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Chemical Characterization of Galactomannans and Arabinogalactans from Two Arabica Coffee Infusions As Affected by the Degree of Roast

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Galactomannans and arabinogalactans compose almost exclusively the polysaccharide fraction of roasted coffee infusions. To increase the knowledge about the effect of the degree of roast (DR) in the amount and chemical structure of the galactomannans and arabinogalactans, two arabica coffees of different geographical origins (Costa Rica and Brazil) were roasted for three degrees of roast (DRs 4.7−5.0, 8.7, and 10% of dry weight loss of green coffee beans, on a dry basis). The high molecular weight material was extracted with hot water and dialyzed (molecular weight cutoff >12 kDa), and the material was separated in three cold-water-soluble fractions by graded addition of ethanol. The degree of polymerization and the degree of branching of the galactomannans decreased with the increase of the DR. As the DR increased, less branched arabinogalactans were extracted. The relative amount of terminally linked arabinosyl residues of the arabinogalactans decreased with the increase in DR, and the terminally linked galactosyl residues increased. Also, the size of the arabinosyl side chains of the arabinogalactans decreased with the increase in DR.

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

INTRODUCTION

The two main polysaccharides present in roasted coffee infusions are galactomannans and type II arabinogalactans (*1*, *2*). NMR (*1*) and methylation (*2*) analysis showed that, for a medium roast coffee infusion, the water-soluble galactomannans had a main backbone of mannosyl residues linked by β-(1→4) linkages, with 4% of them branched at C-6 by single galactosyl residues. Arabinogalactans had a ratio of (1→3)/(1→3,6)-Gal residues near 1.5, with side chains of arabinosyl and galactosyl residues. The roasting process led to a great change in the amount and structure of the hot-water-soluble polysaccharides extracted in relation to the green coffee (*2*). The roasting process changes the amount of polysaccharides extracted, and the relative amount of the component sugars vary with the degree of roasting (*2*−*5*). Thus, the polysaccharides obtained from the different degrees of roast for the same green coffee are expected to have different structural features.

The aim of this work was to determine the changes in structure and amount of hot-water-soluble galactomannans and arabinogalactans extracted from two arabica coffees (*Coffea arabica*) of different geographical origins, Costa Rica (wet-processed) and Brazil (dry-processed) coffees, when submitted to different DRs.

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MATERIALS AND METHODS

Materials. The green coffee samples (*C. 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, 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 (*4*) The two coffees were roasted to DRs of approximately 5%, 8.7%, and 10% and were ground to a particle size of 0.350 ± 0.050 mm and defatted by Soxhlet extraction with petroleum ether (*4*).

Percentage of Water Content. The percentage of water content was determined in duplicate, for the green coffees, according to International Organization for Standardization 1447-1978, by the method of two-stage oven drying at 130 °C and for the ground-roasted coffees, according to the International Organization for Standardization 11294-1993, by the method of oven drying at 105 °C for 4 h (*6*).

Preparation of High Molecular Weight Material (HMWM). With constant stirring, 50 g 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, 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 was fluffy and brown.

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

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at 24 400*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, concentrated by rotary evaporation at 40 °C, and then freeze-dried (*7*).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). The electrophoresis in polyacrylamide gel (12.5% and 4% of acrylamide for the running and stacking gel, respectively) under denaturing conditions (SDS), and under nonreductive and reductive conditions was as described by Shewry et al. (*8*). 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, 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. (*9*).

Sugars Analysis. Neutral sugars were released by Saeman hydrolysis (*10*) and analyzed as their alditol acetates by GLC (*11*, *12*). Hexuronic acids were determined colorimetrically by a modification (*13*) of the method of Blumenkrantz and Asboe-Hansen (*14*). The hydrolysis of all samples was performed in duplicate. Results used had less than 5% variability in the major component sugars.

Methylation Analysis. Polysaccharides were activated with powdered NaOH and methylated with CH_3I (*15*, *16*) as described by Coimbra et al. (*13*), followed by a remethylation to ensure complete methylation of the polysaccharides (*2*). The remethylated material was hydrolyzed with trifluoroacetic acid (*17*), 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 (PMAA) were identified by gas chromatography-mass spectrometry (*2*) and quantified by gas chromatography-flame ionization detection with use the molar response factors of Sweet et al. (*18*).

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 as eluent (*2*). Fractions were assayed for carbohydrate (phenolsulfuric acid method) (*19*), protein (Coomassie staining) (*20*), and phenolic compounds [$FeCl_3 + K_3Fe(CN)_6$] (*21*). 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 2 000, 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 (*2*). Fractions were assayed for absorption at 280 and 400 nm and for carbohydrate.

Statistical Analysis. Significant differences at $p = 0.05$ level were evaluated by using the Student *t* test.

RESULTS AND DISCUSSION

Influence of the DR on the Amount and Chemical Composition of the HMWM. The amount of HMWM extracted with hot water, on a dry and defatted green coffee basis, increased with the increase of DR in Costa Rica (CR) and decreased in Brazil (BR) roasted coffees (**Table 1**).

The amount of polysaccharides extracted, for CR coffee, was fairly constant with the DR in relation to the equivalent dry weight of green and defatted coffee (2.2−2.3 g/100 g green coffee), whereas, for BR coffee, it decreased from 2.6 in the light roast to 2.0 g /100 g of green coffee in the highest DR (**Table 1**). The amount of hot-water-soluble polysaccharides and its variation with the DR are not, apparently, in accordance with the results obtained by Thaler and Arneth (*3*), which reported values of 3.5−4.0 g/100 g of dry green coffee and higher amount of polysaccharides extracted with hot water for the highest DRs. These differences can be caused by the different extraction temperature (100 °C) used and by a different MW cutoff of the dialysis membrane of 5 kDa (*3*). Also, a different degree of grinding of the material, although not stated, could have this effect (*22*). All roasted coffees analyzed had higher amount of polysaccharides in the HMWM than the correspondent green coffees [1.13 and 1.86 g for CR and BR green coffees (*2*)].

Table 1. Chemical Composition of Roasted Coffees HMWM As a Function of DR[a]

origin	DR[b]	HMWM	polysaccharides[c]	protein[d]	phenolics[e]
Costa Rica	5.0	6.31	2.21 ± 0.04[f]	0.55 ± 0.02	1.73 ± 0.08
	8.7	6.96	2.18 ± 0.05	0.67 ± 0.03	2.27 ± 0.06
	10.0	7.27	2.26 ± 0.07	0.58 ± 0.01	2.59 ± 0.10
Brazil	4.7	8.11	2.57 ± 0.05	0.79 ± 0.02	2.30 ± 0.10
	8.7	7.77	2.27 ± 0.02	0.69 ± 0.01	3.31 ± 0.07
	9.9	7.20	1.99 ± 0.06	0.55 ± 0.01	3.41 ± 0.10

[a] Values are in grams per 100 g 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.

For the two coffees, the amount of protein represented 8−10% of the HMWM. The SDS−PAGE patterns obtained were similar to those previously described for the roasted coffees (*2*), with a defined band with ≤14 kDa and a diffuse band with >200 kDa (results not shown).

The amount of phenolic compounds present in the HMWM of the roasted coffees increased with the increase of DR, although the difference from the medium to the high DR was not statistically significant. For the same DRs, the amount of phenolic compounds present in BR was higher than that found in CR.

Influence of the DR on the Amount and Structure of the HMWM Polysaccharides. Table 2 shows the monosaccharide composition of the HMWM polysaccharides obtained for CR and BR roasted coffees. These polysaccharides were mainly composed of mannose (Man) and galactose (Gal). The lightest roasted coffees were also rich in arabinose (Ara).

For CR coffee, in relation the equivalent weight of dry and defatted green coffee, the amount of Man residues extracted increased 22% from the low to the medium DR and was kept constant from the medium to the highest DR. For BR coffee, the amount of Man residues extracted was approximately the same for the low and the medium, DR but a decrease of 14% was observed in the highest DR. Methylation analysis (**Table 3**) showed that these Man residues were the building blocks of the galactomannan polysaccharides, as observed previously (*1*, *2*). The relative amount of terminally linked Man (T-Man*p*) residues obtained for all roasted coffees were higher than that obtained for the correspondent green coffees (*2*). Also, for both coffees, there was a tendency for the increase in the relative amount of T-Man*p* residues with the increase of DR. This fact allowed us to conclude that, with increasing DR, lower molecular weight galactomannans are extracted. The relative amount of the branched residues (1→4,6)-Man*p*, in relation to all Man residues, ranged from 3.5 to 4.8% in CR coffee and from 3.8 to 4.3% in BR coffee. These values were clearly lower than those found for the correspondent green coffees (*2*), where values of 6.3 and 5.2% were reported, respectively, for CR and BR coffees.

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

coffee	DR	Rha	Ara	Man	Gal	Glc	HexA
Costa Rica	5.0	0.041 ± 0.010	0.18 ± 0.01	1.24 ± 0.02	0.70 ± 0.02	0.045 ± 0.020	t
	8.7	0.020 ± 0.010	0.072 ± 0.002	1.52 ± 0.04	0.48 ± 0.02	0.088 ± 0.020	t
	10.0	0.021 ± 0.010	0.075 ± 0.003	1.50 ± 0.06	0.62 ± 0.01	0.046 ± 0.030	t
Brazil	4.7	0.024 ± 0.006	0.26 ± 0.01	1.46 ± 0.05	0.72 ± 0.02	0.11 ± 0.01	t
	8.7	0.021 ± 0.001	0.11 ± 0.01	1.51 ± 0.01	0.58 ± 0.01	0.047 ± 0.007	t
	9.9	0.036 ± 0.003	0.065 ± 0.002	1.29 ± 0.02	0.44 ± 0.04	0.16 ± 0.04	t

[a] Values are in grams of anhydrosugar per 100 g green coffee (on a dry and defatted basis). Mean ± standard deviation; t = traces.

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

	DR					
	Costa Rica			Brazil		
linkage	5.0	8.7	10.0	4.7	8.7	9.9
T-Rha*p*	1.4	0.5	0.5	0.7	0.9	1.8
T-Ara*f*	6.8	2.9	2.7	7.0	4.8	2.9
5-Ara*f*	4.3	1.3	1.1	4.8	2.0	1.3
T-Man*p*	2.8	3.9	4.2	2.8	3.9	3.7
4-Man*p*	50.9	60.3	61.7	52.8	58.5	60.0
4,6-Man*p*	2.7	2.3	2.6	2.2	2.8	2.7
T-Gal*p*	5.9	6.5	7.2	4.7	6.0	6.6
3-Gal*p*	11.4	8.6	10.2	9.7	10.3	9.9
6-Gal*p*	4.3	3.0	2.9	3.3	2.2	2.3
3,6-Gal*p*	8.4	5.3	6.1	8.1	7.1	6.6
T-Glc*p*	nd[a]	nd	nd	nd	nd	nd
4-Glc*p*	1.1	4.8	0.1	2.9	1.2	2.0
6-Glc*p*	0.1	0.6	0.7	0.9	0.4	0.2

[a] nd, not determined by gas chromatography-flame ionization detection, but detected by gas chromatography-mass spectrometry.

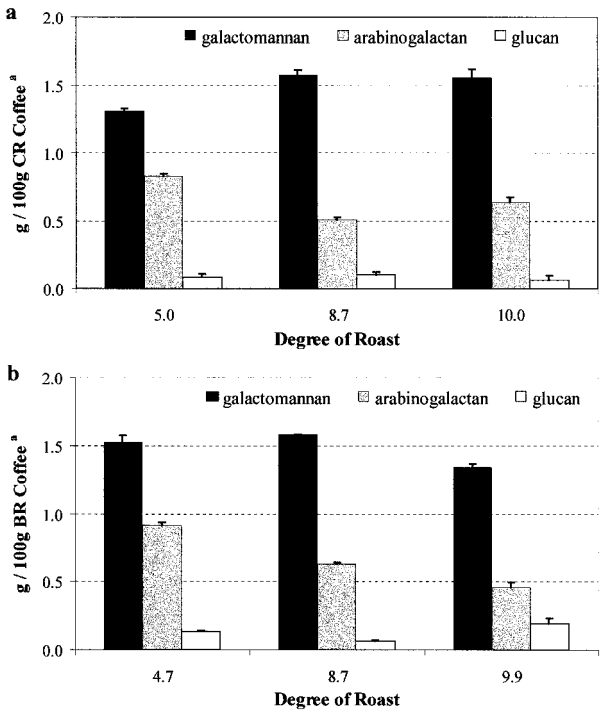

Figure 1. Amount of the individual polysaccharides extracted from the different roasted coffees. (a) CR; (b) BR. Bars represent the standard deviation. [a]In relation to the equivalent weight of dry and defatted green coffee.

For CR coffee, in relation to the equivalent weight of dry and defatted green coffee, the amount of Gal residues extracted decreased from the first to the second DR, and an increase from the second to the third DR (**Table 2**). For BR coffee the amount of Gal residues extracted decreased from the first to the last DR. For all DRs, for both coffees, most of the Gal residues were (1→3)- and (1→3,6)-linked (59−69% of all Gal*p* residues). These galactosyl linkages are the building blocks of type II arabinogalactans (*1, 2*). Also present were the terminally linked Gal (18−28%) and (1→6)-linked residues.

For both coffees, a decrease in the amount of Ara residues extracted with the increase in the DR was observed. Most of the Ara residues were terminally linked (T-Ara*f*). With the increase in DR, the relative amount of these residues, in relation to the (1→5)-Ara*f* residues, increased. This showed that the size of the arabinosyl side chains of the arabinogalactans decreased with the increase in DR.

From the results obtained for the galactosyl and arabinosyl residues by methylation analysis it can be inferred that, with the increase in the DR, a change occurred in the structure of the arabinogalactans extracted to the HMWM. An increase in the ratio (1→3)-/(1→3,6)-Gal with the increase in the DR allowed the inference that less branched arabinogalactans were extracted with the increase in DR. The decrease in the amount of arabinosyl residues was more pronounced than the decrease in the (1→3,6)-Gal*p*, which, taking into consideration that they belong to the arabinogalactan side chains, explains the observed increase in the relative amounts of T-Gal*p*. The less-branched arabinogalactans extracted had side chains with fewer T-Ara*f* residues but with a higher amount of T-Gal*p* residues.

These results show that the structure of the HMWM polysaccharides extracted from the roasted coffees depended on the DR. For the two coffees studied, the structure of the polysaccharides extracted and its variation with the DR were very similar. Because the amount of polysaccharides extracted for the roasted coffees was higher than that extracted for the correspondent green coffees (*2*), these structural changes can be due to a structural change of the polysaccharides present in the green coffee caused by the roasting process and/or by the bean physical changes that occur during the roasting process (*6*, *23*), allowing the extraction of different polysaccharides already present in the green coffee beans.

On the basis of the sugar composition and methylation analysis of the HMWM polysaccharides and of the knowledge of the type of polysaccharides present in the roasted coffee infusions (*1*, *2*), the amount of galactomannan, arabinogalactan, and glucan were estimated for each roasted coffee (**Figure 1**). For CR coffee, in relation to the equivalent weight of dry and defatted green coffee, the amount of galactomannan extracted increased from the first to the second DR and remains constant for the third DR. For BR coffee, the amount of galactomannan extracted was approximately the same in the first and second DR, and decreased in the third DR. Nevertheless, the maximum amount of extractable galactomannans, in the range of DR studied, was approximately the same for both coffees (1.6 g/100 g). For both coffees, the amount of arabinogalactans extracted

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 coffee)[c]
			Rha	Ara	Man	Gal	Glc		
CR 5.0%	WIppt	14.0	2	8	52	23	15	15.6	145
	Et50	35.2	1	3	85	11	1	49.1	1145
	Et75	16.3	2	17	16	63	3	43.0	465
	EtSN	31.4	6	30	17	42	5	17.2	358
CR 8.7%	WIppt	28.5	1	2	78	11	8	34.1	740
	Et50	29.5	0	1	90	8	1	50.3	1130
	Et75	16.8	1	7	37	52	2	31.7	405
	EtSN	19.7	0	17	28	52	4	16.4	246
CR 10%	WIppt	31.1	0	1	90	7	2	42.8	698
	Et50	24.4	2	2	84	12	1	42.4	1085
	Et75	20.1	3	6	38	52	1	36.1	555
	EtSN	22.6	3	12	29	49	8	14.7	287
BR 4.7%	WIppt	28.4	0	0	67	20	13	33.4	808
	Et50	29.2	1	4	82	12	1	46.5	1156
	Et75	18.1	2	21	17	58	2	37.0	571
	EtSN	22.1	4	38	11	45	2	22.7	428
BR 8.7%	WIppt	25.5	0	2	89	6	3	43.3	939
	Et50	24.0	1	2	86	10	1	44.0	899
	Et75	17.3	1	10	35	52	2	34.6	508
	EtSN	23.4	5	18	23	51	4	14.7	293
BR 9.9%	WIppt	26.9	0	4	50	22	25	27.1	581
	Et50	31.6	1	2	86	9	1	44.7	1127
	Et75	17.1	0	10	51	39	0	32.1	438
	EtSN	20.8	5	19	23	49	4	14.2	236

[a] As percent of HMWM weight. [b] Grams of anhydrosugar per 100 g of fraction. [c] Dry and defatted coffee.

for the first DR was higher than for the other DRs and was equivalent to the amount extracted for the corresponding green coffees (*2*). The amount of glucans was less than 0.2%. These results show the average structural features of the overall polysaccharides present in the HMWM. To evaluate the structural heterogeneity of the galactomannan and arabinogalactan, the HMWMs were fractionated by graded addition of absolute ethanol.

Ethanol Fractionation of HMWM. The HMWM of each coffee was fractionated into 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. **Table 4** shows the yield and sugar composition of the fractions and the last column gives the amount of polysaccharides of each fraction in relation to the dry and defatted weight of roasted coffee.

For most of the coffees, the fractions with the higher amount of material were Et50 and WIppt. Most of the polysaccharides solubilized occurred in fractions Et50. These fractions were composed mainly of Man-rich polymers (82−90 mol %) and Gal (8−12 mol %); the relative amount of Ara (1−4 mol %), Glc (1 mol %), and Rha (0−2 mol %) residues was not significant. This sugar composition allows the inference that most of the polysaccharides present were galactomannans. The relative abundance of Man, Gal, and Glc residues in Et50 fractions and their variation with DR was very similar to that obtained by Thaler (*24*) for the copper-precipitated polysaccharides obtained from the aqueous extracts of roasted coffee. In WIppt fractions, the most abundant monosaccharide residue was also Man (50−90 mol %); but some fractions also showed significant amounts of Gal (6−23 mol %) and Glc (2−25 mol %) residues. Ara and Rha residues were found in very low relative amounts (0−8 and 0−2 mol %, respectively). Fractions Et75 and EtSN were mainly composed of Gal-rich polymers, containing in addition a relatively high amount of Ara residues, mainly in the EtSN fraction. These two fractions contained also a relatively high amount of Man residues. The relative amount of Man in these fractions increased with the increase of DR, possibly resulting from the depolymerization of the galactomannans with the roasting. The occurrence of arabinogalactans and galactomannans with different solubility in the aqueous ethanol solutions showed a great heterogeneity in these polysaccharide structures.

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

linkage	DR					
	Costa Rica			Brazil		
	5.0	8.7	10.0	4.7	8.7	9.9
T-Rha*p*	0.6	0.2	0.8	1.0	0.6	0.2
T-Ara*f*	2.0	1.1	1.6	2.6	1.2	0.9
5-Ara*f*	0.8	0.3	0.7	0.9	0.4	0.5
T-Man*p*	3.5	4.4	4.9	3.6	4.2	4.1
4-Man*p*	75.7	81.5	75.1	74.8	79.7	82.0
4,6-Man*p*	5.0	3.8	3.2	4.2	3.1	3.1
T-Gal*p*	5.4	4.1	3.7	4.4	4.0	4.4
3-Gal*p*	4.3	2.2	5.5	3.8	3.1	2.7
6-Gal*p*	0.8	0.9	0.9	1.3	1.0	0.4
3,6-Gal*p*	2.0	1.3	2.3	2.7	1.9	1.5
T-Glc*p*	nd[a]	nd	nd	nd	nd	nd
4-Glc*p*	0.6	0.5	1.1	0.5	0.7	0.3
6-Glc*p*	0.2	t[b]	0.3	0.1	0.2	t

[a] nd, not determined by gas chromatography-flame ionization detection, but detected by gas chromatography-mass spectrometry. [b] t = traces.

Because fraction Et50 contained most of the soluble galactomannans, it was submitted to methylation analysis and size-exclusion chromatography.

Influence of the DR in the Chemical Composition and Structure of the Et50 Fraction. 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, for the two coffees, the major glycosidic linkages found were (1→4)-Man*p* (75−82%), T-Man*p* (4−5%), (1→4,6)-Man*p* (3−5%), and T-Gal*p* (4−5%). Also present were (1→3)-Gal*p* (2−

Table 6. Chemical Composition of Et50 Fractions of Roasted Coffees

coffee	DR	yield[a]	polysaccharides[b,c]	protein[b,d]	phenolics[b,e]
Costa Rica	5.0	2.33	49.1 ± 0.6[f]	8.8 ± 0.4	12.2 ± 0.7
	8.7	2.25	50.3 ± 0.3	15.0 ± 0.9	15.5 ± 1.0
	10.0	2.56	42.4 ± 0.5	19.5 ± 0.7	18.5 ± 0.4
Brazil	4.7	2.49	46.5 ± 0.3	9.2 ± 0.2	15.1 ± 0.5
	8.7	2.04	44.0 ± 0.4	14.6 ± 0.7	19.8 ± 1.5
	10.0	2.52	44.7 ± 1.1	17.0 ± 0.5	20.1 ± 0.7

[a] As a coffee, percent of dry and defatted basis. [b] As percent of Et50. [c] As anhydrosugar. [d] As BSA equivalents by the Coomassie staining procedure. [e] As 3-CQA equivalents by the Prussian blue method. [f] Mean ± standard deviation.

6%) and (1→3,6)-Gal*p* (1−3%) residues and T-Ara*f* (1−3%) and (1→5)-Ara*f* (0.3−0.9%) from arabinogalactans. For the two coffees, and as observed for the HMWM galactomannans, the relative amount of T-Man*p* residues increased with the increase in DR; the exception was the last DR of BR coffee (**Table 5**, last column). The relative amount of (1→4,6)-Man*p* decreased from the first to the second DR for both coffees. The decrease was less pronounced from the second to the third DR.

The polysaccharides present in fraction Et50 represented only 42−50% of the material recovered (**Table 6**). The remaining material was protein and phenolic compounds. In an earlier work (*2*) a coelution of the polysaccharides, protein, phenolic compounds, and brown and UV-absorbing compounds (280 nm) was shown for the roasted CR and BR coffees. For all the DRs studied for both coffees, a coelution of the Et50 components also took place (results not shown). The mean molecular weight of the Et50 material of CR coffee decreased with the increase of DR: 120, 91, and 79 kDa, respectively, for light, medium, and highest DR. The mean molecular weight of the Et50 material from BR coffee showed a decrease from the first to second DR (140−60 kDa); however, for the highest DR, the mean molecular weight determined increased (91 kDa). With the increase of the DR, an increase in the amount of phenolic compounds retained in the Et50 fraction was also observed for both coffees. Size-exclusion chromatography on Sephadex G-25, with strong hydrogen bond (Ureia 7 M) and hydrophobic dissociation (SDS 1%) conditions, allowed us to conclude that the brown and phenolic compounds were covalently linked to the polymeric material present in the Et50 fraction for all the DRs for both coffees (results not shown), as described previously (*2*).

Although close agreement exists between the sugar composition obtained from the sugar analysis and the sugar composition from the methylation analysis, the use of the molar percentage of T-Man*p* residues for the determination of the degree of polymerization (*DP*) of the galactomannans showed *DP*s between 17 and 24. These *DP* figures are very low, not in accordance with the results of molecular weights obtained by size-exclusion chromatography and the retention of these polysaccharides in the dialysis bags. To be sure that the results of methylation were accurate, each sample was analyzed five times, with solubilizations of different lengths (3 h to 2 days), periods of NaOH activation of different lengths (3−6 h), and periods of CH_3I methylation of different lengths (2−12 h). In a last attempt, a remethylation was conducted. Nevertheless, these values were in accordance with the results obtained for the Man-rich polymers isolated from instant coffees (*25*, *26*) that were reported to have *DP*s between 13 and 45. Recently, a work done on green coffee polysaccharide fractions showed similar *DP* values obtained by methylation analysis (*27*).

General Discussion. The structure of the HMWM polysaccharides extracted from the arabica-roasted coffees depends on the DR to which the coffee has been subjected. Although no clear pattern was obtained for the variation of the amount of galactomannans in the HMWM extracted from the roasted coffees, the maximum amount of extractable galactomannans, in the range of DR studied, was about the same for both coffees (1.6 g/100 g). Half of the galactomannans recovered were insoluble in 50% ethanol solutions. The degree of polymerization (DP) of the galactomannans decreased with the increase of the DR. This was shown by methylation analysis and by size-exclusion chromatography. As the galactomannan solutions became less viscous with the decrease of the DP (*28*, *29*), this structural change in the galactomannans could be important to explain differences in the body and foam of the coffee infusions.

The degree of branching of the HMWM galactomannans, for all roasted coffees studied, was lower than that obtained for the green coffees (*2*). No trend was observed for the variation of the degree of branching with DR but, for the galactomannans recovered in Et50 fraction, it was observed a decrease in the degree of branching with increasing DR. The degree of branching of the galactomannans have been associated with the increase of their solubility properties in aqueous solutions (*28*, *29*).

As the DR increased, fewer branched arabinogalactans were extracted. A change in the terminal residues found in the arabinogalactans was also observed, because a decrease in the terminally linked arabinosyl residues occurred with an increase in the terminally linked galactosyl residues. Also the size of the arabinosyl side chains of arabinogalactans decreased with increasing DR.

The size-exclusion fractionation of Et50 fraction of all coffees showed a coelution of polysaccharides, proteins, phenolic, and brown compounds. The use of strong hydrogen bond and hydrophobic dissociation conditions allowed us to conclude that the phenolic compounds and brown compounds present were covalently linked to the polymeric material.

ABBREVIATIONS USED

Ara, arabinose; 5-Ara*f*, 1→5 linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; BR, Brazil; CR, Costa Rica; 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; 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; 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; T-Rha*p*, terminally linked rhamnopyranosyl residues; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; HexA, hexuronic acids; WIppt, water-insoluble precipitate.

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