Melanoidins from Coffee Infusions. Fractionation, Chemical Characterization, and Effect of the Degree of Roast

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A method involving fractionation in ethanol aqueous solutions, anion exchange chromatography, and immobilized copper chelating chromatography was developed to obtain high molecular weight anionic melanoidin populations from coffee infusions. Six anionic fractions with different physicochemical properties (ethanol solubility and chelating ability) and chemical composition regarding carbohydrate as well as protein nature and content were isolated. Fractions with similar chemical composition were obtained for light-, medium-, and dark-roasted coffee infusions. These melanoidin fractions accounted for 30−33% of the cold-water soluble high molecular weight material, independently of the degree of roast in coffee. The nature and abundance of the different polysaccharides in each fraction were dependent on their ethanol solubility. The 50% ethanol insoluble melanoidin populations contained mostly galactomannan-like carbohydrates, and the fractions obtained with 75% ethanol contained mostly arabinogalactan-like carbohydrates. The melanoidin populations with chelating properties presented significantly lower carbohydrate content and, from these, the 75% ethanol soluble fractions were almost devoid of carbohydrate material. The results obtained suggest that the chelating ability of these coffee melanoidins is modulated by their carbohydrates.

KEYWORDS: Coffee infusions; roasting; melanoidins; Maillard reaction; phenolic; polysaccharides; protein, CML; CEL; furosine

INTRODUCTION

Coffee infusions are one of the main sources of melanoidin intake in human nutrition, accounting for up to 25% of the total solids (*1*). Melanoidins have been shown to present not only nutritional and health benefits but also risks (*2−4*). These are brown in color and composed of a heterogeneous macromolecular material containing nitrogen (*1−5*); their structural features, even according to model systems, are still uncertain (*6−9*).

The formation of coffee melanoidins during roasting has been linked to the diversity of chemical components present in green coffee beans: sucrose (after inversion), polysaccharides (galactomannans and arabinogalactans), amino acids, proteins (11S storage and cell wall proteins), and chlorogenic acids. All of these can increase the chemical complexity and heterogeneity of coffee melanoidins. Furthermore, the nature of melanoidins of coffee infusions is also dependent on their water solubility and extractability. There is increasing evidence that polysaccharides (*10*, *11*), proteins (*12*, *13*), and phenolic compounds (*14−18*) are components of coffee infusion melanoidins. However, the presence of phenolic compounds chemically linked to coffee melanoidins has always been questioned (*19*).

Recently, it was demonstrated that phenolic compounds can be non-covalently linked to coffee melanoidins (*20*, *21*). From the information obtained, and despite the use of crude fractionation procedures, different melanoidin populations with dissimilar structural, physical, and biological properties are expected to be present in coffee infusions (*22−25*). The high amount of melanoidins in thermally processed foods and their relevance already demonstrated for their biological properties require a deep knowledge of their chemical structure. To achieve that, targeted fractionation procedures need to be developed.

The purpose of this work was to exploit the anionic nature of coffee melanoidins (*26*, *27*) and their metal chelating capacity (*28−30*) in the development of a fractionation procedure suitable for allowing the knowledge of (1) the chemical and structural features of coffee infusion melanoidins, (2) their heterogeneity, and (3) the influence of the degree of roast in the abundance and structure of the melanoidins present in roasted coffee infusions.

MATERIALS AND METHODS

Reagents. Glucose, mannose, galactose, arabinose, ribose, xylose, rhamnose 2-deoxyglucose, fucose, ferulic acid, caffeic acid, 3,4-dihydroxybenzoic acid, benzoic acid, catechol, and vanillic acid were from Merck (Darmstadt, Germany). Alanine, glycine, valine, serine, threonine, leucine, isoleucine, norleucine, proline, 4-hydroxyproline, methionine, aspartic acid, phenylalanine, glutamic acid, lysine, tyrosine,

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Scheme 1.   Fractionation of the HMWM of Coffee Infusions
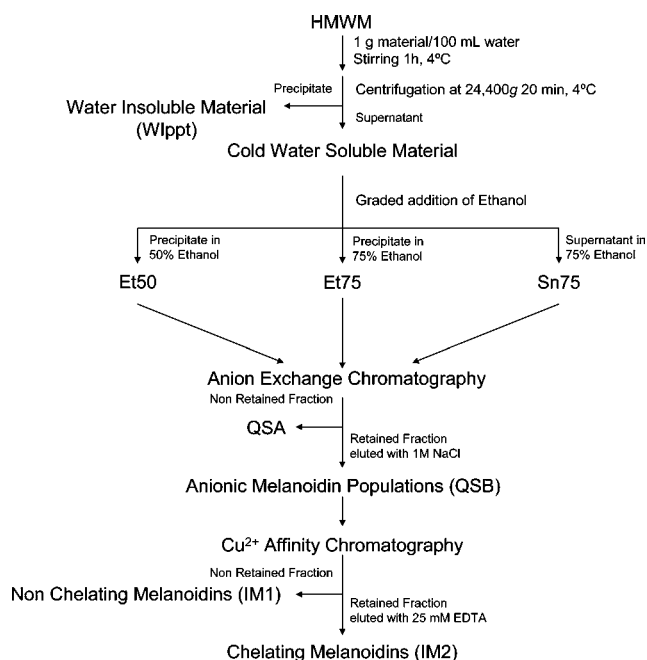
histidine, arginine, gallic acid, 3,5-dihydroxybenzoic acid, 4-hydroxy-benzoic acid, glucuronic acid, and galacturonic acid were from Sigma-Aldrich (St. Louis, MO). Q-Sepharose Fast Flow and Iminodiacetic Acid Immobilized Sepharose FF were from Amersham Biosciences (Uppsala, Sweden). Furosine was from Neosystem Laboratoire (Strasbourg, France). Other reagents were of analytical grade or higher available purity.

Samples and General Procedures. Coffee infusions were prepared from Arabica Brazil coffee (dry processed) from three degrees of roast: a light roast, with 5.0% dry matter loss on a dry basis in relation to the green coffee weight (DR 5%); a medium roast, with 8.7% dry matter loss (DR 8%); and a dark roast, with 10% dry matter loss (DR 10%). With constant stirring, 50 g of each ground and defatted coffee was 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 high molecular weight material (HMWM).

Sugars were determined by gas chromatography as alditol acetates (GC-FID) after hydrolysis for 2.5 h with 1 M sulfuric acid at 100 °C or by hydrolysis for 1 h with 2 M TFA 2 M at 120 °C. Uronic acids were determined colorimetrically according to a modification (*31*) of the method of Blumenkrantz and Asboe-Hansen (*32*). Methylation analysis was performed as described previously (*22−24*).

Protein content was determined after acid hydrolysis with 6 M HCl during 24 h. After removal of the acid by centrifugal evaporation (Univapo 100 ECH, UniEquip, Munich, Germany) at 40 °C under vacuum, the solid residue was dissolved in 3 mL of 0.1 M HCl and filtered through a 45 μm membrane. Amino acids were determined by GC-FID after derivatization as heptafluorobutylisobutyl derivatives (*33*).

Color dilution (CD) analysis of the HMWM and melanoidin fractions was performed according to the method described by Hofmann (*34*). The color contribution of melanoidin populations to the overall HMWM color was calculated by multiplying the CD factor of the sample by the percentage of material recovered from the HMWM and then dividing by the CD factor of the HMWM.

Fractionation Scheme of the HMWM. The fractionation procedure of the HMWM is presented in **Scheme 1**. Briefly, 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 (Sn75). To remove the ethanol completely, each precipitate was dissolved in water, concentrated by rotary evaporation at 40 °C, and then freeze-dried (*22−24*). Due to its insolubility in cold water, the WIppt fractions were not further fractionated. Fractions Et50, Et75, and Sn75 were fractionated by anion exchange chromatography on a Q-Sepharose FF stationary phase. The eluent was a pH 6.5 100 mM sodium phosphate buffer containing 3 M urea and 0.02% sodium azide. Fractions (150 mg) were dissolved in the initial buffer (1 mg/mL), applied to the column, and eluted at a flow rate of 0.20 mL/min with a minimum of 4 column volumes of the initial buffer or until the absorbance at 280 nm reached the initial level, yielding a nonretained fraction (QSA). The retained material (QSB fraction) was eluted with buffer containing 3 M urea and 1 M NaCl. Fractions (2 mL) were collected and assayed for sugars according to the phenol−sulfuric acid method, and the column eluent was continuously monitored at 280 nm. The polysaccharide and brown material-rich fractions were pooled, concentrated on a rotary evaporator (40 °C), dialyzed (12−14 kDa cutoff, 5 water renewals), and freeze-dried. The anionic fractions (QSB) were further fractionated by immobilized copper affinity chromatography on an iminodiacetic acid immobilized Sepharose FF (Sigma) medium loaded with copper ions. Before each run, the column was reloaded with copper ions and washed with the equilibration buffer. The material retained in the anion exchange chromatography was dissolved in the initial buffer (100−120 mL) to give an approximate concentration of 0.5 mg/mL and eluted at a flow rate of 0.20 mL/min with a minimum of 4 column volumes of the initial buffer or until the absorbance at 280 nm reached the initial level, yielding a nonretained fraction (IM1). The retained material (IM2 fraction) was selectively eluted with the initial buffer containing 25 mM EDTA, with a minimum of 4 column volumes. Fractions (2 mL) were assayed for sugars with the phenol−sulfuric acid method and continuously monitored for absorbance at 280 nm. The polysaccharide and brown material-rich fractions were pooled, dialyzed (12 kDa cutoff, 5 water renewals), concentrated on a rotary evaporator (40 °C) to approximately 10 mL, dialyzed again (12 kDa cutoff, 5 water renewals), and freeze-dried.

Analysis of Furosine, CML, and CEL. The quantification of furosine, CML, and CEL of the HMWM and melanoidin fractions was performed as heptafluorobutyl isobutyl derivatives by gas chromatography−quadrupole mass spectrometry (GC-MS, Agilent) after hydrolysis by 7.8 M HCl for 24 h at 110 °C, under nitrogen (*35*). Selected ion monitoring (SIM) was used for quantification. The ions at *m/z* 110, 621, and 578 were used, respectively, for quantification of furosine, CML, and CEL. For qualification, additional ions at *m/z* 280 and 240, *m/z* 565 and 519, and *m/z* 533 and 511, respectively, were used. Quantification was performed by a calibration curve using the lysine residues as internal standard. The MS was operated in the electron impact mode with an electron impact energy of 70 eV and data collected at a rate of 1 scan s^{-1}. The ion source was kept at 180 °C and the transfer line at 280 °C. CML and CEL, used as reference, were prepared by reductive amination of glyoxylic acid and pyruvate (*36*), respectively, with N^α-formyllysine (*37*) in the presence of NaCNBH$_3$ at room temperature. The purity of the products obtained was confirmed by GC-MS analysis after derivatization, the heptafluorobutyl isobutyl derivatives being the only products detected.

Alkaline Fusion. In a nickel crucible, 1 g of NaOH (s) and 100 mg of zinc dust were weighed, and, after fusion of the mixture at 350 °C (*38*), 10 mg of HMWM was also added (or 1 mg of melanoidin fractions). After 10 s, the nickel crucible was removed and rapidly cooled on ice. The fusion cake was solubilized by adding 6 M HCl, and 200 μL of internal standard solution was added (3,4-dimethoxy-benzoic acid, 1 mg/mL) and acidified with 6 M HCl to pH 1−2. The acidic mixture was extracted four times with 30 mL of ethyl ether. After evaporation of the organic solvent under vacuum, the residue was derivatized and analyzed by GC-EI/MS. Samples were derivatized with 500 μL of pyridine and 500 μL of *N,O*-bis(trimethylsilyl)-

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Table 1. Yield and Sugar and Amino Acid Compositions of Melanoidin Populations Obtained by Fractionation of the HMWM from Brazilian Roasted Coffee with DR 5%

	Et50			Et75			Sn75		
	QSB	IM1	IM2	QSB	IM1	IM2	QSB	IM1	IM2
yield[a] (%)	29	45	41	51	49	31	51	46	57
% HMWM[b]		3.6	3.3		4.3	2.7		4.9	6.1
sugars[c]									
Rha	1.44 (0.35)	4.95 (0.89)	1.41 (0.55)	1.93 (0.26)	3.93 (0.95)	1.36 (0.38)	1.50 (0.13)	3.67 (0.65)	0.42 (0.17)
Ara	3.09 (0.16)	5.65 (0.59)	2.48 (0.54)	6.62 (0.44)	7.91 (1.12)	3.48 (0.55)	5.02 (0.03)	7.35 (0.49)	1.17 (0.24)
Man	15.5 (0.5)	22.7 (0.8)	14.4 (0.9)	1.18 (0.05)	2.14 (0.24)	1.23 (0.17)	0.47 (0.13)	1.23 (0.33)	0.45 (0.07)
Gal	12.6 (0.9)	15.5 (0.9)	7.52 (0.54)	14.7 (0.3)	22.2 (0.7)	8.24 (0.30)	4.00 (0.42)	6.72 (0.59)	1.40 (0.42)
Glc	1.10 (0.17)	2.54 (0.56)	1.82 (0.17)	0.48 (0.02)	1.27 (0.75)	0.49 (0.10)	0.31 (0.06)	1.34 (0.34)	0.43 (0.18)
UA	Tr[e]	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**33.6 (2.1)**	**51.3 (3.7)**	**27.6 (2.4)**	**24.9 (1.6)**	**37.4 (2.3)**	**14.8 (1.5)**	**11.3 (0.5)**	**20.3 (1.2)**	**3.87 (0.58)**
amino acids[d]									
Ala	0.35 (0.03)	0.35 (0.03)	0.72 (0.02)	0.44 (0.07)	0.35 (0.03)	0.57 (0.04)	0.98 (0.01)	2.33 (0.17)	0.50 (0.02)
Gly	0.54 (0.06)	0.46 (0.02)	0.69 (0.01)	0.58 (0.07)	0.49 (0.03)	0.95 (0.02)	1.20 (0.01)	1.31 (0.08)	0.60 (0.02)
Val	0.30 (0.03)	0.38 (0.04)	0.41 (0.02)	0.29 (0.04)	0.37 (0.03)	0.53 (0.04)	0.81 (0.03)	1.42 (0.10)	0.43 (0.03)
Thr	0.22 (0.01)	0.18 (0.02)	0.24 (0.02)	0.13 (0.02)	0.18 (0.01)	0.30 (0.02)	0.35 (0.00)	0.74 (0.09)	0.26 (0.05)
Ser	0.27 (0.00)	0.23 (0.02)	0.62 (0.02)	0.49 (0.06)	0.28 (0.03)	0.52 (0.02)	0.56 (0.02)	0.83 (0.08)	0.59 (0.02)
Leu	0.46 (0.04)	0.44 (0.03)	0.56 (0.01)	0.38 (0.04)	0.48 (0.04)	0.78 (0.04)	1.32 (0.06)	2.23 (0.14)	0.69 (0.00)
Ile	0.37 (0.04)	0.15 (0.02)	0.24 (0.01)	1.03 (0.02)	0.25 (0.01)	0.43 (0.02)	0.67 (0.09)	0.77 (0.03)	0.30 (0.01)
Pro	0.35 (0.02)	0.29 (0.01)	0.35 (0.01)	0.29 (0.01)	0.39 (0.03)	0.62 (0.01)	0.90 (0.04)	0.76 (0.02)	0.44 (0.02)
Hyp	0.29 (0.03)	0.27 (0.01)	0.17 (0.01)	0.26 (0.01)	0.32 (0.03)	0.36 (0.01)	0.15 (0.01)	0.22 (0.01)	0.12 (0.01)
Met	0.04 (0.00)	0.08 (0.04)	0.04 (0.00)		0.04 (0.02)	0.18 (0.05)			0.01 (0.01)
Asx	0.72 (0.05)	0.59 (0.04)	0.52 (0.01)	0.51 (0.05)	0.51 (0.05)	0.79 (0.03)	1.56 (0.04)	1.05 (0.09)	0.55 (0.05)
Phe	0.24 (0.02)	0.27 (0.01)	0.76 (0.02)	0.23 (0.02)	0.21 (0.03)	0.42 (0.03)	0.72 (0.05)	1.00 (0.04)	0.51 (0.02)
Glx	2.03 (0.20)	1.82 (0.13)	2.05 (0.11)	1.72 (0.18)	1.74 (0.14)	2.86 (0.17)	4.96 (0.38)	3.90 (0.20)	2.88 (0.19)
Lys	0.12 (0.01)	0.11 (0.01)	0.17 (0.01)	0.12 (0.01)	0.10 (0.01)	0.15 (0.01)	0.22 (0.03)	0.06 (0.01)	0.13 (0.00)
Tyr	0.10 (0.00)	0.11 (0.02)	0.51 (0.03)	0.08 (0.03)	0.12 (0.02)	0.28 (0.02)	0.39 (0.04)	0.18 (0.02)	0.14 (0.00)
His	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**6.39 (0.54)**	**5.71 (0.41)**	**8.03 (0.29)**	**6.79 (0.59)**	**5.82 (0.47)**	**9.75 (0.52)**	**14.8 (0.8)**	**16.8 (1.1)**	**8.15 (0.42)**

[a] For QSB, percentages (w/w) in relation to the applied material; for IM1 and IM2, percentages (w/w) in relation to QSB fraction. [b] Percentages (w/w) in relation to the high molecular weight material (HMWM). [c] Percentage (w/w) of anhydrosugars in fraction; values in parentheses are the standard deviation. [d] Percentage (w/w) of anhydroamino acids in fraction; values in parentheses are the standard deviation. [e] Traces.

trifluoroacetamide and heated at 70 °C for 30 min (*39*). After cooling to room temperature, the silyl derivatives of phenolic compounds were analyzed by GC-MS (Agilent) and quantified by GC-FID (Perkin-Elmer) using a DB-1 column (30 m length, 0.23 mm internal diameter, and 0.2 μm film thickness) and injecting 1 μL in splitless mode (time of splitless = 0.75 min). Both the injector and the transfer line were set at 250 °C. The initial column temperature was 70 °C, held for 1 min, increasing at 5 °C/min until 250 °C. The MS source temperature was set at 180 °C and the electron ionization energy was set at 70 eV, with scans from *m/z* 40 to 600. Retention time and EI spectra of pure standards were used to identify phenolic compounds. Quantification was performed by the internal standard method using 3,4-dimethoxy-benzoic acid.

Alkaline Hydrolysis. After the addition of approximately 10 mg of HMWM and 5 mL of 2 M NaOH (previously purged with nitrogen), the reaction proceeded for 48 h in the dark at room temperature (*40*). At the end of the hydrolysis, 200 μL of internal standard solution was added (3,4-dimethoxybenzoic acid, 1 mg/mL), and the mixture was acidified with 6 M HCl to pH 1−2. The acidic mixture was extracted four times with 30 mL of ethyl ether. After evaporation of the organic solvent under vacuum, the residue was derivatized and analyzed by GC-EI/MS as described above. As no phenolic compounds could be detected by this method, the conditions were changed and the hydrolysis was carried out at 100 °C for 12 h, and the samples, after cooling, were treated in the same way as above.

Pyrolysis−Gas Chromatography−Mass Spectrometry. Analytical pyrolysis was performed on duplicates (0.4 mg of sample) using a CDS Analytical Pyroprobe 1000 pyrolyser interfaced to a Trace GC 2000 gas chromatograph and a mass selective detector Finnigan Trace MS. Pyrolysis was carried out at 550 °C for 5 s (reached at 1 °C/ms), and the Py-GC-MS interface was kept at 250 °C. Helium was used as gas carrier (35 cm s^{-1}), and a DB-5 J&W capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used (*41*). The chromatographic conditions were as follows: initial temperature, 45 °C (4 min); heating at 3 °C/min to 213 °C and then heating at 30 °C/min to 300 °C (5

min); injector temperature, 250 °C; transfer line temperature, 300 °C. The MS was operated in the electron impact mode with an electron impact energy of 70 eV, and data were collected at a rate of 1 scan s^{-1} over a range of *m/z* 33−800. The ion source was kept at 200 °C.

Analysis of the Non-covalently Linked Phenolic Compounds. The method of Delgado-Andrade and Morales (*20*) was used to assess the presence of ionically bound phenolic compounds in the high molecular weight material. Solutions containing 10 mg/mL of the high molecular weight material in 2 M NaCl were prepared and incubated overnight. These solutions were ultrafiltered (Microcon YM-10, regenerated cellulose 10 kDa) at 14000*g* for 50 min. The ultrafiltered solutions were analyzed by injection of 30 μL by reverse phase (C$_{18}$) high-performance liquid chromatography (HPLC) coupled to a diode array detector (Thermo-Finnigan Surveyor HPLC system) set to collect data from 200 to 600 nm overall; data for phenolics extracted at 270 nm were from total scan data (*42*). Solvent A was 0.1% v/v trifluoroacetic acid in ultrapure water, and solvent B was 0.1% v/v trifluoroacetic acid in HPLC grade acetonitrile; flow rate was 1 mL/min. Peak identifications were performed from retention time, UV spectral data, and direct comparison to pure standards.

Statistical Analysis. All chemical analyses obtained for each melanoidin population were analyzed by one-way ANOVA for significant differences ($p = 0.05$), using the degree of roast as the main effect. Scheffe post hoc test was performed for detecting significantly different means ($p = 0.05$).

RESULTS AND DISCUSSION

Fractionation of the High Molecular Weight Material from Coffee Infusions. The HMWM extracted from Brazilian roasted coffees with three different degrees of roast (DR) were obtained by extensive dialysis at 4 °C in the dark and sequential precipitation of the freeze-dried material in ethanol solutions, as previously described (*22, 23*). The three cold-water soluble

Table 2. Yield and Sugar and Amino Acid Compositions of Melanoidin Populations Obtained by Fractionation of the HMWM from Brazilian Roasted Coffee with DR 8%

	Et50			Et75			Sn75		
	QSB	IM1	IM2	QSB	IM1	IM2	QSB	IM1	IM2
yield[a] (%)	28	50	53	52	61	34	48	41	51
% HMWM[b]		3.1	3.3		5.1	2.8		4.2	5.3
sugars[c]									
Rha	0.80 (0.13)	2.36 (0.48)	0.51 (0.17)	0.97 (0.06)	1.97 (0.62)	1.12 (0.17)	0.30 (0.10)	1.45 (0.63)	0.18 (0.10)
Ara	2.50 (0.18)	3.97 (0.36)	2.38 (0.13)	4.10 (0.16)	5.14 (0.18)	3.17 (0.24)	2.15 (0.40)	3.48 (0.68)	1.01 (0.04)
Man	24.8 (2.9)	27.4 (2.9)	18.2 (1.2)	3.98 (0.33)	4.34 (0.31)	2.85 (0.34)	0.86 (0.09)	1.23 (0.16)	0.35 (0.17)
Gal	10.3 (1.9)	14.5 (0.5)	9.14 (0.10)	15.9 (0.8)	21.4 (0.6)	8.74 (0.21)	1.88 (0.06)	4.18 (0.25)	1.01 (0.07)
Glc	1.80 (0.04)	1.52 (0.40)	0.96 (0.11)	0.25 (0.02)	1.37 (0.51)	1.04 (0.06)	0.18 (0.03)	0.59 (0.13)	0.13 (0.06)
UA	Tr[e]	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**40.2 (5.2)**	**49.8 (1.24)**	**31.2 (1.7)**	**25.1 (0.5)**	**34.2 (1.6)**	**16.9 (1.0)**	**5.38 (0.07)**	**10.9 (1.3)**	**2.68 (0.33)**
amino acids[d]									
Ala	0.65 (0.00)	0.49 (0.01)	0.39 (0.03)	0.54 (0.00)	0.57 (0.02)	0.46 (0.02)	0.75 (0.05)	0.82 (0.04)	0.34 (0.01)
Gly	0.66 (0.02)	0.60 (0.02)	0.50 (0.02)	0.68 (0.02)	0.48 (0.02)	0.65 (0.01)	0.72 (0.02)	0.80 (0.05)	0.45 (0.02)
Val	0.23 (0.01)	0.47 (0.03)	0.34 (0.02)	0.41 (0.02)	0.47 (0.06)	0.43 (0.01)	0.53 (0.18)	0.79 (0.03)	0.33 (0.02)
Thr	0.30 (0.02)	0.13 (0.03)	0.07 (0.02)	0.08 (0.01)	0.20 (0.01)	0.07 (0.02)	0.11 (0.02)	0.24 (0.01)	0.12 (0.01)
Ser	2.17 (0.05)	0.50 (0.02)	0.10 (0.02)	0.21 (0.03)	0.22 (0.02)	0.10 (0.05)	0.20 (0.02)	0.22 (0.01)	0.37 (0.02)
Leu	0.38 (0.01)	0.53 (0.02)	0.60 (0.02)	0.50 (0.03)	0.63 (0.04)	0.61 (0.02)	0.96 (0.03)	1.45 (0.04)	0.60 (0.02)
Ile	0.34 (0.02)	0.30 (0.01)	1.27 (0.03)	0.82 (0.07)	0.33 (0.03)	0.29 (0.02)	0.43 (0.23)	0.82 (0.04)	0.36 (0.02)
Pro	0.33 (0.00)	0.25 (0.01)	0.33 (0.02)	0.40 (0.04)	0.37 (0.03)	0.41 (0.02)	0.59 (0.06)	0.79 (0.05)	0.32 (0.01)
Hyp	0.21 (0.01)	0.18 (0.01)	0.13 (0.01)	0.24 (0.00)	0.26 (0.02)	0.14 (0.01)	0.08 (0.02)	0.11 (0.01)	0.05 (0.04)
Met	0.06 (0.00)	0.03 (0.01)	0.01 (0.01)					0.11 (0.02)	0.02 (0.02)
Asx	0.59 (0.02)	0.47 (0.03)	0.38 (0.04)	0.97 (0.08)	0.64 (0.05)	0.58 (0.03)	1.23 (0.40)	1.25 (0.06)	0.61 (0.05)
Phe	0.22 (0.00)	0.30 (0.02)	0.41 (0.04)	0.28 (0.08)	0.28 (0.02)	0.35 (0.01)	0.52 (0.03)	0.70 (0.04)	0.40 (0.02)
Glx	1.75 (0.05)	1.84 (0.21)	1.90 (0.11)	1.95 (0.08)	1.61 (0.20)	2.15 (0.11)	2.58 (0.34)	3.98 (0.23)	1.73 (0.16)
Lys	0.15 (0.00)	0.09 (0.01)	0.12 (0.01)	0.12 (0.01)	0.29 (0.02)	0.18 (0.02)	0.11 (0.01)	0.34 (0.04)	0.13 (0.02)
Tyr	0.08 (0.00)	0.06 (0.02)	0.18 (0.02)	0.20 (0.05)	0.14 (0.01)	0.21 (0.01)	0.23 (0.04)	0.46 (0.05)	0.27 (0.02)
His	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**8.10 (0.67)**	**6.23 (0.40)**	**6.72 (0.37)**	**7.29 (0.30)**	**6.48 (0.53)**	**6.63 (0.36)**	**9.04 (0.41)**	**12.9 (0.72)**	**6.07 (0.36)**

[a] For QSB, percentages (w/w) in relation to the applied material; for IM1 and IM2, percentages (w/w) in relation to QSB fraction. [b] Percentages (w/w) in relation to the high molecular weight material (HMWM). [c] Percentage (w/w) of anhydrosugars in fraction; values in parentheses are the standard deviation. [d] Percentage (w/w) of anhydroamino acids in fraction; values in parentheses are the standard deviation. [e] Traces.

fractions were recovered from the precipitates in 50% (Et50) and 75% ethanol solutions (Et75) and from the supernatant in 75% ethanol (Sn75, **Scheme 1**). They were further fractionated by anion exchange chromatography on Q-Sepharose FF, allowing to be obtained, from each one of them, two fractions: a nonretained (QSA) and a retained fraction (QSB), recovered from the column by elution with 1 M NaCl (**Scheme 1**). For Et50, Et75, and Sn75, independent of the degree of roast of the coffees, 28−33, 51−52, and 48−51% of the applied material was, respectively, retained in the anion exchange column (**Tables 1−3**). The deep brown color of these QSB fractions allowed us to conclude that a significant number of the melanoidins present in these coffee infusions have an anionic character. The anionic character of melanoidins has been shown, by capillary electrophoresis, in both model studies (*27*) and roasted coffee high molecular weight melanoidins (*26*). The amount of carbohydrate material in QSB fractions ranged from 40 to 5% (**Tables 1−3**), decreasing with the increase of the solubility of the fractions in ethanol solutions (Et50 > Et75 > Sn75). The observation of anionically bound polysaccharides in these roasted fractions, which contrasted with the negligible retention observed for the green coffee polysaccharides under the same chromatographic conditions, allows us to conclude that the polysaccharides present in QSB fractions are linked to the retained melanoidins. This is reinforced by the fact that it is known that during the roasting process the uronic acid content of arabinogalactans decreases (*43*). In addition, the uronic acid analysis of QSA and QSB fractions showed only vestigial amounts of uronic acids.

The polysaccharides present in roasted coffee infusions are almost exclusively composed by galactomannans and arabinogalactans (*22−24*). The galactomannans are neutral linear polysaccharides composed by a backbone of β-(1→4)-linked D-mannose residues, which can be O-acetylated and substituted in O-6 by single galactose and arabinose residues (*44*). The arabinogalactans are highly branched anionic polymers composed by a backbone of β-(1→3)-linked D-galactose residues, highly substituted in O-6 by short chains of other β-(1→6)-linked D-galactose residues. These side-chain residues can also be substituted in O-3 by single L-arabinose or α-(1→5)-linked L-arabinose disaccharide residues (*22−24, 45*). Also present, although in much lower amounts, are terminally linked D-glucuronic acid residues. **Tables 1−3** show that the galactomannan-like carbohydrates were the main carbohydrates present in Et50 fractions, although for the light-roasted coffee also significant relative amounts of arabinogalactan-like carbohydrates were present. For Et75QSB and Sn75QSB fractions the arabinogalactan-like carbohydrates were the main carbohydrate components, although the relative amount of galactomannan-like carbohydrates increased with the increase of the degree of roast.

It has been shown that, during roasting, proteins change considerably (*13, 22*) by acquiring negative charge density (*13*). To assess the importance of the protein material in these anionic melanoidin fractions, amino acid analysis was performed, and the results are shown in **Tables 1−3** for DR5, DR8, and DR10, respectively. The Et50 and Et75 retained fractions had higher contents of proteic material than the nonretained fractions (Et50QSA, 3.2−3.3%, vs Et50QSB, 6.4−8.6%, and Et75QSA, 3.8−5.9%, vs Et75QSB, 6.8−8.0%), whereas for Sn75 fractions the opposite was observed, as the protein content in the Sn75QSA fraction was higher (11.5−19.9%) than in Sn75QSB (9.0−14.8%). Although there were differences found in the amount of proteic material when the retained fractions were

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Table 3. Yield and Sugar and Amino Acid Compositions of Melanoidin Populations Obtained by Fractionation of the HMWM from Brazilian Roasted Coffee with DR 10%

	Et50			Et75			Sn75		
	QSB	IM1	IM2	QSB	IM1	IM2	QSB	IM1	IM2
yield[a] (%)	33	42	57	51	45	50	48	60	30
% HMWM[b]		3.9	5.3		3.5	3.9		5.4	2.7
sugars[c]									
Rha	0.54 (0.24)	1.86 (0.48)	0.38 (0.16)	0.49 (0.05)	2.08 (0.11)	0.30 (0.14)	0.31 (0.01)	1.12 (0.13)	0.20 (0.10)
Ara	1.21 (0.81)	2.66 (0.24)	1.60 (0.36)	2.94 (0.06)	4.46 (0.65)	2.28 (0.16)	1.68 (0.08)	3.15 (0.08)	1.13 (0.14)
Man	16.6 (0.5)	15.5 (0.8)	15.4 (0.81)	3.72 (0.26)	5.44 (0.79)	3.43 (0.61)	0.63 (0.02)	1.10 (0.17)	0.29 (0.11)
Gal	5.58 (0.55)	8.73 (0.71)	4.29 (0.45)	13.0 (1.3)	18.8 (1.1)	7.81 (0.27)	2.57 (0.01)	1.90 (0.30)	0.78 (0.11)
Glc	0.93 (0.23)	1.35 (0.21)	0.88 (0.14)	0.77 (0.03)		0.15 (0.07)	0.51 (0.01)	0.91 (0.06)	1.37 (0.28)
UA	Tr[e]	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**24.8 (2.3)**	**30.1 (2.0)**	**22.6 (0.7)**	**20.9 (1.1)**	**30.7 (0.8)**	**14.0 (0.7)**	**5.71 (0.30)**	**8.17 (0.62)**	**3.77 (0.75)**
amino acids[d]									
Ala	0.53 (0.03)	0.51 (0.04)	0.60 (0.02)	0.50 (0.00)	0.40 (0.02)	0.37 (0.02)	0.92 (0.07)	0.73 (0.05)	0.46 (0.04)
Gly	0.83 (0.08)	0.66 (0.04)	0.54 (0.02)	0.58 (0.01)	0.49 (0.02)	0.51 (0.03)	0.83 (0.06)	0.81 (0.06)	0.58 (0.05)
Val	0.37 (0.02)	0.44 (0.03)	0.48 (0.02)	0.41 (0.00)	0.41 (0.02)	0.38 (0.07)	0.68 (0.07)	0.80 (0.01)	0.46 (0.07)
Thr	0.22 (0.02)	0.10 (0.01)	0.09 (0.01)	0.07 (0.00)	0.07 (0.04)	0.05 (0.04)	0.09 (0.01)	0.12 (0.01)	0.10 (0.03)
Ser	1.20 (0.08)	0.12 (0.01)	0.15 (0.01)	0.30 (0.00)	0.06 (0.02)	0.08 (0.03)	0.31 (0.01)	0.11 (0.03)	0.24 (0.04)
Leu	0.61 (0.03)	0.83 (0.04)	0.76 (0.03)	0.53 (0.00)	0.60 (0.02)	0.60 (0.00)	0.97 (0.05)	1.30 (0.07)	0.77 (0.04)
Ile	0.39 (0.02)	0.45 (0.01)	0.36 (0.02)	1.47 (0.17)	0.37 (0.02)	0.33 (0.03)	0.48 (0.03)	0.55(0.02)	0.39 (0.05)
Pro	0.48 (0.02)	0.49 (0.03)	0.42 (0.04)	0.38 (0.02)	0.48 (0.02)	0.39 (0.04)	0.58 (0.04)	0.74 (0.07)	0.45 (0.03)
Hyp	0.20 (0.02)	0.16 (0.01)	0.19 (0.02)	0.15 (0.01)	0.28 (0.03)	0.13 (0.01)	0.11 (0.00)	0.11 (0.01)	0.09 (0.01)
Met	0.07 (0.00)								
Asx	0.80 (0.02)	0.89 (0.02)	0.50 (0.04)	0.97 (0.01)	0.26 (0.01)	0.57 (0.05)	1.10 (0.02)	0.94 (0.04)	0.51 (0.05)
Phe	0.37 (0.02)	0.45 (0.02)	0.43 (0.02)	0.37 (0.01)	0.14 (0.01)	0.44 (0.09)	0.56 (0.02)	0.64 (0.02)	0.51 (0.03)
Glx	2.30 (0.13)	2.85 (0.20)	1.84 (0.07)	1.92 (0.05)	2.05 (0.13)	1.62 (0.09)	2.72 (0.03)	3.33 (0.19)	1.84 (0.13)
Lys	0.15 (0.01)	0.23 (0.01)	0.10 (0.09)	0.14 (0.01)	0.17 (0.01)	0.09 (0.00)	0.16 (0.01)	0.22 (0.02)	0.14 (0.01)
Tyr	0.12 (0.01)	0.14 (0.00)	0.18 (0.02)	0.16 (0.01)	0.08 (0.01)	0.19 (0.01)	0.31 (0.05)	0.33 (0.01)	0.34 (0.04)
His	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
total	**8.61 (0.50)**	**8.31 (0.41)**	**6.65 (0.32)**	**7.95 (0.27)**	**5.83 (0.40)**	**5.76 (0.39)**	**9.81 (0.39)**	**10.7 (0.6)**	**6.97 (0.61)**

[a] For QSB, percentages (w/w) in relation to the applied material; for IM1 and IM2, percentages (w/w) in relation to QSB fraction. [b] Percentages (w/w) in relation to the high molecular weight material (HMWM). [c] Percentage (w/w) of anhydrosugars in fraction; values in parentheses are the standard deviation. [d] Percentage (w/w) of anhydroamino acids in fraction; values in parentheses are the standard deviation. [e] Traces.

compared with the nonretained fractions, the amino acid relative abundances were similar (results not shown). The most abundant amino acids were Glx, Gly, Ala, Asx, Ile, Leu, and Pro, whereas the least abundant were the hydrophilic ones, that is, Met, Lys, and Tyr. Arg residues were not found, and only vestigial amounts of His were present. Also encountered in relatively high amounts was hydroxyproline (Hyp), an amino acid residue found in the arabinogalactan proteins isolated from green coffee cell walls. The presence of Hyp, as well as galactose and arabinose residues, allows us to conclude that arabinogalactan-protein-like structures were retained in QSB fractions. The composition in amino acids of the retained fractions were in accordance with the values found for roasted coffees (25, 46, 47) and for roasted coffee infusions (25).

Fractionation and Chemical Characterization of Melanoidin Populations. The metal chelating ability of melanoidins from model systems (28, 30) and coffee (17, 29) are well documented. This property was exploited to further fractionation of the anionic melanoidin fractions (QSB fractions) by developing a methodology based on Cu(II) affinity chromatography. As these fractions were negatively charged, 1 M NaCl was always included in the eluent to suppress the electrostatic interactions with the stationary phase. All anionic melanoidin fractions (QSB) yielded a nonretained (IM1) fraction and a fraction that was retained and eluted with the buffer containing 25 mM EDTA (IM2) (**Figure 1**). The relative amount of polymeric material and content in carbohydrate as well as the amino acids present in these fractions are shown in **Tables 1−3**. The carbohydrate content of the nonchelating fractions was significantly higher than that of chelating fractions for all fractions yield and degrees of roast. Fractions Et50IM1 from DR5 and DR8 contained the highest levels of carbohydrates, whereas

fraction Sn75IM2 contained the lowest levels. To characterize the glycosidic linkage composition of the carbohydrate material present in the different nonchelating and chelating melanoidin populations and the effect of the degree of roast in their structural features, methylation analysis was performed (**Tables 4−6**).

Et50. For Et50IM1 and Et50IM2 melanoidin populations as well as for the three degrees of roast, the high percentages of (1→4)- and (1→4,6)-linked Man residues (**Table 4**) allowed us to confirm that the carbohydrates present were structurally related to the galactomannans. These polymers had a degree of branching (% 4,6-Man$p$/ManTotal, **Table 4**) in the same range found for the galactomannans present in the HMWM (22, 23). The presence in samples from all degrees of roast of (1→3)- and (1→3,6)-linked Gal residues and terminally and (1→5)-linked arabinose residues (**Table 4**), which are structural elements of type II arabinogalactans (22−24), allowed us to confirm that the arabinogalactan-like carbohydrates were also components of these melanoidin populations. The structural features of the arabinogalactan-like carbohydrates found in these melanoidin populations (**Table 4**) were in accordance with the changes observed for the polysaccharides present in the HMWM of coffee infusions (22−24) and whole coffee polysaccharides (48). Thus, these structural changes can be attributed to roasting-induced changes of the polysaccharides present in coffee beans.

The amount of galactomannan-like and arabinogalactan-like carbohydrates present in the melanoidin populations was estimated on the basis of their sugar composition and methylation analysis (**Figure 2**). These estimates were performed by taking into account not only that all mannose residues present in the melanoidin populations were components of galactomannan-like carbohydrates but also that an amount of galactose

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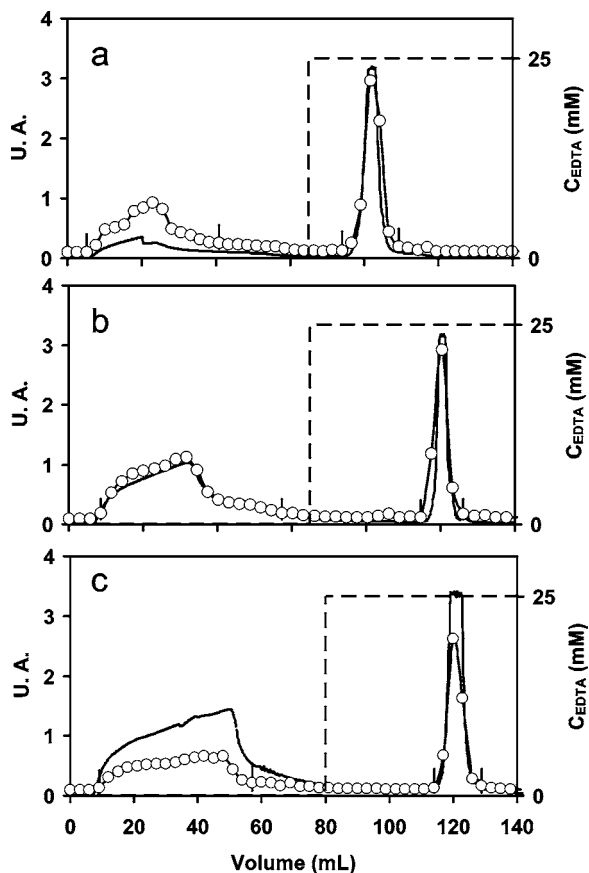
Figure 1. Chromatographic profile on Cu(II) metal affinity chromatography of anionic melanoidin populations (QSB) of Et50 for DR 8% (**a**), Et75 for DR 10% (**b**), and Sn75 for DR 5% (**c**): (○) sugars (absorbance at 490 nm); (−) absorbance at 280 nm.

residues equal to the amount of (1→4,6)-Man*p* was a component of galactomannan-like carbohydrates. Furthermore, all arabinose and galactose residues, except the T-Gal*p* previously attributed to the galactomannan-like carbohydrates, were assumed to be component sugars of the arabinogalactan-like carbohydrates (*23, 24*). As shown in **Figure 2**, for Et50 anionic melanoidin populations, the amount of both galactomannan-like and arabinogalactan-like carbohydrates decreased with the increase of the degree of roast. The content of protein-like material of the Et50IM1 fraction increased for the dark-roasted coffee, whereas the reverse was observed for the Et50IM2 (**Figure 2**).

Et75. The melanoidins recovered in fractions Et75IM1 and Et75IM2 had a different carbohydrate profile from those recovered in fractions Et50IM1 and Et50IM2 (**Table 5** and **Figure 2**). The arabinogalactan-like carbohydrates were the main carbohydrates present in both fractions (>70%). The amount of arabinogalactan-like carbohydrates was approximately the same for the different degrees of roast, even though the amount of galactomannan-like carbohydrates increased with increasing degree of roast (**Figure 2**). The structural features and changes conferred by the roast on these arabinogalactan-like carbohydrates (**Table 5**) were very similar to those recovered in the Et50IM1 and Et50IM2 fractions (**Table 4**).

The amount of protein-like material for Et75IM1 fractions remained approximately constant with the increase of the degree of roast, whereas for the melanoidin population recovered in fraction Et75IM2, the content of protein-like material decreased with the increase of the degree of roast (**Tables 1−3**).

Sn75. The two melanoidin populations recovered from Sn75 were both very low in carbohydrates, the chelating fraction

(Sn75IM2) being the poorest one (**Tables 1−3**). The carbohydrate content of the nonchelating fractions (Sn75IM1) decreased significantly with the increase in the degree of roast from the light to the medium and dark degrees of roast (**Figure 2**). Because the amount of galactomannan-like carbohydrates remained constant, this can be attributed to the decrease in arabinogalactan-like carbohydrates. The structural features of the arabinogalactan-like carbohydrates recovered in these fractions (**Table 6**) were substantially different from those recovered in Et50 and Et75 melanoidin populations. These fractions had similar 3-/3,6-Gal ratios but higher Ara/Gal and lower T-Ara/5-Ara ratios, allowing us to infer the occurrence of a higher abundance of arabinose disaccharide residues in the arabinogalactan-like carbohydrate side chains. Nevertheless, the structural changes due to the increase of degree of roast observed for the arabinogalactan-like carbohydrates recovered in Et50 and Et75 melanoidin populations were also observed for the arabinogalactan-like carbohydrates recovered in fractions Sn75.

The melanoidins of the Sn75 fractions were those that presented the higher amount of protein-like material of the entire melanoidin fractions recovered. Furthermore, an increase in the degree of roast led to a decrease in the amount of protein-like material recovered in these fractions (**Figure 2**).

The chelating melanoidin population recovered from the Sn75 fraction (Sn75IM2) presented the lower carbohydrate and protein contents of all recovered fractions. The similarity between the physicochemical properties of the Sn75IM2 found in this study, at all degrees of roast, and the fractions isolated from instant coffe by zinc precipitation by Takenaka et al. (*17*) is remarkable. The amount and molar abundances of the different sugars and amino acids were almost identical, although the amount of protein obtained by zinc precipitation was lower.

Color Dilution Analysis. To estimate the importance of these six melanoidin populations to the total color of the HMWM, the color potency of the HMWM and melanoidin populations was determined by color dilution analysis (*34*). As can be observed in **Table 7**, the color potency of the HMWM increased with the increase of the degree of roast, which was in accordance with the color presented by the HMWM lyophilized powders. The color potency of the six different melanoidin populations also increased with the increase of the degree of roast, although not all with the same trend observed for the HMWM (**Table 7**). For the least roasted coffee, these six anionic melanoidin populations accounted for 65% of the color potency of the HMWM (**Table 7**). Nevertheless, for the highest degree of roast, although with an increase in color potency of all fractions, the same six anionic melanoidin populations accounted for only 39% of the color potency of the corresponding HMWM. These results allow us to conclude that, for the medium- and dark-roasted coffees, the nonretained fractions in the anion exchange column (QSA), together with the cold-water insoluble precipitates (WIppt), accounted for a significant amount of the color potency of the HMWM.

Quantification of Maillard Reaction Products in the HMWM and Melanoidin Populations. The presence of a significant amount of melanoidins with chelating ability prompts the investigation of the probable cause for this chelating capacity. In a first approach, the HMWM was characterized for the presence of compounds with chelating properties. It has been previously shown that nearly 17% of the nitrogen present in high molecular weight fractions (*25*) is present in a non-amino-acid form. It has also been shown that other non-amino-acid-nitrogen-containing compounds, derived from Maillard reaction, are present in coffee melanoidins (*49*). To ascertain the non-

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Table 4. Methylation Analysis (Molar Percentage) of Melanoidin Populations Obtained by Fractionation of the Et50QSB Fraction from Brazilian Roasted Coffee

	DR 5%		DR 8%		DR 10%	
	IM1	IM2	IM1	IM2	IM1	IM2
T-Rha$p$	2.0	2.0	1.7	0.7	1.1	0.7
total	**2.0 (10)[a]**	**2.0 (6)**	**1.7 (5)**	**0.7 (2)**	**1.1 (7)**	**0.7 (2)**
T-Ara$f$	7.2	6.2	4.4	5.4	5.0	4.3
T-Ara$p$	0.5	0.5				
5-Ara$f$	3.5	3.4	1.8	2.4	2.1	1.7
total	**11.2 (13)**	**10.1 (11)**	**6.2 (10)**	**7.8 (9)**	**7.1 (11)**	**6.0 (9)**
T-Man$p$	3.7	3.5	5.4	4.3	8.3	6.1
4-Man$p$	47.3	53.9	54.6	53.4	41.9	60.8
4,6-Man$p$	2.1	2.2	2.3	1.9	2.9	2.2
total	**53.1 (43)**	**59.6 (51)**	**62.3 (54)**	**59.6 (57)**	**53.1 (50)**	**69.1 (67)**
T-Gal$p$	9.0	7.2	8.7	10.0	11.6	9.2
6-Gal$p$	3.6	3.0	3.2	3.8	4.4	2.9
3-Gal$p$	10.3	7.0	9.4	10.2	10.6	6.9
3,6-Gal$p$	7.3	5.6	5.9	6.7	6.0	4.0
total	**30.2 (29)**	**22.8 (27)**	**27.2 (29)**	**30.7 (29)**	**32.6 (28)**	**23.0 (19)**
4-Glc$p$	3.6	5.5	2.7	1.3	6.1	1.1
total	**3.6 (5)**	**5.5 (6)**	**2.7 (3)**	**1.3 (3)**	**6.1 (4)**	**1.1 (4)**
% 4,6-/total Man$p$	4.0	3.7	3.7	3.2	5.5	3.2
Ara/Gal	0.40	0.49	0.25	0.27	0.24	0.29
T-/5-Ara$f$	2.1	1.8	2.4	2.3	2.4	2.5
T-Ara/3,6-Gal$p$	1.0	1.1	0.8	0.8	0.8	1.1
3-/3,6-Gal$p$	1.4	1.3	1.6	1.5	1.8	1.7

[a] Molar sugar composition determined by alditol acetates.

Table 5. Methylation Analysis (Molar Percentage) of Melanoidin Populations Obtained by Fractionation of the Et75QSB Fraction from Brazilian Roasted Coffee

	DR 5%		DR 8%		DR 10%	
	IM1	IM2	IM1	IM2	IM1	IM2
T-Rha$p$	7.4	7.2	4.2	5.4	1.9	1.6
total	**7.4 (11)[a]**	**7.2 (10)**	**4.2 (6)**	**5.4 (7)**	**1.9 (7)**	**1.6 (2)**
T-Ara$f$	18.7	14.4	12.8	10.6	9.4	9.5
T-Ara$p$	0.9	1.1				
5-Ara$f$	11.5	12.7	5.6	6.7	3.6	4.1
total	**31.1 (24)**	**28.2 (27)**	**18.4 (18)**	**(22)**	**13.0 (17)**	**13.6 (19)**
T-Man$p$				1.7	2.0	2.7
4-Man$p$	5.9	7.8	13.1	16.3	18.5	24.2
4,6-Man$p$				0.7	0.6	0.9
total	**5.9 (5)**	**7.8 (8)**	**13.1 (12)**	**18.7 (16)**	**21.0 (17)**	**27.8 (24)**
T-Gal$p$	13.3	12.4	18.7	14.4	18.1	16.7
6-Gal$p$	7.6	7.3	9.0	6.4	8.1	7.1
3-Gal$p$	18.6	21.3	21.9	17.1	21.5	19.3
3,6-Gal$p$	15.8	15.9	17.4	11.2	15.5	13.0
total	**55.3 (56)**	**56.9 (52)**	**67.0 (60)**	**49.1 (49)**	**63.2 (59)**	**56.1 (54)**
4-Glc$p$	0.3		1.6	9.2	0.8	1.0
total	**0.3 (3)**	**(3)**	**1.6 (4)**	**9.2 (6)**	**0.8**	**1.0 (1)**
Ara/Gal	0.56	0.50	0.28	0.45	0.21	0.24
T-/5-Ara$f$	1.6	1.1	2.3	1.6	2.6	2.3
T-Ara/3,6-Gal$p$	1.2	1.1	0.7	0.9	0.7	0.7
3-/3,6-Gal$p$	1.2	1.3	1.3	1.5	1.4	1.5

[a] Molar sugar composition determined by alditol acetates.

amino-acid-nitrogen content of the HMWM, the quantification of amino acids was performed. The amino acid content of the HMWM (grams of anhydroamino acids/100 g of HMWM) decreased with the increase of the degree of roast (10.27 ± 0.92, 9.22 ± 0.54, and 6.09 ± 0.30 for DR 5, 8, and 10%, respectively). These values corresponded, on average, to 94, 92, and 70% of the total nitrogen of roasted coffee HMWM, determined by the Kjeldahl procedure (*22, 23*).

N^ϵ-(Fructosyl)lysine (FL) and N^ϵ-(carboxymethyl)lysine (CML) (*50*) are common Maillard reaction products (MRP) with chelating properties. The analysis of the HMWM for FL, determined as furosine (Fur) after acid hydrolysis, CML, and N^ϵ-(carboxyethyl)lysine (CEL) allowed us to determine their amounts in the HMWM of the coffees with different degrees of roast (**Table 8**). The content of FL increased significantly from the light to the medium degree of roast and decreased from the medium to the dark degree of roast. This trend, although not so accentuated, was also observed for CML and CEL. When the same analysis was performed for all melanoidin populations obtained, only CML and CEL were quantified, as
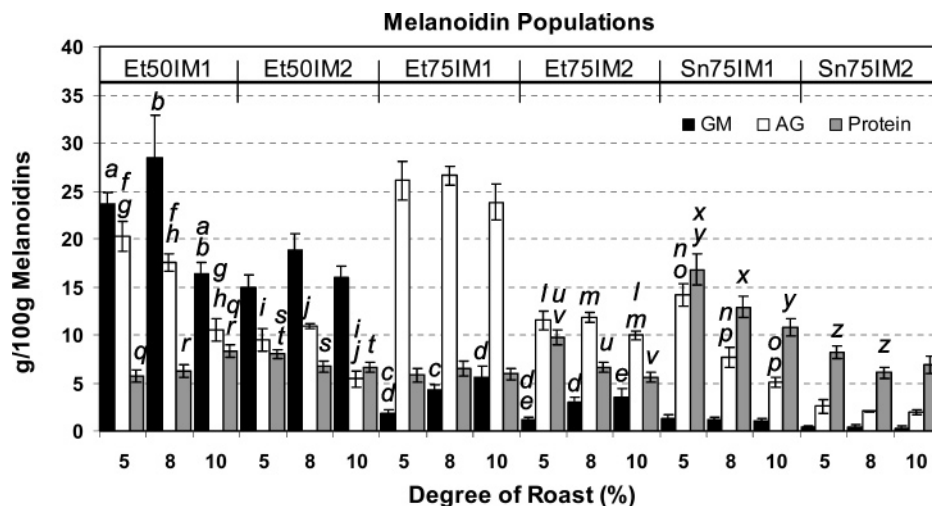
Figure 2. Galactomannan-like (GM) and arabinogalactan-like (AG) carbohydrates and protein content of melanoidin populations purified from roasted coffee HMWM with different degrees of roast. The same levels in each melanoidin population represent significantly different ($p = 0.05$) content between pairs.

Table 6. Methylation Analysis (Molar Percentage) of Melanoidin Populations Obtained by Fractionation of the Sn75QSB Fraction from Brazilian Roasted Coffee

	DR 5%		DR 8%		DR 10%	
	IM1	IM2	IM1	IM2	IM1	IM2
T-Rha*p*	14.8	11.1	9.5	8.2	5.9	3.1
total	**14.8 (18)**[a]	**11.1 (11)**	**9.5 (14)**	**8.2 (7)**	**5.9 (14)**	**3.1 (5)**
T-Ara*f*	21.9	20.5	17.5	15.6	16.0	12.6
T-Ara*p*	1.9	2.2				
5-Ara*f*	22.1	19.1	15.4	16.9	13.7	10.1
total	**45.9 (40)**	**41.8 (34)**	**32.9 (36)**	**33.5 (42)**	**29.7 (36)**	**22.7 (34)**
T-Man*p*			4.1	5.2	2.4	3.2
4-Man*p*	6.2	7.6	13.2	15.8	13.7	13.6
4,6-Man*p*						
total	**6.2 (5)**	**7.6 (11)**	**17.3 (10)**	**21.0 (12)**	**16.1 (10)**	**16.8 (7)**
T-Gal*p*	7.3	8.5	12.3	10.1	14.1	11.5
6-Gal*p*	4.2	4.3	5.6	4.1	5.9	4.2
3-Gal*p*	9.5	10.7	6.9	10.5	13.1	13.1
3,6-Gal*p*	8.2	8.4	5.1	2.8	7.8	4.5
total	**29.2 (30)**	**31.9 (33)**	**29.9 (35)**	**27.5 (34)**	**40.9 (35)**	**33.3 (19)**
4-Glc*p*	3.9	7.6	10.4	9.8	7.7	24.1
total	**3.9 (6)**	**7.6 (10)**	**10.4 (5)**	**9.8 (4)**	**7.7 (5)**	**24.1 (34)**
Ara/Gal	1.33		1.10		1.17	
T-/5-Ara*f*	1.0		1.1		1.2	
T-Ara/3,6-Gal*p*	2.7		2.1		2.1	
3-/3,6-Gal*p*	1.2		1.4		1.7	

[a] Molar sugar composition determined by alditol acetates.

the FL level was below the signal/noise ratio of 10, not allowing its accurate quantification. The values found for CML and CEL for all studied melanoidin populations ranged from 46 to 534 mg of CML/kg of protein and from 65 to 527 mg of CEL/kg of protein. No significant differences were observed between CML and CEL values for either chelating (IM2) or nonchelating (IM1) fractions (results not shown).

Evaluation of the Presence of Phenolic Compounds in the HMWM and Melanoidin Populations. Apart from MRP, the only other class of compounds claimed to be present in the HMWM, or any other similar fraction, is phenolic compounds. Their presence and quantification has been ascertained by a large range of techniques (*14−17*, *22−25*). To investigate the occurrence of phenolic compounds in coffee HMWM, various procedures were applied to the HMWM recovered from the coffee infusions with different degrees of roast. The alkaline hydrolysis with 2 M NaOH at room temperature, which is an

efficient method for releasing esterified phenolic compounds from lignins (*40*), did not allow the release of monomeric phenolic compounds from any of the studied HMWM. Likewise, no monomeric phenolic compounds could be detected when the hydrolysis temperature was raised to 100 °C. The same fractions were analyzed by pyrolysis−GC-MS, using the conditions described by Henrich and Baltes (*15*), and once more no phenolic compounds could be detected. Similar results were obtained after applying the method of Delgado-Andrade and Morales (*20*) for releasing ionic-bound phenolic compounds to the HMWM, that is, no monomeric phenolic compounds were detected. However, when the alkaline fusion method was applied to all HMWM samples, between 2.6 and 3.2% of monomeric phenolic compounds could be recovered (**Table 8**). The compounds released were in accordance with those reported by Takenaka et al. (*17*) for the melanoidin fraction obtained by zinc chelating precipitation.

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Table 7. Contribution of the Purified Melanoidin Populations from Roasted Coffee Infusions with Different Degrees of Roast to the Overall Color of the HMWM

sample	DR 5%		DR 8%		DR 10%	
	CD_{total} (2^n)	contribution to HMWM color[a] (%)	CD_{total} (2^n)	contribution to HMWM color (%)	CD_{total} (2^n)	contribution to HMWM color (%)
HMWM	6[b]		7		8	
Et50IM1	7	7.2	7	3.1	8	3.9
Et50IM2	7	6.6	8	6.6	9	10.3
Et75IM1	7	8.6	7	5.1	8	3.5
Et75IM2	8	10.8	8	5.6	9	7.8
Sn75IM1	8	19.6	8	8.4	9	10.8
Sn75IM2	7	12.2	8	10.6	8	2.7
total (%)		65.0		39.4		39.0

[a] Color contribution of the melanoidin population present in the sample to the color of HMWM. [b] Number of dilutions of the 1 mg/mL sample solution (*n*).

Table 8. Maillard Reaction Products (Milligrams per Kilogram of Protein) and Phenolic Compounds (Grams per 100 g) Present in the HMWM of Brazilian Coffee

	DR 5%	DR 8%	DR 10%
Maillard Reaction Products			
FL[a]	94 (17)	237 (21)	71 (14)
CML	116 (16)	232 (37)	99 (14)
CEL	161 (22)	281 (49)	123 (22)
Phenol Derivatives			
phenol	+		
3-methylphenol	0.040 (0.013)	0.060 (0.005)	0.067 (0.007)
2-hydroxyphenol	0.20 (0.04)	0.32 (0.10)	0.22 (0.10)
4-hydroxyphenol	0.22 (0.07)	0.26 (0.03)	0.30 (0.03)
2-hydroxy-4-methylphenol[b]	0.030 (0.011)	0.041 (0.004)	0.030 (0.01)
2,3-dihydroxyphenol	0.036 (0.013)	0.011 (0.007)	0.007 (0.003)
Benzoic Acid Derivatives			
benzoic acid	0.14 (0.02)	0.17 (0.05)	0.16 (0.02)
2-methylbenzoic acid[b]	0.021 (0.006)	0.027 (0.007)	0.021 (0.004)
2-hydroxybenzoic acid	+	+	+
3-hydroxybenzoic acid	0.34 (0.09)	0.36 (0.04)	0.26 (0.07)
4-hydroxybenzoic acid	0.24 (0.04)	0.24 (0.04)	0.23 (0.06)
2,3-dihydroxybenzoic acid	0.027 (0.008)	0.015 (0.005)	0.015 (0.003)
2,5-dihydroxybenzoic acid	0.012 (0.001)		0.008 (0.002)
3,4-dihydroxybenzoic acid	1.61 (0.06)	1.57 (0.17)	1.33 (0.04)
3,4,5-trihydroxybenzoic acid	0.11 (0.01)	0.11 (0.02)	0.038 (0.010)
Other Phenolic Derivatives			
4-hydroxybenzenacetic acid[b]	+	+	+
4-hydroxyphenylpropanoic acid[b]	+	+	+
total	**3.02** (0.20)	**3.19** (0.31)	**2.69** (0.19)

[a] Obtained by multiplying the furosine levels found by 3.2; values in parentheses are standard deviation (*n* = 2). [b] Identified by GC-MS; +, detected by GC-MS but not quantified by GC-FID; values in parentheses are standard deviation (*n* = 2).

It is known that strong alkali conditions can convert carbohydrates into phenolic compounds (*51*) that retain the original six carbons of the hexoses. To exclude the hypothesis that the phenolic compounds detected could derive from the coffee carbohydrates, commercial locust bean gum was also submitted to the strong alkali conditions applied to the HMWM roasted coffee fractions. No phenolic compound could be detected by GC-MS. Because only hydroxyl-containing phenolics and benzoic acids were released from the melanoidin fractions, and because high temperatures can effect the demethylation of phenolic compounds (*52*), the same procedure was also applied to ferulic, caffeic, and gallic acid standards. The alkaline fusion of ferulic acid yielded 24.4% of 3,4-dihydroxybenzoic acid, 2.7% of 2-hydroxyphenol, 0.12% of 2-hydroxy-4-methylphenol, and 0.08% of benzoic acid. The alkaline fusion products of

caffeic acid were similar to those found for ferulic acid, although with a significantly higher yield: 72.2% of 3,4-dihydroxybenzoic acid, 7.8% of 2-hydroxyphenol, 0.26% of 2-hydroxy-4-methylphenol, and 0.04% of benzoic acid. The majority (93.5%) of the original gallic acid was recovered, and the products contained only 3.0% of 2,3-dihydroxyphenol and vestigial amounts of benzoic acid. As no other applied method was able to release phenolic compounds from the HMWM, besides alkaline fusion, which is a method known to release condensed phenolic structures (*38, 52*), it can be concluded that the phenolic compounds were covalently linked to the melanoidins. Moreover, if these phenolic compounds have been derived from ferulic acid, their amount should be underestimated.

The Sn75 melanoidin fractions that contained a higher abundance of chemically unknown material were analyzed for phenolic compounds by the alkaline fusion method. In all fractions, monomeric phenolic compounds were found. The major phenolic compound detected was 3,4-dihydroxybenzoic acid (0.43−0.81% of the fraction) followed by 3-hydroxybenzoic acid (0.19−0.34%), 4-dihydroxybenzoic acid (0.12−0.38%), benzoic acid (0.07−0.26%), and 2-hydroxyphenol (0.04−0.11%). The amounts of phenolic compounds recovered from chelating and nonchelating Sn75 melanoidin populations were not significantly different, ranging from 1.52 to 1.89% for Sn75IM1 and from 1.04 to 1.39% for Sn75IM2.

Significance and Possible Origin of Anionic Melanoidins Populations of Roasted Coffee Infusions. The six anionic melanoidin populations, Et50IM1, Et50IM2, Et75IM1, Et75IM2, Sn75IM1, and Sn75IM2, when isolated from the HMWM of roasted coffee infusions prepared from light-, medium-, and dark-roasted coffees, revealed not only distinct physical−chemical properties (ethanol solubility and chelating ability) but also chemical composition regarding carbohydrate and protein nature and content, independent of the degree of roast. These melanoidin populations represented between 30 and 33% of the cold-water soluble HMWM, and half of this material had chelating ability for immobilized copper ions. The amount of carbohydrates associated with these melanoidin populations accounted for 20−26% of the cold-water soluble HMWM polysaccharides: the galactomannan-like carbohydrates accounted for 16−23% of the galactomannans extracted from roasted coffee, and the arabinogalactan-like carbohydrates accounted for 33−43% of the arabinogalactans extracted. The protein recovered in these melanoidin populations accounted for 32−40% of the protein-like material present in the cold-water soluble HMWM.

The amount of carbohydrates recovered in fractions Et50IM1 and Et50IM2, Et75IM1 and Et75IM2, and Sn75IM1, which had structural features similar to those isolated from green coffee infusions (*22, 24*) and cell walls (*53, 54*), allowed us to conclude that a significant amount of coffee melanoidins have their origin in the cell walls of coffee. This does not exclude the fact that other cellular components can be involved in their formation, as a close contact may occur among them when cell collapse takes place during roasting (*55*). The different proportions of the galactomannan- and arabinogalactan-like carbohydrates for Et50, Et75, and Sn75IM1 melanoidin populations may represent different origins, as the cell walls of coffee have a nonhomogeneous environment (*56*). Nevertheless, the melanoidin population derived from the Sn75 fractions, due to their much higher protein and lower carbohydrate contents, appears to be formed by a different mechanism. Also, it can arise from a different location, possibly from the inner cellular materials, where the 11S storage proteins are located (*57*). What seems rather striking

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is the fact that even for the light roast, for which nearly all nitrogen of the HMWM was recovered as amino acid nitrogen, these six melanoidin fractions were present in high amounts (33%) and the HMWM presented a significant brown color. These results suggest that phenolic condensation may also play a significant role in the browning process and melanoidin formation during coffee roasting, despite the low amount of recoverable phenolic compounds by alkaline fusion from all HMWM. Thus, more work is needed to find out how and how much the phenolic compounds are linked to these melanoidins.

The presence of the same amount of chelating CML in all nonchelating and chelating melanoidin populations and recoverable phenolic compounds in Sn75IM1 and Sn75IM2 populations suggests that these compounds are not the only ones responsible for the different chelating abilities of these melanoidin populations. It is very likely that the different structural/spatial arrangement of carbohydrates within the melanoidin populations is in the origin of their chelating ability as all nonchelating populations contained higher amounts of carbohydrates than the chelating ones.

ABBREVIATIONS USED

Ara, arabinose; CML, N^ϵ-(carboxymethyl)lysine; CEL, N^ϵ-(carboxyethyl)lysine; DR, degree of roast; Et50, material precipitated in 50% ethanol solution; Et75, material precipitated in 75% ethanol solution; Sn75, material soluble in 75% ethanol solution; FL, N^ϵ-(fructosyl)lysine; Fur, furosine; Gal, galactose; GC-EIMS, gas chromatography–electron ionization mass spectrometry; Glc, glucose; HMWM, high molecular weight material; Man, mannose; IM1, nonchelating anionic melanoidins; IM2, chelating anionic melanoidins; MRP, Maillard reaction products; QSA, material not retained in the anion exchange column; QSB, material retained in the anion exchange column; UA, uronic acids; WIppt, material insoluble in cold water.

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LITERATURE CITED

(1) Borrelli, R. C.; Visconti, A.; Mennella, C.; Anese, M.; Fogliano, V. Chemical characterization and antioxidant properties of coffee melanoidins. *J. Agric. Food Chem.* **2002**, *50*, 6527–6533.

(2) Ames, J. M.; Wynne, A.; Hofmann, A.; Plos, S.; Gibson, G. R. The effect of a model melanoidin mixture on faecal bacterial populations in vitro. *Br. J. Nutr.* **1999**, *82*, 489–495.

(3) Hiramoto, S.; Itoh, K.; Shizuuchi, S.; Kawachi, Y.; Morishita, Y.; Nagase, M.; Suzuki, Y.; Nobuta, Y.; Sudou, Y.; Nakamura, O.; Kagaya, I.; Goshima, H.; Kodama, Y.; Icatro, F. C.; Koizumi, W.; Saigenji, K.; Miura, S.; Sugiyama, T.; Kimura, N. Melanoidin, a food protein-derived advanced maillard reaction product, suppresses *Helicobacter pylori in vitro* and *in vivo*. *Helicobacter* **2004**, *9*, 429–435.

(4) Somoza, V. Five years of research on health risks and benefits of Maillard reaction products: an update. *Mol. Nutr. Food Res.* **2005**, *49*, 663–672.

(5) Ledl, F.; Scheicher, E. New aspects of the maillard reaction in food and in the human body. *Angew. Chem. Int. Ed.* **1990**, *29*, 565–594.

(6) Kato, H.; Tsuchida, H. Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Food Nutr. Sci.* **1981**, *5*, 147–156.

(7) Tressl, R.; Wondrak, G. T.; Garbe, L. A.; Kruger, R. P.; Rewicki, D. Pentoses and hexoses as sources of new melanoidin-like Maillard polymers. *J. Agric. Food Chem.* **1998**, *46*, 1765–1776.

(8) Hofmann, T. Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde–chemical characterisation of a red coloured domaine. *Eur. Food Res. Technol.* **1998**, *206*, 251–258.

(9) Cämmerer, B.; Kroh, L. W. Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chem.* **1995**, *53*, 55–59.

(10) Maier, H. G.; Dietmair, W.; Ganssman, J. Isolation and characterization of brown roast compounds of coffee (In German). *Z. Lebensm. Unters. Forsch. A* **1968**, *137*, 282–292.

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

(12) Maier, H. G. E; Buttle, H. Isolation and characterization of brown roast compounds of coffee. 2. (in German). *Z. Lebensm. Unters. Forsch. A* **1973**, *150*, 331–334.

(13) Montavon, P.; Mauron, A.-F.; Duruz, E. Changes in green coffee protein profiles during roasting. *J. Agric. Food Chem.* **2003**, *51*, 2335–2343.

(14) Klocking, R.; Hofmann, R.; Mucke, D. Substances of humic acid type occurring in extracts of roasted coffee. 3. Detection of phenols in hydrolysates of humic acids from coffee. *Z. Lebensm. Unters. Forsch. A* **1971**, *146*, 79–82.

(15) Henrich, L.; Baltes, W. Occurrence of phenols in coffee melanoidins. *Z. Lebensm. Unters. Forsch. A* **1987**, *185*, 366–370.

(16) Rawel, H. M.; Rohn, S.; Kroll, J. Characterization of 11S protein fractions and phenolic compounds from green coffee beans under special consideration of their interactions–a review. *Dtsch. Lebensmitt. Rundsch.* **2005**, *101*, 148–160.

(17) Takenaka, M.; Sato, N.; Asakawa, H.; Wen, X.; Murata, M.; Homma, S. Characterization of a metal-chelating substance in coffee. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 26–30.

(18) Terasawa, N.; Murata, M.; Homma, S. Comparison of brown pigments in food by microbial descolorization. *J. Food Sci.* **1996**, *61*, 669–672.

(19) Clifford, M. N. Chemical and physical aspects of green coffee and coffee products. In *Coffee: Botany, Biochemistry and Production of Beans and Beverage*; Clifford, M. N., Wilson, K. C., Eds.; Croom Helm Publishers: London, U.K., 1985; pp 305–374.

(20) Delgado-Andrade, C.; Morales, F. J. Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *J. Agric. Food Chem.* **2005**, *53*, 1403–1407.

(21) D'Agostina, A.; Boschin, G.; Bacchini, F.; Arnoldi, A. Investigations on the high molecular weight foaming fractions of espresso coffee. *J. Agric. Food Chem.* **2004**, *52*, 7118–7125.

(22) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted arabica coffee. *J. Agric. Food Chem.* **2001**, *49*, 1773–1782.

(23) Nunes, F. M.; Coimbra, M. A. Chemical characterization of galactomannans and arabinogalactans from two arabica coffee infusions as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 1429–1434.

(24) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high molecular weight material extracted with hot water from green and roasted robusta coffee as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 7046–7052.

(25) Bekedam, E. K.; Schols, H. A.; Van Boekel, M. A. J. S.; Smit, G. High molecular weight melanoidins from coffee brew. *J. Agric. Food Chem.* **2006**, *54*, 7658–7666.

(26) Morales, F. J. Application of capillary zone electrophoresis to the study of food and food-model melanoidins. *Food Chem.* **2002**, *76*, 363–369.

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(27) Royle, L.; Bailey, R. G.; Ames, J. M. Separation of maillard reaction products from xylose−glycine and glucose−glycine model systems by capillary electrophoresis and comparison to reverse phase HPLC. *Food Chem.* **1998**, *62*, 425−430.

(28) Gomyo, T.; Horikoshi, M. Interaction of melanoidins with metallic-ions. *Agric. Biol. Chem.* **1976**, *40*, 33−40.

(29) Morales, F. J.; Fernandez-Fraguas, C.; Jimenez-Perez, S. Iron-binding ability of melanoidins from food and model systems. *Food Chem.* **2005**, *90*, 821−827.

(30) Terasawa, N.; Murata, M.; Homma, S. Separation of model melanoidin into components with copper chelating Sepharose-6B column chromatography and comparision of chelating activity. *Agric. Biol. Chem.* **1991**, *55*, 1507−1514.

(31) Coimbra, M. A.; Delgadillo, I.; Waldron, K. W.; Selvendran, R. R. Isolation and analysis of cell wall polymers from olive pulp. In *Plant Cell Wall Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Modern Methods of Plant Analysis 17; Springer-Verlag: Berlin, Germany, 1996; pp 19−44.

(32) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

(33) MacKenzie, S. L. Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 151−160.

(34) Hofmann, T. Studies on the influence of the solvent on the contribution of single Maillard reaction products to the total color of browned pentose/alanine solutions. A quantitative correlation using the color activity concept. *J. Agric. Food Chem.* **1998**, *46*, 3912−3917.

(35) Knecht, K. J.; Dunn, J. A.; McFarland, K. F.; McCance, D. R.; Lyons, T. J.; Thorpe, S. R.; Baynes, J. W. Effect of diabetes and aging on carboxymethyllysine levels in human urine. *Diabetes* **1991**, *40*, 190−196.

(36) Nagai, R.; Araki, T.; Hayashi, C. M.; Hayase, F.; Horiuchi, S. Identification of *N*$^\epsilon$-(carboxyethyl)lysine, one of the methylglyoxal-derived AGE structures, in glucose-modified protein: mechanism for protein modification by reactive aldehydes. *J. Chromatogr. B* **2003**, *788*, 75−84.

(37) Hofmann, K.; Stutz, E.; Spuhler, G.; Yajima, H.; Schwartz, E. T. Studies on polypeptides. XVI. The preparation of *N*-ε-formyl-L-lysine and its application on the synthesis of peptides. *J. Chem. Soc.* **1960**, *82*, 3727−3732.

(38) Batistic, L.; Mayaudon, J. Étude chromatographic des acides phénoliques et des phenols obtenus par la fusion alcaline des acides humiques. *Plant Soil* **1970**, *33*, 473−477.

(39) Soleas, G. J.; Diamandis, E. P.; Karumanchiri, A.; Goldberg, D. M. A multiresidue derivatization gas chromatographic assay of fifteen phenolic constituents with mass selective detection. *Anal. Chem.* **1997**, *69*, 4405−4409.

(40) Chen, C.-L. Nitrobenzene and cupric oxid oxidations. In *Methods in Lignin Chemistry*; Lin, S. Y., Dence, C. W., Eds.; Springer-Verlag: Berlin, Germany, 1992; Chapter 6.2, pp 301−319.

(41) Silvestre, A. J. D.; Pereira, C. C. L.; Neto, C. P.; Evtuguin, D. V.; Duarte, A. C.; Cavaleiro, J. A. S.; Furtado, F. P. Chemical composition of pitch deposits from an ECF Eucalyptus globulus bleached kraft pulp mill: its relationship with wood extractives and additives in process streams. *Appita J.* **1999**, *52*, 375−382.

(42) Bennet, R. N.; Rosa, E. A. S.; Mellon, F. A.; Kroon, P. A. Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (turkish rocket). *J. Agric. Food Chem.* **2006**, *54*, 4005−4015.

(43) Oosterveld, A.; Voragen, A. G. J.; Schols, H. A. Effect of roasting on the carbohydrate composition of *Coffea arabica* beans. *Carbohydr. Polym.* **2003**, *54*, 183−192.

(44) Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. *Carbohydr. Res.* **2005**, *340*, 1689−1698.

(45) Redgwell, R. J.; Curti, D.; Fischer, M.; Nicolas, P.; Fay, L. B. Coffee bean arabinogalactans: acidic polymers covalently linked to protein. *Carbohydr. Res.* **2002**, *337*, 239−253.

(46) Feldman, R. S.; Ryder, W. S.; Kung, J. T. Importance of nonvolatile compounds to the flavor of coffee. *J. Agric. Food Chem.* **1969**, *17*, 733−739.

(47) Casal, S.; Mendes, E.; Oliveira, M. B. P. P.; Ferreira, M. A. Roast effects on coffee amino acid enantiomers. *Food Chem.* **2005**, *89*, 333−340.

(48) Redgwell, R. J.; Trovato, V.; Curti, D.; Fischer, M. Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans. *Carbohydr. Res.* **2002**, *337*, 421−431.

(49) Henle, T.; Schwarzenbolz, U.; Klostermeyer, H. Detection and quantification of pentosidine in foods. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 95−98.

(50) Seifert, S. T.; Krause, R.; Gloe, K.; Henle, T. Metal complexation by the peptide-bound *N*-ε-fructoselysine and *N*-ε-carboxymethyllysine. *J. Agric. Food Chem.* **2004**, *52*, 2347−2350.

(51) Knill, C. J.; Kennedy, J. F. Degradation of cellulose under alkaline conditions. *Carbohydr. Polym.* **2003**, *51*, 281−300.

(52) Newhall, F. W.; Ting, S. V. Degradation of hesperetin and naringenin to phloroglucinol. *J. Agric. Food Chem.* **1967**, *15*, 776−777.

(53) Fischer, M.; Reimann, S.; Trovato, V.; Redgwell, R. J. Polysaccharides of green Arabica and robusta coffee beans. *Carbohydr. Res.* **2001**, *330*, 93−101.

(54) Oosterveld, A.; Harmsen, J. S.; Voragen, A. G. J.; Schols H. A. Extraction and characterization of polysaccharides from green and roasted *Coffea arabica* beans. *Carbohydr. Polym.* **2003**, *52*, 285−296.

(55) Illy, A.; Viani, R. *Espresso Coffee. The Chemistry of Quality*; Academic Press: London, U.K., 1995; Chapter 5.

(56) Sutherland, P. W.; Hallett, I. C.; MacRae, E.; Fischer, M.; Redgwell, R. J. Cytochemistry and immunolocalisation of polysaccharides and proteoglycans in the endosperm of green arabica coffee beans. *Protoplasma* **2004**, *223*, 203−211.

(57) Rogers, W. J.; Bezard, G.; Dashayes, A.; Meyer, I.; Petiard, V.; Marraccini, P. Biochemical and molecular characterization and expression of the 11S-type storage proteins from *Coffea arabica* endosperm. *Plant Physiol. Biochem.* **1999**, *37*, 361−272.