated with powdered NaOH and methylated with CH_3I (*23*, *24*) as described by Coimbra et al. (*21*), followed by a remethylation to ensure complete methylation of the polysaccharides (*9*). The remethylated material was hydrolyzed with 2 M trifluoroacetic acid (*25*), and the partially methylated sugars were reduced with $NaBD_4$ and acetylated with acetic anhydride with 1-methylimidazole as catalyst. The partially methylated alditol acetates (PMAAs) were identified by gas chromatography−mass spectrometry (*9*) and quantified by gas chromatography−flame ionization detection using the molar response factors of Sweet et al. (*26*).

Gel-Filtration Chromatography. Gel-filtration chromatography on Sephacryl S-400 HR (Pharmacia) was performed on a 100 × 1.6 cm (XK 16/100, Pharmacia) column at a flow rate of 2.5 mL/min, with use of phosphate−urea buffer at pH 6.5 as eluent (*9*). Fractions were assayed for carbohydrate (phenol−sulfuric acid method) (*27*), protein (Coomassie staining) (*28*), and phenolic compounds ($FeCl_3$ + $K_3Fe(CN)_6$) (*17*). The absorption at 400 nm for detection of brown compounds was also measured, and the eluent was monitored continuously at 280 nm. Standard dextrans of 2000, 487, 266, and 72 kDa (Sigma) and glucose were used for column calibration.

Gel-filtration chromatography on Sephadex G-25 was performed on a 25 × 1.6 cm (XK 16/25 Pharmacia) column at a flow rate of 0.5 mL/min, with a solution of 0.1 M acetate buffer at pH 5 containing 1% SDS, 5 M triethanolamine, and 7 M urea as eluent (*9*). Fractions were assayed for absorption at 280 and 400 nm and for carbohydrate.

Statistical Analysis. Significant differences at the $p = 0.05$ level were evaluated by using Student's *t* test.

Table 1. Amount and Chemical Composition of Green and Roasted Coffees' HMWM as a Function of DR[a]

DR[b]	HMWM	polysaccharides[c]	protein[d]	phenolics[e]
green	11.80	1.85 ± 0.025[f]	4.20 ± 0.07	2.40 ± 0.06
5.3	4.91	1.42 ± 0.034	0.66 ± 0.02	1.76 ± 0.10
8.2	6.84	1.56 ± 0.027	0.77 ± 0.03	2.71 ± 0.07
10.3	6.74	1.48 ± 0.023	0.84 ± 0.02	2.94 ± 0.06

[a] Values are in grams per 100 g of green coffee (dry and defatted). [b] Percent of dry weight lost, on a dry basis. [c] Anhydrosugar. [d] %N × 6.25. [e] As caffeoylquinic acid (3-CQA) equivalents by the Prussian blue method. [f] Mean ± standard deviation. DR, degree of roasting; HMWM, high-molecular-weight material.

RESULTS AND DISCUSSION

Characterization of the High-Molecular-Weight Material (HMWM) of Green and Roasted Coffees. Table 1 shows the amount and chemical composition of the HMWM extracted with hot water from Ugandan green and roasted coffees. The amount

Table 2. Sugar Composition of the HMWM for the Green and Roasted Coffees[a]

DR	HMWM	Rha	Ara	Man	Gal	Glc	HexA
green	11.80	0.035 ± 0.015	0.37 ± 0.01	0.41 ± 0.01	0.91 ± 0.01	0.14 ± 0.01	tr
5.3	4.91	0.053 ± 0.021	0.20 ± 0.01	0.59 ± 0.03	0.56 ± 0.01	0.029 ± 0.01	tr
8.2	6.84	0.029 ± 0.014	0.11 ± 0.01	0.86 ± 0.01	0.54 ± 0.02	0.032 ± 0.01	tr
10.3	6.74	0.027 ± 0.016	0.075 ± 0.003	0.88 ± 0.01	0.47 ± 0.01	0.030 ± 0.01	tr

[a] Values are in grams of anhydrosugar per 100 g of green coffee (dry and defatted basis). Mean ± standard deviation. tr, traces; DR, degree of roasting; HMWM, high-molecular-weight material; Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose; HexA, hexuronic acids.

of polysaccharides extracted from the green coffee, on a dry and defatted weight basis, was 1.85%. This value is within the 0.8−2.1% range reported in the literature (*29*−*31*) and is similar to the value reported for green arabica coffee from Brazil, obtained by using the same extraction procedure (*9*). However, it is much smaller than that obtained by Fischer et al. (*32*) for a robusta coffee sequentially extracted with phenol/acetic acid/water and cold water, where a combined value of 5.18 g/100 g of coffee was obtained. This could be the result of the differences in the extraction procedures: treatment with phenol/acetic acid/water, higher time of water extraction (2 h), use of a lower molecular weight cutoff dialysis bag (3.5 kDa) for the recovery of the polysaccharides, and/or use of a more finely ground coffee.

The amount of polysaccharides extracted from the roasted coffees, when recalculated for the equivalent weight of dry and defatted green coffee (1.42−1.56%), was smaller than that extracted for the corresponding green coffee. The decrease in the amount of polysaccharides extracted after the roasting process was in contrast to the observation for arabica coffees (*9*), where the amount of polysaccharides extracted with hot water from the roasted coffees was higher than that observed for the corresponding green coffees.

Protein accounted for 36% of the green coffee's HMWM and 11−13% of the roasted coffees' HMWM. The SDS−PAGE patterns obtained for the green and roasted coffees (results not shown) were similar to those obtained for arabica coffees (*9*). Under nonreducing conditions, the green coffee HMWM presented a major protein band at 58 kDa and a second one at 38 kDa, with other less intense bands at 170, 150, 16, and ≤14 kDa. For the roasted coffees, under nonreducing conditions, there was a defined band at ≤14 kDa and diffused bands for the higher molecular weights.

Although the HMWMs were obtained after extensive dialysis (7 days, 12−14 kDa membrane cutoff), the HMWMs from the green and roasted coffees contained phenolic compounds (**Table 1**). After an initial decrease in phenolic compounds in the HMWM compared to green coffee, the phenolic compounds content increased with increasing DR. The exact nature of the linkage between phenolic compounds and the HMWM is still uncertain; some authors proposed an ester linkage between the quinic acid residue of the 5-caffeoylquinic acid and the polysaccharides and proteins (*33*). However, according to the results obtained by degradation methods (*34*), the linkage between phenolic compounds seems to occur trough radical mechanisms.

Characterization of Green Coffee HMWM Polysaccharides. The major monosaccharide residue of the HMWM of Ugandan green coffee was galactose (47 mol %). Arabinose (23 mol %), mannose (21 mol %), and glucose (7 mol %) were also present in considerable amounts (**Table 2**). This sugars composition reflects the known polysaccharide composition obtained from other studies on arabica (*9*, *32*) and robusta green coffees (*32*): arabinogalactans occurred as the main hot-water-soluble polysaccharides, in a mixture with galactomannans. From the results of methylation analysis of the galactose and arabinose residues (**Table 3**), the glycosidic linkage composition of the arabinogalactans extracted from this robusta coffee was inferred. The majority of the galactose residues were (1→3)- (50%) and (1→3,6)-linked (35%). The remaining galactose residues were terminally linked and (1→6)-linked (11% and 5%, respectively). The arabinose residues were terminally linked (65%) and (1→5)-linked (35%). The ratio (1→3)-Gal*p*/(1→3,6)-Gal*p* of 1.4 indicates highly substituted arabinogalactans, with terminally linked arabinose residues (T-Ara*f*/(1→3,6)-Gal*p* = 0.9), most of them present as single arabinose residues (T-Ara*f*/(1→5)-Ara*f* = 1.8). These results showed that the structures of the arabinogalactans extracted from this robusta coffee were very similar to those found in arabica coffees extracted by the same procedure (*9*); the exception is the ratio (1→3)-Gal*p*/(1→3,6)-Gal*p*, which was approximately 1 in arabica coffees.

Table 3. Glycosidic Linkage Composition (Mole Percent) of the Polysaccharides Present in the HMWM of Green and Roasted Coffees

	DR			
linkage	green	5.3	8.2	10.3
T-Rha*p*	0.8	1.8	0.6	0.3
T-Ara*f*	14.3	7.8	4.8	3.9
5-Ara*f*	7.8	8.0	2.6	1.8
T-Man*p*	0.8	1.8	2.6	3.8
4-Man*p*	20.2	38.4	49.7	53.9
4,6-Man*p*	1.4	2.3	2.5	2.6
T-Gal*p*	5.1	6.2	7.1	6.8
3-Gal*p*	23.8	16.1	17.9	16.1
6-Gal*p*	2.3	4.4	2.7	2.4
3,6-Gal*p*	16.7	11.2	8.0	6.5
T-Glc*p*	nd[a]	nd	nd	nd
4-Glc*p*	2.2	2.0	1.4	1.8
6-Glc*p*	4.6	0.1	0.1	0.1

[a] nd, not determined by gas chromatography–flame ionization detection but detected by gas chromatography–mass spectrometry. DR, degree of roasting; HMWM, high-molecular-weight material; 5-Ara*f*, (1→5)-linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; 3-Gal*p*, (1→3)-linked galactopyranosyl residues; 6-Gal*p*, (1→6)-linked galactopyranosyl residues; 3,6-Gal*p*, (1→3,6)-linked galactopyranosyl residues; T-Gal*p*, terminally linked galactopyranosyl residues; 4-Glc*p*, (1→4)-linked glucopyranosyl residues; 6-Glc*p*, (1→6)-linked glucopyranosyl residues; T-Glc*p*, terminally linked glucopyranosyl residues; 4-Man*p*, (1→4)-linked mannopyranosyl residues; 4,6-Man*p*, (1→4,6)-linked mannopyranosyl residues; T-Man*p*, terminally linked mannopyranosyl residues.

The galactomannans extracted from Ugandan green coffee contained 90% of (1→4)-linked mannose residues, 6% of (1→4,6)-linked mannose residues, and 4% of terminally linked mannose residues. The percentage of T-Man*p* was higher for this robusta coffee than for the arabica coffees (2.2−2.6%), indicating the presence of shorter galactomannans in Ugandan green coffee. The percentage of substituted mannosyl residues found for the HMWM galactomannans from robusta coffee was similar to that found from arabica coffee (*9*) and was close to

that found in the water-soluble galactomananas isolated by Fischer et al. (*32*).

On the basis of the fact that all mannose residues present in the HMWM were components of the galactomannans and that an amount of galactose residues equal to the amount of (1→4,6)-Man*p* was also a component of galactomannans, the amount of galactomannans extracted from Ugandan green coffee was estimated to be 0.43%, on a dry weight basis of green and defatted coffee. This value is in the range of 0.29−0.45% found for Costa Rican and Brazilian green coffees (*9*). Fischer et al. (*32*) showed that robusta green coffee contained a higher amount of galactomannans with a higher degree of branching than arabica green coffees. As a higher molecular weight cutoff was used in our study, this difference could mean that robusta green coffee contained a higher proportion of lower molecular weight polysaccharides (galactomannans and arabinogalactans) than arabica coffees, that can be recovered in a 3.5 kDa cutoff dialysis bag but not at 12 kDa.

On the basis of the fact that all arabinose and galactose residues, except for the T-Gal*p* previously calculated to belong to the galactomannans, are component sugars of the arabinogalactans, the amount of arabinogalactans was estimated as 1.24%. This value was higher than that obtained for the arabica green coffees (0.74% and 0.97%) (*9*). This result is consistent with the results of Fischer et al. (*32*), who found that the amount of arabinogalactans extracted from a robusta green coffee was higher than the amount extracted from arabica coffee.

Influence of the DR on the Amount and Structure of the HMWM Polysaccharides. For the roasted coffees, mannose (40−59 mol %) was the major sugar residue of the HMWM polysaccharides, followed by galactose (31−38 mol %) and arabinose (6−16 mol %) (**Table 2**). These sugars compositions showed that, in contrast to what was observed for the green coffees, the main hot-water-soluble polysaccharides in the roasted coffees were galactomannans, as was observed in arabica coffees (*9*, *10*). The amount of mannose residues extracted for the lightest roasted coffee increased 64% (on an equivalent weight of dry and defatted coffee) in relation to the green coffee. This increase was smaller than that observed for the arabica coffees (260% and 370%) (*10*). With increasing DR, there was a 57% increase in the amount of mannose residues extracted from the light to the medium roasted coffees. The amount of galactose and arabinose residues decreased from the green to roasted coffees and decreased also with increasing DR.

Methylation analysis of mannose residues showed the characteristic glycosidic linkage composition of galactomannans (**Table 3**). The percentage of terminally linked mannose residues from all roasted coffees was higher than that found for the galactomannans extracted from the green coffee, showing the extraction of shorter galactomannans from roasted coffees. This percentage was approximately the same for the light and medium roasted coffees (4.2% and 4.7%, respectively, of the total Man), but it was higher for the darkest roast (6.3%). The percentage of (1→4,6)-Man in relation to total Man decreased from green to roasted coffees and from the lightest to the darkest roast. This mannosyl linkage composition showed that, with roasting, less branched and shorter galactomannans were extracted.

From the results obtained for the galactose and arabinose residues by methylation analysis, it can be inferred that, with increasing DR, there is a change in the structure of the arabinogalactans extracted to the HMWM. An increase was observed in the ratio (1→3)-Gal*p*/(1→3,6)-Gal*p*, that varied from 1.4 for the light to 2.5 for the dark roasted coffee. This means that the degree of branching of the arabinogalactans extracted

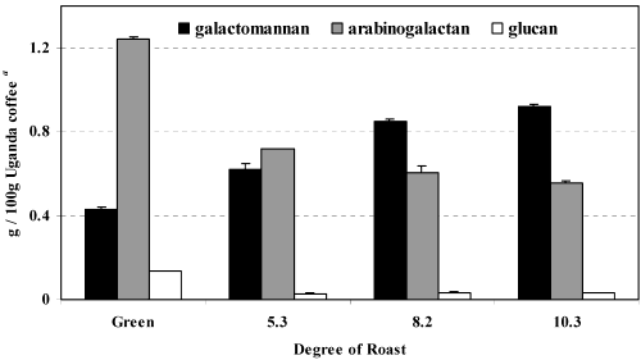

Figure 1. Amount of the individual polysaccharides extracted from green and roasted Ugandan coffees. Bars represent the standard deviation. [a]In relation to the equivalent weight of dry and defatted green coffee.

decreased with increasing DR. Also, the decrease of the ratio T-Ara*f*/total Gal*p* and the increase of the ratio (1→5)-Ara*f*/T-Ara*f* with increasing DR showed the occurrence of less branched arabinogalactans with higher relative amount of single arabinosyl residues as side chains. The relative amount of (1→6)-Gal was higher in the HMWM of roasted coffees (7.5−14.8% of total Gal residues) than in the green coffee (4.8%). This fact, together with the observed relative increase of (1→3)-Gal*p*, seems to indicate that the loss of (1→3,6)-linked galactosyl residues could operate by two mechanisms: by the loss of (1→6)-linkage, rendering (1→3)-Gal*p*, and by the loss of (1→3)-linkage, rendering (1→6)-Gal*p*. This was also observed for the arabinogalactans extracted for the arabica coffee varieties (*9*, *10*).

The degree of branching of the galactomannans extracted for the roasted coffee was similar to the degree of branching of the galactomannans found by Fischer et al. (*32*) in the cell wall material of green coffees. This fact supports the already proposed hypothesis (*9*, *10*) that the increase in the extraction of galactomannans from roasted coffees is due to the extraction of galactomannans already present in the green coffee, and that the roasting process causes a change in the structure of the cell walls of the coffee beans (*13*, *14*) that most probably allows a higher extraction of polysaccharides present in the roasted coffee beans. Nevertheless, a structural change in the green coffee polysaccharides is also observed, as the structure of the arabinogalactans extracted for the roasted coffees is substantially different from those of all the arabinogalactans present in the green coffee beans (*35*).

On the basis of the sugar composition and methylation analysis of the HMWM polysaccharides and the knowledge of the type of polysaccharides present in the coffee infusions, the amounts of galactomannan, arabinogalactan, and glucan were estimated, and the results were expressed in relation to the equivalent weight of dry and defatted green coffee (**Figure 1**). There was an increase in the amount of galactomannan extracted with increasing DR. The amount extracted from the roasted coffees ranged from 0.66% to 0.92%. These values were lower than those obtained for the arabica coffees using the same extraction procedure (1.31−1.58%) (*10*). The amount of arabinogalactans extracted for the roasted coffees ranged from 0.56% to 0.72% and decreased with increasing DR. Similar values were obtained for the arabica coffees (0.46−0.92%) (*10*). These results show that the decrease in the amount of polysaccharides extracted from the green to the roasted coffees was due to a large decrease in the amount of arabinogalactans extracted to the HMWM and a small increase in the amount of galactomannans extracted when compared to the arabica coffees (*9*, *10*).

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

coffee and fraction		yield (%)[a]	sugars (mol %)					total sugars (%)[b]	total sugars (mg/100 g of coffee)[c]
			Rha	Ara	Man	Gal	Glc		
green	WIppt	52.2	2	9	32	39	18	8.7	536
	Et50	9.6	1	14	42	40	3	37.5	424
	Et75	22.0	2	31	7	58	1	37.5	973
	EtSN	12.0	4	9	32	34	21	9.0	127
DR5.3	WIppt	23.8	0	5	41	40	14	11.8	138
	Et50	25.5	1	4	79	14	1	43.7	546
	Et75	21.5	3	23	13	60	0	44.9	474
	EtSN	24.7	7	34	10	47	2	15.9	193
DR8.2	WIppt	16.5	3	5	47	28	17	15.7	177
	Et50	26.7	1	2	80	16	1	45.3	826
	Et75	20.8	1	10	30	58	1	36.5	520
	EtSN	30.1	6	20	16	52	6	13.9	286
DR10.3	WIppt	20.2	1	3	67	19	10	17.4	237
	Et50	31.7	1	1	84	12	2	37.4	799
	Et75	19.0	0	8	40	52	0	36.9	474
	EtSN	24.2	7	18	19	54	2	12.1	197

[a] As percent of HMWM weight. [b] Grams of anhydrosugar per 100 g of fraction. [c] Dry and defatted coffee. DR, degree of roasting; HMWM, high-molecular-weight material; Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose; HexA, hexuronic acids.

The analysis of the HMWM showed the average structural features of the overall polysaccharides extracted with hot water. To evaluate the structural heterogeneity of the galactomannans and arabinogalactans, the polymers present in the HMWM were fractionated by graded addition of absolute ethanol.

Ethanol Fractionation of HMWM. The HMWM of each coffee was fractionated by a graded addition of ethanol in four fractions: WIppt, material insoluble in cold water; Et50, material precipitated with 50% ethanol; Et75, material precipitated with 75% ethanol; and EtSN, material that remained soluble in 75% ethanol solutions. Previous results of the use of this fractionation technique for green and roasted coffee extracts (*6*, *9*, *10*) showed Et50 as galactomannan-enriched fractions and Et75 as fractions containing the majority of arabinogalactans. **Table 4** shows the yield and sugar composition of the fractions, and the last column gives the amount of polysaccharides in each fraction in relation to the dry and defatted weight of coffee. As fractions Et50 contained the majority of the soluble galactomannans, they were submitted to methylation analysis and size-exclusion chromatography.

Influence of the DR on the Chemical Composition and Structure of Et50 Polysaccharides. Table 5 shows the glycosidic linkage composition of the polysaccharides present in Et50 fractions. As expected from the methylation analysis of the HMWM and from the sugar composition of fraction Et50, the main glycosidic linkages found were (1→4)-Man*p* (35% for green coffee and 73−77% for roasted coffees), T-Man*p* (1.4% for green coffee and 3.1−4.1% for roasted coffees), (1→4,6)-Man*p* (4.6% for green coffee and 3.1−3.7% for roasted coffees), and T-Gal*p* (10.2% for green coffees and 4.5−5.8% for roasted coffees). No differences were obtained for the degree of branching and degree of polymerization of the Et50 Ugandan coffee galactomannans and those obtained for Brazilian and Costa Rican coffees (*9*, *10*). As observed for the HMWM, the galactomannans recovered in Et50 had higher percentages of T-Man*p* and lower percentages of (1→4)-Man*p* residues with increasing DR.

The polysaccharides present in fractions Et50 represent only 37−45% of the material recovered (**Table 4**). The remaining material was protein (45% and 11−22% for green and roasted coffees, respectively) and phenolic compounds (3% and 19−27%). Size-exclusion chromatography of these fractions, monitored for polysaccharides, proteins, phenolics, UV-absorbing compounds (280 nm), and brown color, showed, as previously observed for arabica coffees (*9*), that the elution of the polysaccharide material was closely followed by peaks at 280 and 400 nm, protein and phenolics (data not shown). The molecular weight of the polysaccharide fraction obtained was 180 kDa for the light roasted coffee fraction, 140 kDa for the medium roasted coffee, and 79 kDa for the darkest roasted coffee fraction. This material was also eluted in a Sephadex G-25 gel column using strong dissociating conditions in the eluent (7 M urea, 1% SDS, and 5 M triethanolamine), and only one band was in the exclusion limit of the gel, as determined by the absorbances at 280 and 400 nm, and by the phenol−sulfuric acid reaction (data not shown). This allowed us to infer, as for arabica roasted coffee Et50 fractions, that the phenolic compounds and brown material present in roasted coffee Et50

Table 5. Glycosidic Linkage Composition (Mole Percent) of the Polysaccharides Present in Et50 Fractions of Green and Roasted Coffees

linkage	DR			
	green	5.30	8.20	10.3
T-Rha*p*	1.2	0.4	0.2	0.6
T-Ara*f*	8.9	2.0	1.3	0.7
5-Ara*f*	5.7	1.6	0.7	0.2
T-Man*p*	1.4	3.1	4.1	4.1
4-Man*p*	35.1	73.5	73.0	77.7
4,6-Man*p*	4.6	3.7	3.6	3.1
T-Gal*p*	10.2	4.8	5.8	4.5
3-Gal*p*	17.0	6.2	6.2	5.1
6-Gal*p*	1.6	1.1	1.6	0.5
3,6-Gal*p*	12.2	2.5	2.6	2.1
T-Glc*p*	nd[a]	nd	nd	nd
4-Glc*p*	0.6	1.0	0.7	1.2
6-Glc*p*	1.5	0.1	0.1	0.2

[a] nd, not determined by gas chromatography–flame ionization detection, but detected by gas chromatography–mass spectrometry. DR, degree of roasting; HMWM, high-molecular-weight material; 5-Ara*f*, (1→5)-linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; 3-Gal*p*, (1→3)-linked galactopyranosyl residues; 6-Gal*p*, (1→6)-linked galactopyranosyl residues; 3,6-Gal*p*, (1→3,6)-linked galactopyranosyl residues; T-Gal*p*, terminally linked galactopyranosyl residues; 4-Glc*p*, (1→4)-linked glucopyranosyl residues; 6-Glc*p*, (1→6)-linked glucopyranosyl residues; T-Glc*p*, terminally linked glucopyranosyl residues; 4-Man*p*, (1→4)-linked mannopyranosyl residues; 4,6-Man*p*, (1→4,6)-linked mannopyranosyl residues; T-Man*p*, terminally linked mannopyranosyl residues.

fractions were strongly linked, possibly by covalent bonds, as was previously observed for arabica coffees (*10*).

CONCLUSIONS

The content of arabinogalactans extracted with water from robusta green coffee was higher than that extracted from arabica coffees (*9*). As this was also observed in other studies (*32*), this seems to be a characteristic that distinguishes robusta from arabica green coffees. For roasted coffees, the amount of galactomannans extracted with hot water from Ugandan robusta coffee ranged from 0.66% to 0.92%. These values were clearly lower than those obtained for the arabica coffees using the same extraction procedure (1.31−1.58%) (*10*). However, the amount of arabinogalactans extracted from robusta coffees (0.56−0.72%) was in the range obtained from arabica (0.46−0.92%) (*10*). Nevertheless, the structures of arabinogalactans and galactomannans extracted from green and roasted coffees were not sufficiently different between robusta and arabica coffees to explain the observed differences in extraction yields for the arabinogalactans from green coffees and for the galactomannans from roasted coffees. Also, no marked difference in the total polysaccharide content was observed between green arabica and robusta coffee beans: neither the structures of the galactomannans nor the arabinogalactans in the two green coffee varieties differ significantly (*32*, *36*).

Because during the roasting process the cell walls of the arabica coffees are more degraded and are known to contain a more pores in the cell walls than the robusta coffee (*13*), it is possible that a different cell wall structure could confer a different behavior to the two coffee species during the roasting process, causing a different extraction yield of polysaccharides, although with the same overall structure. Also, it is possible that the observed difference could be the result of a higher degradation of the cell walls of polysaccharides from robusta coffee, reducing the molecular weight, that could be lost through the dialysis membrane; alternatively, the observed difference could be the result of a higher cross-linking of green coffee components during the roasting process that could lower the extraction yield. These suggestions arise from the fact that, although robusta green coffee contained a higher amount of water-soluble arabinogalactans, the roasting process leads to a decrease in the amount of arabinogalactans extracted to the same level found for the arabica green coffees (*10*).

ABREVIATIONS USED

Ara, arabinose; 5-Ara*f*, (1→5)-linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; DR, degree of roasting; Et50, material precipitated with 50% ethanol; Et75, material precipitated with 75% ethanol; EtOH, ethanol; EtSN, material soluble in 75% ethanol; Gal, galactose; 3-Gal*p*, (1→3)-linked galactopyranosyl residues; 6-Gal*p*, (1→6)-linked galactopyranosyl residues; 3,6-Gal*p*, (1→3,6)-linked galactopyranosyl residues; T-Gal*p*, terminally linked galactopyranosyl residues; 4-Glc*p*, (1→4)-linked glucopyranosyl residues; 6-Glc*p*, (1→6)-linked glucopyranosyl residues; T-Glc*p*, terminally linked glucopyranosyl residues; HexA, hexuronic acids; HMWM, high-molecular-weight material; Man, mannose; 4-Man*p*, (1→4)-linked mannopyranosyl residues; 4,6-Man*p*, (1→4,6)-linked mannopyranosyl residues; T-Man*p*, terminally linked mannopyranosyl residues; Rha, rhamnose; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; WIppt, water-insoluble precipitate.

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