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Insight into the Mechanism of Coffee Melanoidin Formation Using Modified "in Bean" Models

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Supporting Information

ABSTRACT: To study the mechanism of coffee melanoidin formation, green coffee beans were prepared by (1) removal of the hot water extractable components (WECoffee); (2) direct incorporation of sucrose (SucCoffee); and (3) direct incorporation of type II arabinogalactan-proteins (AGPCoffee). As a control of sucrose and AGP incorporation, lyophilized green coffee beans were also immersed in water (control). The original coffee and the four modified "in bean" coffee models were roasted and their chemical characteristics compared. The formation of material not identified as carbohydrates or protein, usually referred to as "unknown material" and related to melanoidins, and the development of the brown color during coffee roasting have distinct origins. Therefore, a new parameter for coffee melanoidin evaluation, named the "melanoidin browning index" (MBI), was introduced to handle simultaneously the two concepts. Sucrose is important for the formation of colored structures but not to the formation of "unknown material". Type II AGPs also increase the brown color of the melanoidins, but did not increase the amount of "unknown material". The green coffee hot water extractable components are essential for coffee melanoidin formation during roasting. The cell wall material was able to generate a large amount of "unknown material". The galactomannans modified by the roasting and the melanoidin populations enriched in galactomannans accounted for 47% of the high molecular weight brown color material, showing that these polysaccharides are very relevant for coffee melanoidin formation.

KEYWORDS: *coffee, infusion, melanoidins, sucrose, type II arabinogalactan-proteins*

INTRODUCTION

Melanoidins are the high molecular weight (HMW) brown products containing nitrogen, end products of the Maillard reaction.[1,2] They are a heterogeneous group of food polymers with a structural diversity that has hindered a definitive chemical definition. There are several proposals for the mechanisms of melanoidin formation and structures.[3−14] Polysaccharides have a key role in the formation of the most abundant population of coffee brew melanoidins.[15−19] A central role has been attributed to arabinogalactan-proteins (AGPs) in coffee melanoidin formation,[15,16] although there is a clear involvement of galactomannans.[17,18] Nevertheless, there are melanoidin populations that contain only negligible amounts of polysaccharides.[17,20−22] Beyond polysaccharides, coffee melanoidins incorporate chlorogenic acids (CGA) and amino acid/protein fragments. The different chemical compositions of melanoidin populations isolated from roasted coffee brews[15−24] strongly suggest that there are several pathways through which coffee melanoidins are formed.

The amount of melanoidins in coffee brews is usually quantified gravimetrically as the difference between all known HMW components (sugars and amino acids) and the total amount of material. Alternatively, the amount of melanoidins can be estimated through the measurement of the brown color of the material, by determination of either the color dilution factor[17,25] or the $K_{\text{mix 405nm}}$ parameter, which is the specific extinction coefficient at 405 nm.[15,16,23,24] The gravimetric determination of the unknown components and the measure of the brown color of the HMW fractions are direct measures of the formation of new compounds during the roasting process, either as HMW chemically undefined backbones or as colored structures appended to the HMW polysaccharides or proteins.

One way to gain insights into the mechanism of coffee melanoidin formation is through the evaluation of the relative importance of the different coffee components on the yield and color of the unknown material. This could be done by the use of the "in bean" model approach.[26−28] These in bean model experiments allow more realistic conditions, as green coffee beans exhibit a cellular structure with thick cell walls that can withstand the large pressure buildup during roasting due to water evaporation and carbon dioxide evolution, allowing the view of coffee cells as pressurized mini-reactors. Also, there is strong evidence that coffee cell walls participate in coffee melanoidin formation.[17,18,21,23,24] The use of the in bean models allow one to take into account the effect of the cell wall structure of the roasted coffee powders on the extraction procedure during the coffee brew preparation.[29] To prepare an in bean model, the green coffee beans, without grinding, are exhaustively extracted with hot water to obtain the green coffee water extractable material, leaving the insoluble, intact coffee bean shell. Coffee bean shells, before roasting, are then reincorporated with the initial water soluble extract or with

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the extract after removal or fortification with one or several original components.[26,27]

The coffee cell components are present in compartmentalized arrangements, with proteins distributed evenly throughout the cytoplasm, the oil located in vacuoles, and chlorogenic acids occurring in deposits near the cell walls.[30] With the purpose of minimizing the disturbance of the distribution of the components in the coffee cells, a modified in bean model was developed by the direct incorporation of the component under evaluation. The objective of this work was to develop and validate a modified in bean model and apply it to gain further insights into the role of sucrose and type II AGPs in the mechanism of coffee melanoidin formation. In parallel, the relative importance of green coffee soluble solids and cell walls on melanoidin formation was also evaluated.

MATERIALS AND METHODS

Preparation of the Modified in Bean Green Coffee Models. For preparation of the modified in bean coffee models, Brazil green arabica coffee beans were used. The beans were initially freeze-dried until constant weight (10% weight loss). For the preparation of the sucrose-enriched coffee model (SucCoffee), to 200 g of the freeze-dried green coffee beans was added 200 mL of a sucrose-saturated aqueous solution, and the mixture was shaken overnight at room temperature. After incorporation, the coffee beans were washed with distilled water for removal of the surface adhering solution and freeze-dried. For the preparation of type II arabinogalactan-protein-enriched coffee model (AGPCoffee), the same procedure was used, replacing the sucrose solution by 200 mL of a solution containing 0.1 g/mL of type II AGP, from gum arabic. As a control, a 200 g batch of freeze-dried coffee beans was incorporated with 200 mL of water under the same conditions.

Preparation of Coffee Bean Shells. Freeze-dried green coffee (1 kg) was extracted with water (2 L) during 1 h at 95 °C. The extraction was repeated three more times.[26] After freeze-drying, the water-extracted model coffee (WECoffee) was obtained.

Roasting of Coffee Bean Model Samples. Prior to roasting, all samples, including the original freeze-dried coffee, were adjusted to the same moisture of 10% by spraying enough water and checking coffee weight after 1 week. Then the raw coffee beans (50 g) were roasted (Probat Pre 1Z 88) at 200 °C during approximately 5 min to obtain an organic roasting loss of 8%. After roasting, the coffee was degassed during 2 days at room temperature.

Preparation of Coffee Brews and Isolation of the High Molecular Weight Material (HMWM). Roasted coffee beans were ground (250−450 μm), and infusions were prepared by extraction of 30 g of powdered coffee with 1 L of water at 80 °C during 20 min. After filtration and vacuum concentration to 200 mL, 50 mL of coffee infusion was freeze-dried to calculate the total soluble solids. The other 150 mL of concentrated coffee infusion was extensively dialyzed at 4 °C (six water renewals, MW cutoff 12−14 kDa). After dialysis, the retentate was freeze-dried, giving the HMWM.

Fractionation of the HMWM by Graded Precipitation in Ethanol. For the fractionation of HMWM material for each coffee infusion, the method previously developed[31] was used with a modification. To solubilize all of the HMWM after freeze-drying, a solution of 6 M urea was used instead of water. The material in the Et50 and Et75 precipitated fractions was resolubilized in water and dialyzed (six water renewals, MW cutoff 12−14 kDa). After dialysis, the retentate was freeze-dried. The material soluble in the EtSn fraction was recovered after concentration, dialysis, and freeze-drying.

Chlorogenic Acid and Caffeine Content of Green and Roasted Coffees. Chlorogenic acids (CGA) were extracted overnight from green and roasted ground coffees (1 g) with 100 mL of a solution of methanol/water (70:30 v/v) containing 0.5% Na_2SO_3 with constant stirring (125 rpm) in the dark. To the solution was added 1 mL of verartric acid as internal standard (100 mg/mL, in methanol). After filtration of the coffee powder, the colloidal material present in the extracts was precipitated by the addition of 1 mL of Carrez I and II solutions.[32] The extracts were analyzed by HPLC (Dionex, Ultimate 3000) by injection of 25 μL of the sample on a reversed-phase HPLC column (C18-ACE; 25 cm length, 0.45 cm internal diameter, and 5 μm particle diameter). Eluent A was a 5% formic acid aqueous solution, and eluent B was methanol. The eluent program was as follows: 0−5 min, 5% eluent B; 5−45 min, 40% B; 45−65 min, 70% B; 65−75 min, 5% B. The column temperature was set at 25 °C, and the flow was 0.8 mL/min. The eluent was continuously monitored from 200 to 600 nm with a photodiode array detector (PDA-100, Dionex). The identification of caffeoylquinic acids (CQA) and cinnamoyl-1,5-γ-quinolactones (CGL) was performed by comparison with the retention time of the respective standards and literature values.[33,34] CGAs were quantified by the internal standard method using a 5-CQA calibration. Caffeine was quantified in the same way by using a caffeine calibration curve.

Characterization of Coffee Bean Samples and Fractions. Green and roasted coffee and coffee models were characterized regarding their sucrose, glucose, and fructose contents by anion exchange chromatography. Total sugars content was determined by anion exchange chromatography after Saeman and acid hydrolysis.[31] Protein content was determined according to the Dumas method (PRIMACS, Carbon-Nitrogen/Protein analyzer, Skalar, The Netherlands), by multiplying the nitrogen content by 6.25. Amino acid analysis was performed by gas chromatography of the heptafluorobutyl isobutyl derivatives after acid hydrolysis.[35] The condensed phenolic content of the HMWM and ethanol-precipitated fractions were determined after alkaline fusion and analysis by GC-MS as described previously.[17] The melanoidin content of fractions was determined by the $K_{\text{mix }405\text{nm}}$ as previously described.[15]

Color of Roasted Coffee Powder. Roasted coffee powder colors obtained for the original, control, and in bean coffee models were directly measured with a Minolta chroma meter (model CR-400, Minolta, Tokyo, Japan). The equipment was set up for illuminant D65 and 10° observer. The equipment was calibrated with a white standard (L^* = 97:71; a^* = −0:59; and b^* = 2:31), and each sample was put on a Petri dish and reed in five different locations. Numerical values of chroma (C^*), hue (h), and color difference (ΔE^*) were calculated according to the following formulas:

$$h = \arctan \frac{b^*}{a^*}$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Statistical Analysis. All chemical analyses of green coffee, roasted coffee, soluble solids, and HMWM were performed in duplicate. Significant differences ($p < 0.05$) in chemical composition were analyzed by one-way ANOVA using the software Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA). A Tukey HSD post hoc test was performed for detecting significantly different means ($p < 0.05$).

RESULTS AND DISCUSSION

Chemical Characterization of Green Coffee and in Bean Green Coffee Models. To evaluate the changes occurring in green coffee bean chemical composition due to the incorporation process, the compositions of the nonmodified green coffee (original), freeze-dried green coffee immersed in water (control), and in bean green coffee models produced by the incorporation of an aqueous solution of sucrose (SucCoffee) and an aqueous solution of type II arabinogalactan-proteins (AGPCoffee) were analyzed. Green bean coffee shell produced by the extraction of the hot water extractable green coffee components (WECoffee) was also analyzed.

Total Soluble Sugars. Original green coffee had a total soluble sugar content of 11.7%, mainly composed by sucrose

Table 1. Soluble Sugar Content (Grams per 100 g Dry Basis) of Original, Control, Hot Water Extracted, and "in Bean" Model Green and Roasted Coffees

	green				roasted			
coffee	glucose	fructose	sucrose	total	glucose	fructose	sucrose	total
original	0.38 ± 0.19	0.20 ± 0.71	11.2 ± 1.01	11.7 ± 0.41	0.023 ± 0.17	0.004 ± 0.03	0.171 ± 0.21	0.20 ± 0.11
control	1.74 ± 0.17	1.2 ± 0.30	5.42 ± 0.81	8.42 ± 0.39	0.054 ± 0.03	0.007 ± 0.02	0.235 ± 0.12	0.29 ± 0.21
SucCoffee	2.31 ± 0.04	2.85 ± 0.15	13.3 ± 1.15	18.4 ± 0.73	0.086 ± 0.04	0.021 ± 0.08	0.313 ± 0.18	0.42 ± 0.07
AGPCoffee	1.98 ± 1.03	3.20 ± 0.87	3.66 ± 0.11	8.83 ± 0.69	0.103 ± 0.09	0.044 ± 0.11	0.299 ± 0.13	0.45 ± 0.10
WECoffee	0.05 ± 0.23	0.01 ± 0.02	0.92 ± 0.54	0.98 ± 0.20	0.005 ± 0.01	0.001 ± 0.03	0.058 ± 0.08	0.06 ± 0.5

Table 2. Chlorogenic Acid Composition of Original, Control, and Hot Water Extracted Coffee and "in Bean" Model Green Coffees and Roasted Coffees (Grams per 100 g Dry Basis)[a]

	green					roasted				
CGA	original	control	SucCoffee	AGPCoffee	WECoffee	original	control	SucCoffee	AGPCoffee	WECoffee
3-CQA	0.41	0.32	0.30	0.31	0.08	0.33	0.21	0.18	0.32	0.03
5-CQA	5.26	4.12	3.96	4.07	0.24	0.91	0.75	0.66	1.19	0.12
4-CQA	0.89	0.76	0.73	0.71	0.16	0.03	0.04	0.06	nd[b]	nd
total CQA	6.56	5.20	4.99	5.09	0.48	1.27	0.99	0.90	1.51	0.15
1-FQA	nd	nd	nd	nd	nd	0.002	0.01	0.01	nd	nd
3-FQA	0.29	0.26	0.24	0.25	nd	0.48	0.35	0.25	0.51	0.05
5-FQA	0.37	0.31	0.27	0.28	0.01	nd	nd	nd	nd	nd
4-FQA	0.04	0.04	0.03	0.03	0.01	nd	nd	nd	nd	nd
total FQA	0.70	0.61	0.54	0.56	0.02	0.48	0.36	0.26	0.51	0.05
3-CoQA	0.06	0.05	0.05	0.05	0.01	0.06	0.02	0.03	0.01	nd
5-CoQA	0.05	0.04	0.04	0.04	nd	0.18	0.04	0.05	0.10	0.03
4-CoQA	0.01	0.01	0.01	0.01	0.01	nd	nd	nd	nd	nd
total CoQA	0.12	0.10	0.10	0.10	0.02	0.23	0.05	0.07	0.11	0.03
3-CQL	nd	nd	nd	nd	nd	0.004	nd	nd	0.002	nd
4-CQL	nd	nd	nd	nd	nd	nd	0.01	0.003	nd	nd
3-FQL	nd	nd	nd	nd	nd	0.23	0.08	nd	0.12	0.03
1-FQL	nd	nd	nd	nd	nd	0.01	0.01	0.01	0.01	0.004
3,4-diCQA	0.21	0.16	0.16	0.16	nd	0.05	0.03	0.03	0.06	0.01
3,5-diCQA	0.48	0.38	0.36	0.38	0.04	0.03	0.03	0.02	0.07	0.01
4,5-diCQA	0.40	0.32	0.32	0.31	0.05	0.05	0.05	0.04	0.10	0.01
total diCQA	1.09	0.86	0.84	0.85	0.09	0.13	0.12	0.09	0.23	0.04
total (CGA)	8.47	6.75	6.46	6.64	0.60	2.11	1.52	1.35	2.36	0.26

[a]Duplicate analyses had variation coefficients under 5%. [b]Not detected.

(96%) and low amounts of glucose and fructose (Table 1), which is in accordance with the literature.[36−38] For the control coffee, a decrease in 28% of the total soluble sugar content was observed, as well as a change in the soluble sugar profile due to the increase in the relative amounts of glucose and fructose and a decrease in the relative amount of sucrose. This change may be the result of the activity of sucrose-metabolizing enzymes[39] during the overnight immersion of the freeze-dried green coffee beans in water. The incorporation of sucrose into the green coffee beans (SucCoffee) increased the total soluble sugar content of coffee beans to 18.4% (Table 1). This incorporation resulted also in an increase in glucose and fructose content, in accordance with the control coffee. For the AGPCoffee, the total soluble sugar content was similar to that found for the control coffee. The hot water extraction of the green coffee beans decreased the total soluble sugar content of the WECoffee by 92% in relation to the original coffee (Table 1).

Chlorogenic Acids. Twelve chlorogenic acids (CGA) were identified in all green coffees (Table 2). The most abundant CGA group in green coffee samples was the caffeoylquinic acids (CQA), which represented about 77% of the total CGA in green coffee. Total levels of feruloylquinic acids (FQA) represented 9% of the CGA, and coumaroylquinic acids (CoQA) represented 0.7%. Total CGA content of the original coffee was 8.47 g/100 g, which is in agreement with the literature.[32−42] For the control coffee, the total CGA content decreased to 6.75 g/100 g, possibly due to the activity of polyphenol oxidase during the hydration process.[43] The total CGA contents of SucCoffee and AGPCoffee were similar to that of the control coffee. On the other hand, the hot water extraction of green coffee beans resulted in a 93% decrease of the total CGA content in relation to the original coffee (Table 2).

Caffeine and Total Protein. The caffeine content of the original coffee was 1.67 g/100 g (Table 3), which is in accordance with literature values[44] and similar to that found for the control coffee. The caffeine content was slightly lower for SucCoffee and AGPCoffee models, and for WECoffee a

Table 3. Caffeine and Protein Content (Grams per 100 g Dry Basis) of Original, Control, and Hot Water Extracted Coffee and "in Bean" Green and Roasted Coffee Models

	green		roasted	
coffee	caffeine	protein[a]	caffeine	protein[a]
original	1.67 ± 0.11	12.1 ± 0.5	1.15 ± 0.08	12.5 ± 0.8
control	1.68 ± 0.09	11.1 ± 0.7	0.86 ± 0.07	11.8 ± 0.7
SucCoffee	1.40 ± 0.11	11.1 ± 0.3	0.85 ± 0.06	12.6 ± 0.2
AGPCoffee	1.55 ± 0.07	11.3 ± 0.5	0.94 ± 0.08	11.9 ± 0.7
WECoffee	0.10 ± 0.01	11.9 ± 0.7	0.08 ± 0.02	13.4 ± 1.0

[a] $(N_{total} - N_{caffeine}) \times 6.25$.

decrease of 95% in the caffeine content was observed. The protein content of green coffee beans and green coffee models was estimated on the basis of the nitrogen content after correction for caffeine nitrogen (Table 3). The protein content of the original coffee was 12.1%, a value slightly higher than that found for the control coffee (11.1%). The protein content of SucCoffee and AGPCoffee was similar to that of the control coffee and to the literature.[45] For WECoffee, the protein content was also similar to that found for the original coffee. This result shows that although there was an extensive hot water extraction of the green coffee beans, only 33% of the original protein content was extracted (corrected for the 32% weight loss observed for the WECoffee). This may be due to the denaturation of proteins during the extraction process, decreasing their solubility, and also due to the fact that a high amount of coffee protein is associated with the cell wall arabinogalactans.[46]

Polymeric Sugars. The sugar composition of green coffee polysaccharides was determined by subtracting from the total sugar content the soluble sugars glucose and sucrose contents (Table 4). The sugar content and composition were not significantly different for the original and control green coffees. Also, the sugar content and composition of SucCoffee were not significantly different from the control coffee. For the AGPCoffee, although the total polymeric sugar composition was not significantly different from control coffee, the contents of rhamnose, arabinose, and galactose were significantly higher as a result of the incorporation of AGP. In the AGPCoffee model, the amount of AGP related sugars increased by 2.2 g/100 g in relation to control coffee, corresponding to an increase of 15.2%. The amount of AGPs can be estimated by taking into account that green coffee galactomannans contain galactose in approximately a 1:10 ratio in relation to mannose[47] and that the amount of arabinose present in galactomannans is very small when compared with the amount of arabinose from AGPs.[48] These calculations revealed an increase in the amount of AGPs from 11.4 g/100 g for the control coffee to 13.4 g/100 g for AGPCoffee, corresponding to an increase of AGPs of 18%.

In the WECoffee model a significantly higher content (34%) of all polymeric sugars was observed in relation to the original coffee. This increase is in accordance with the decrease in the soluble sugar content and the decrease in chlorogenic acids, and also of caffeine and protein contents previously described, as well as the insolubility of the majority of the green coffee cell wall polysaccharides.[47,49]

Reliability of the in Bean Coffee Model. The production of the modified in bean model coffees resulted in a change in the total soluble sugar content and total chlorogenic acid content, but the cell wall sugar composition and content and the amounts of caffeine and protein did not change. These results show that although control and model coffees do not reflect the chemical composition of the original coffee, with the exception of the component incorporated, the remaining chemical constituents were not different from the control coffee. However, because during storage of green coffee significant chemical changes in its composition can occur,[50,51] the original coffee sample was analyzed at the end of this study, after 9 months of storage at room temperature. A significant decrease in the CGA content of the coffee beans to 5.8 g/100 g was observed, corresponding to a 31% decrease, as was a 7% decrease in the total soluble sugar content to 10.9 g/100 g. Although control coffee is not representative of the original coffee used in this study, its chemical composition is still representative of a commercial coffee that can be bought after a certain storage period. On the other hand, the WECoffee model, which is enriched in cell wall material, can give some insights into the relative contribution of the green coffee soluble material and insoluble cell wall material upon roasting.

Table 4. Total Sugar Composition (Grams per 100 g Dry Basis) of Original, Control, and Hot Water Extracted Coffee and "in Bean" Model Green and Roasted Coffees[a]

coffee	Rha	Ara	Man	Gal	Glc	$Glc_{polymeric}$	total	$total_{polymeric}$
				Green				
original	0.14 ± 0.01a	3.47 ± 0.24a	28.9 ± 1.5a	10.2 ± 0.6a	14.9 ± 0.7	8.98 ± 0.65a	57.7 ± 3.9	51.4 ± 3.9a
control	0.10 ± 0.02b	3.57 ± 0.21b	31.1 ± 1.4	10.8 ± 0.3b	13.0 ± 0.8	8.72 ± 0.83	58.6 ± 2.6	54.3 ± 2.6
SucCoffee	0.11 ± 0.02	3.57 ± 0.07	31.8 ± 0.1	10.6 ± 0.1	18.9 ± 0.2	10.1 ± 0.2	65.0 ± 0.1	56.3 ± 0.1
AGPCoffee	0.34 ± 0.03b	4.03 ± 0.09b	32.4 ± 1.3	12.3 ± 0.1b	12.4 ± 1.3	8.79 ± 1.32	61.5 ± 3.4	57.9 ± 3.4
WECoffee	0.19 ± 0.02a	4.79 ± 0.00a	38.2 ± 0.6a	14.2 ± 0.2a	11.9 ± 0.2	11.4 ± 0.2a	69.2 ± 1.5	68.7 ± 1.5a
ANOVA	$p < 0.00145$	$p < 0.00281$	$p < 0.00997$	$p < 0.0011$		$p < 0.06567$		$p < 0.00933$
				Roasted				
original	0.12 ± 0.02a	2.32 ± 0.11a	25.6 ± 0.13a	8.43 ± 0.09a	8.51 ± 0.31	8.40 ± 0.35a	45.0 ± 0.92	44.9 ± 0.92a
control	0.10 ± 0.02b	2.43 ± 0.00bc	26.7 ± 0.82b	8.83 ± 0.11bc	8.76 ± 0.08	8.55 ± 0.08b	46.8 ± 0.99	46.7 ± 0.99b
SucCoffee	0.17 ± 0.01	1.97 ± 0.01b	23.3 ± 0.03b	7.51 ± 0.04b	8.22 ± 0.03	7.99 ± 0.03b	41.1 ± 0.17	40.9 ± 0.17b
AGPCoffee	0.24 ± 0.03b	2.89 ± 0.04c	26.9 ± 0.46	9.42 ± 0.14c	9.18 ± 0.04	8.94 ± 0.04	48.6 ± 0.91	48.4 ± 0.92
WECoffee	0.26 ± 0.02a	2.93 ± 0.04a	28.3 ± 0.47a	9.24 ± 0.12a	9.87 ± 0.18	9.83 ± 0.18a	50.6 ± 0.79	50.5 ± 0.79a
ANOVA	$p < 0.00967$	$p < 0.00025$	$p < 0.00156$	$p < 0.00014$		$p < 0.0026$		$p < 0.00054$

[a] For simplicity, significant differences are shown only for the original, control coffee and WECoffee model and for the control, SucCoffee, and AGPCoffee coffee models. Identical letters in the same column are significantly different.

Chemical Characterization of Roasted Coffee and in Bean Roasted Coffee Models. Original, control, and in bean model coffees were roasted to 8.0 ± 0.1% of organic matter loss, and their chemical composition was determined. The roasting process resulted in a 98% loss of total soluble sugars content of the original coffee (Table 1). The same decrease was observed for the control coffee (97%), SucCoffee (98%), AGPCoffee (95%), and also for the WECoffee (94%). This decrease is similar to that described in the literature[52,53] and shows that all coffees behave similarly with regard to the total soluble sugar degradation during the roasting process.

Roasting also decreased the amount of total CGA in all coffees (Table 2). For the original coffee, a 75% decrease in the amount of total CGA was observed. For the control, SucCoffee, and AGPCoffee, a similar decrease was observed. This decrease is in accordance with a coffee submitted to a degree of roast of 8%.[42] The caffeine content also decreased with the roasting process for all coffees and model coffees studied (Table 3). A decrease of caffeine during roasting has been observed previously, although not as high as observed in our study;[44] nevertheless, a caffeine reduction of 30% during roasting has already been described.[54] The protein content of roasted control coffee is similar to that of the original coffee and AGPCoffee and decreased slightly with roasting. For SucCoffee and WECoffee a slight increase in the protein content was observed upon roasting.

The roasting procedure also promoted a significant decrease in the content of all polymeric sugars (Table 4). The original and control coffees behaved in a similar way during the roasting process with regard to the cell wall sugar degradation, with a 20% decrease in the total cell wall sugars (corrected for the organic weight loss during roasting), whereas the polymeric glucose had a lower decrease (12%), followed by mannose (20%), galactose (25%), and arabinose (37%). This sugar degradation profile is in accordance with previous studies on the coffee cell wall degradation during roasting.[49,55]The AGPCoffee model also behaves similarly to control coffee, for which was observed a 23% decrease in total sugar (glucose, 6%; mannose, 24%; galactose, 30%; arabinose, 33%). For the SucCoffee model, the total polymeric sugar lost was 33% (glucose, 27%; mannose, 33%; galactose, 35%; arabinose, 49%). This roasted model contained significantly lower amounts of all cell wall sugars. At the moment we do not have a plausible explanation for this behavior. Also, for WECoffee, a large decrease (32%) of total cell wall sugars (glucose, 21%; mannose, 32%; galactose, 40%, and arabinose 49%) was observed. This greater decrease in polymeric sugars is explained by the fact that the WECoffee model did not contain the hot water extractable components. In this case the 8% weight loss was accomplished by a higher degradation of the polymeric sugars.

The coffee brown color is an indicator of the browning reactions taking place during roasting. The color of roasted coffee beans was monitored visually on the intact beans, allowing the observation in all coffees of an even distribution of the brown color throughout the beans. To compare the brown color developed during the roasting of the different coffees, the CIELAB color space was used to characterize the coffee powders. Only a minor difference between the color of the original coffee and control coffee (ΔE^* = 1.8) was observed (Supporting Information, Table S1). Although all of the coffees were roasted to the same weight loss, the SucCoffee was the darkest brown coffee (ΔE^* = 8.8), followed by AGPCoffee (ΔE^* = 6.9), and the WECoffee was the lightest brown coffee (ΔE^* = 14.1). The lighter color of WECoffee is explained by its low amounts of soluble sugars and CGA. The darkest color of the SucCoffee model is due to the high total soluble sugar content that was almost completely transformed during the roasting process. This is in accordance with the range of caramelization products known to be derived from sucrose.[56] In addition, the glucose and fructose formed by hydrothermolysis or caramelization of sucrose[57,58] can react through the Maillard reaction with proteins and free amino acids. The identical color of the original and control coffees, and also the similar degradation behavior of the diverse chemical components evaluated, led to the conclusion that the freeze-drying of green coffee beans and their immersion in water did not change significantly the behavior and properties of coffee during the roasting process.

Chemical Composition of Coffee Infusions. Coffee infusions were prepared from the original, control, and in bean model coffees, and the HMWM was isolated from each of the coffee brews.[32] The amount of soluble solids extracted from the original roasted coffee was higher than that extracted for the control coffee (less 35%; Supporting Information, Table S2), although both coffees were in the range of the values described in the literature.[15,31,59,60] For the SucCoffee model, there was a significant increase in the amount of soluble solids extracted when compared to the control coffee (42% increase), whereas for the AGPCoffee model only a slight increase was observed (13%) in relation to the control coffee. For the WECoffee model there was a decrease of 73% in relation to the original coffee. The sugar contents of the soluble solids extracted from the original and control coffees were not significantly different (Supporting Information, Table S3). However, the arabinose, galactose, and mannose contents of control coffee soluble solids were significantly lower than those obtained for the original coffee. SucCoffee model soluble solids contained a significantly higher amount of total sugars (22%) when compared to control coffee. This increase was related to a significant increase in the amount of mannose, arabinose, and galactose. For this coffee model, on average, 12% of the roasted coffee cell wall sugars were extracted, representing an increase of 98% in the cell wall sugar extraction in relation to control coffee. The sugar content of the AGPCoffee model soluble solids was significantly lower (11%) than that observed for the control coffee, and it was related to a significant decrease in the amount of mannose extracted. Although the amount of soluble solids extracted from the WECoffee was the lowest, it presented the highest sugar content of all soluble solids obtained, with an increase in the amount of all sugars in relation to the original coffee and especially in the amounts of rhamnose, arabinose, and galactose. This result shows that, upon roasting, the insoluble cell wall material becomes extractable, leading to the conclusion that the hot water insoluble cell wall polysaccharides of green coffee make a significant contribution to the coffee brew sugar content. The amount of sugars extracted to this coffee infusion represents 28% of the sugars extracted to the original coffee infusion, taking into account the weight loss observed for the WECoffee model during the hot water extraction for building the model. Using the $K_{\text{mix 405nm}}$ for the estimation of the melanoidin content of the soluble solids, it can be observed that control coffee contained a slightly lower $K_{\text{mix 405nm}}$ value (0.53 ± 0.01) than the original coffee (0.60 ± 0.00). The incorporation of sucrose in the SucCoffee model led to an increase in $K_{\text{mix 405nm}}$ (0.77 ± 0.03), and the incorporation of

Table 5. Yield and Chemical Composition (Grams per 100 g Dry Basis) of the High Molecular Weight and Resulting Ethanol Precipitated Fractions of the Original, Control, and Hot Water Extracted Coffee and "in Bean" Model Coffees

	g/100 g coffee	g/100 g HMWM	Rha	Ara	Man	Gal	Glc	total sugars	protein	$K_{mix\ 405nm}$	MBI	phenolics
Original												
HMWM	3.74		1.55	3.02	33.8	14.6	1.16	54.1	16.4	0.64	1.8	6.5
Et50	1.12	30.0	1.11	1.02	53.0	3.46	1.03	58.6	14.0	1.1	3.4	1.5
Et75	0.70	18.6	0.73	5.19	33.4	26.4	0.83	67.2	10.7	0.46	2.0	5.0
EtSn	1.53	40.8	3.21	7.55	11.1	15.4	2.31	39.5	26.8	0.73	1.7	4.6
Control												
HMWM	2.64		2.29	8.65	22.3	22.0	2.72	57.9	17.1	0.67	2.0	7.0
Et50	0.79	30.0	0.26	1.22	60.1	3.26	1.19	66.1	14.8	1.0	3.1	4.0
Et75	0.40	15.3	2.09	11.1	33.6	26.4	3.60	76.8	28.4	0.40	1.3	5.5
EtSn	1.35	51.0	3.91	12.1	2.02	21.4	1.91	41.3	17.5	0.74	2.2	3.6
SucCoffee												
HMWM	3.37		1.99	7.71	43.6	16.4	1.72	66.9	13.6	0.91	3.8	8.4
Et50	0.99	29.5	0.20	1.22	69.9	3.35	5.35	80.0	11.0	1.4	16	4.2
Et75	0.41	12.2	1.90	10.6	23.6	33.9	1.54	71.5	14.5	0.64	3.3	2.6
EtSn	1.67	49.9	3.75	18.3	22.6	18.2	2.11	65.0	18.3	1.1	5.0	7.0
AGPCoffee												
HMWM	2.95		2.72	11.6	20.0	17.6	4.15	56.0	19.4	1.1	3.1	7.4
Et50	0.88	29.9	0.43	2.04	49.4	5.46	4.72	62.1	10.1	1.2	3.9	2.4
Et75	1.12	37.8	2.53	15.9	21.2	32.5	5.51	77.7	5.63	0.80	4.4	2.9
EtSn	0.65	22.1	5.24	14.3	4.41	15.4	5.83	45.2	27.0	2.5	6.8	6.4
WECoffee												
HMWM	0.68		1.33	2.43	25.6	13.0	1.09	43.5	10.8	0.52	1.1	3.1
Et50	0.25	36.4	1.80	0.88	48.4	3.27	1.90	52.9		0.93	2.4	2.4
Et75	0.22	32.1	0.54	4.84	17.3	23.5	0.41	28.6	10.4	0.10	0.2	3.4
EtSn	0.08	11.7	2.98	8.48	9.05	14.6	2.00	32.8	10.9	2.4	4.3	1.9

type II AGPs led to a decrease in the $K_{mix\ 405nm}$ (0.42 ± 0.01) of the soluble solids. The extraction of green coffee with hot water (WECoffee) also resulted in a decrease of the $K_{mix\ 405nm}$ (0.46 ± 0.02) of the soluble solids in relation to the original coffee.

Chemical Composition of the HMWM. The amounts of HMWM for all roasted coffee soluble solids, with the exception of the WECoffee model, were similar (15−16%). For the WECoffee model, the HMWM represented only 10% of the soluble solids (Supporting Information, Table S2). When recalculated in relation to the mass of roasted coffee, the amount of HMWM for the original coffee was closer to the values reported in the literature.[15,31] For the SucCoffee and AGPCoffee models, the amount of HMWM isolated was higher than that obtained for the control coffee (12 and 28%, respectively). The amount of HMWM isolated from the WECoffee roasted coffee was 82% lower than the amount extracted from the original coffee. For the control and original coffees, 16 and 21%, respectively, of the brown color of the soluble solids was recovered in the HMWM (Supporting Information, Table S2). The HMWM from SucCoffee and AGPCoffee models presented significantly higher $K_{mix\ 405nm}$ values when compared to the control coffee (Table 5). For the SucCoffee model only 17% of the soluble solids brown color was recovered in the HMWM, but for the AGPCoffee model, the brown color recovered in the HMWM represented 42% of the soluble solids brown color. Both sucrose and AGPs are responsible for the browning of the HMWM, although much of the color developed in the SucCoffee model was from low molecular weight compounds. For the WECoffee, the HMWM presented the lower $K_{mix\ 405nm}$, and it was also the coffee model with the lower amount of brown color recovered in the HMWM (11%).

Sugars Composition. The chemical composition of the HMWM was determined with regard to their total sugar content (Table 5) and protein, based on amino acid composition (Supporting Information, Table S4). The total sugar contents of the original and control coffees were nearly the same (54 and 58%, respectively), whereas for the original coffee mannose was the main sugar (34%), followed by galactose (15%) and arabinose (3%), and for the control coffee the amounts of mannose (22%) and galactose (22%) were identical and the amount of arabinose (9%) was higher than that found for the original coffee. This change in composition was mainly due to the decrease in the extraction of mannose from the control coffee (53%) in relation to the original coffee, although a slight increase in the amounts of galactose and arabinose (18%) extracted was also observed.

The HMWM obtained for the SucCoffee model was the richest in sugars (67%). Its sugar composition was significantly different from that of the control coffee, with mannose as the main sugar (44%), followed by galactose (16%) and arabinose (8%). This change in composition of the HMWM of SucCoffee was due to a 2.6 increase in the extraction of mannose from roasted SucCoffee model in relation to the control coffee, as the amounts of galactose and arabinose extracted were the same as observed for the control coffee (0.80 g/100 g roasted coffee).

The HMWM obtained for the AGPCoffee model presented a total sugar amount and composition similar to the control coffee. Nevertheless, the amount of arabinose was higher. The relative amount of sugars distributed between the high and low molecular weight fractions (calculated by difference between total sugars in total solids and total sugar in the HMWM) was for the original coffee, control coffee, and SucCoffee HMWM of approximately 50% (44−51%) of the total sugars in soluble solids. The AGPCoffee showed a higher amount of sugars

recovered in the HMWM (58%). This coffee model was distinct from the other ones, as the amounts of galactose and arabinose recovered in the HMWM accounted for 62 and 78%, respectively, of total galactose and arabinose. For the other samples, only 40% on average of the galactose and arabinose was recovered in the HMWM. These results showed that for the AGPcoffee model, the galactose and arabinose, upon roasting, were still mostly extracted in a high molecular weight form. For the WECoffee, the sugars recovered in the HMWM were only 16% of the total sugars in soluble solids.

Protein and Melanoidins. The amount of protein of the HMWM of the original and control coffee was similar (Supporting Information, Table S4), representing nearly 92% of the total nitrogen present in the HMWM (Table 5). The amount of melanoidins in the HMWM, estimated by subtracting from the total material the amount of polysaccharides and protein determined (unknown material), were nearly the same in the original and control HMWM. The same amount of melanoidins was also found in the AGPCoffee model, but a lower amount was observed for the SucCoffee model and an increase was observed for the WECoffee model. No correlation was observed between the $K_{\text{mix 405nm}}$ and the amount of melanoidins, defined as the amount of unknown material in the HMWM. For the SucCoffee and AGPCoffee models, although they had the highest $K_{\text{mix 405nm}}$ values, the amount of unknown material was lower compared to the control coffee. Although no increase in the amount of melanoidins in the HMWM for SucCoffee and AGPCoffee occurred, the enrichment of the coffees with sucrose and AGP increased significantly the brown color. This shows that in both models the HMWM did not contain more melanoidins than the control coffee, but contained browner melanoidins. These results allow one to infer that these two components (sucrose and AGP) are important for the formation of melanoidin brown color upon coffee roasting.

Melanoidin Browning Index (MBI). To integrate brown color (by measurement of the $K_{\text{mix 405nm}}$) and HMWM unknown material (estimated by the difference of the material found to be polysaccharides and proteins) of coffee melanoidins and to infer the relative importance of sucrose, AGPs, and water-soluble green coffee components on coffee melanoidin formation, the MBI, calculated by dividing the $K_{\text{mix 405nm}}$ values by the relative amount of unknown material, is proposed (Table 5). The MBI corresponds to the absorbance at 405 nm by unit amount of unknown material. Sucrose and AGP had a higher effect on the color of melanoidins, with a 1.9 times increase of the MBI for the SucCoffee and a 1.6 times increase of the MBI for the AGPCoffee, when compared to control coffee. For the WECoffee, the MBI decreased in relation to the original coffee, although the HMWM of the WECoffee model contained the highest amount of unknown material of all the coffees and models studied.

Phenolic Compounds. The amount of recovered phenolic compounds after alkaline fusion of the original coffee HMWM, expressed as 3,4-dihydroxybenzoic acid equivalents, was similar to that released for the control coffee, corresponding to nearly 20% of the HMWM unknown material (Table 5). The amount of phenolic compounds recovered in the HMWM of the original and control coffee correspond, on average, to 2.8% of the green coffee CGA. For the SucCoffee and AGPCoffee models, the amount of condensed phenolics released after alkaline fusion of the HMWM was not significantly different from that recovered from the control coffee, corresponding to 35 and 21% of the HMWM unknown material and to 4.4 and 3.3% of the green coffee CGA, respectively. It is evident from these results that condensed phenolic compounds constitute a significant amount of the HMWM unknown material, but a direct relationship between the amount of released phenolic compounds and the MBI does not exist.

Fractionation of the HWMW by Ethanol Precipitation. This fractionation procedure allows a crude differentiation of the HMWM polysaccharide and melanoidins with different carbohydrate compositions in galactomannans and galactomannan-enriched melanoidins (Et50), arabinogalactans and arabinogalactan-enriched melanoidins (Et75 and EtSn), and melanoidins with low amount of carbohydrates (EtSn). For the original, control and SucCoffee coffees the EtSn was the major fraction (41−51% of the HMWM) followed by Et50 (30%) and Et75 (15−17%). The $K_{\text{mix 405nm}}$ values observed for each of the three fractions were similar for the original and control coffees (Table 5). For the SucCoffee, the $K_{\text{mix 405nm}}$ values of all fractions were consistently higher than that of the control coffee. For the AGPCoffee model a change in the relative amount of the three fractions was observed, with Et75 representing the major fraction (38% of the HMWM), followed by Et50 (30%) and EtSn (22%). For this model, only the fractions enriched with AGP (Et75 and EtSn) presented higher $K_{\text{mix 405nm}}$ values (Table 5). For the WECoffee model, the Et50 and Et75 fractions represented almost an equal amount of the HMWM, with EtSn representing only 12% of the HMWM. The color of the Et50 and Et75 fractions was lighter than the original coffee, and the EtSn was darker.

For the original and control coffees, the majority of the color was recovered in the Et50 (45−52%) and EtSn (45−58%) fractions and in very low percentage in the Et75 fraction (8−12%). The MBI of the Et50 fractions showed the highest values (3.1−3.4), and the Et75 and EtSn fractions showed MBI values close to each other (1.3−2.2) and lower than those observed for the Et50 fraction (Table 5). For the SucCoffee model, an increase in the amount of sugars in each fraction was observed. A significant increase in the MBI was observed for all fractions in relation to control coffee. Sucrose had a high effect in the color of the HMWM and ethanol-precipitated fractions but did not increase the amount of melanoidins calculated by difference.

The chemical composition of the ethanol-precipitated fractions of AGPCoffee model was similar to that obtained for the control coffee, except for the Et75 fraction, for which was observed an increase in the amount of arabinose + galactose, corresponding to an enrichment of AGPs, as expected considering the incorporation of AGP. Although there was a slight increase in the MBI of the Et50 fraction when compared with the control coffee, the highest increase in the MBI was observed for the Et75 and EtSn fractions, the fractions rich in arabinogalactans. These increases were even higher than those observed for the SucCoffee for these two fractions. For the WECoffee, the composition of the Et50 fraction was similar to that of the original coffee, although presenting a lower MBI. Nevertheless, the sugar contents of the Et75 and EtSn fractions were significantly lower than those observed for the corresponding fractions obtained for the original coffee. The MBI value for the Et75 fraction was the smallest of all values obtained. The amount of the EtSn fraction was very low, representing only 11% of the HMWM and 0.08 g/100 g of roasted coffee. The amount of condensed phenolic structures released by alkaline fusion of the ethanol-precipitated fractions

was not significantly different ($p < 0.05$) for the control, SucCoffee, and AGPCoffee models.

Insight into the Mechanism of Coffee Melanoidin Formation. The amount of unknown material and the intensity of the brown color of the HMWM are the two main parameters used for the evaluation of melanoidin formation during coffee roasting. The use of these two parameters has its own drawbacks, as some of the unknown material may not be related to the coffee melanoidin structure and amount. On the other hand, the $K_{mix\ 405nm}$ has a direct relationship to the amount of different melanoidin populations only if the different structures have the same specific extinction coefficient, which seems improbable in the case of the coffee heterogeneous matrix. The results obtained in this work show that the formation of unknown material and the development of the brown color during coffee roasting can have distinct origins; therefore, to handle simultaneously the two concepts used for coffee melanoidin evaluation, the MBI was proposed.

The total soluble sugar content of coffee, including sucrose, had a strong effect on the color formation of the HMWM and all of the ethanol-precipitated fractions. This is reflected in the significant increase of the MBI for all fractions when compared with the control coffee. On the other hand, the incorporation of sucrose did not result in an increase in the amount of unknown material present in the HMWM or in the amount of HMW unknown material extracted from the roasted coffee beans. These results show that the contribution of sucrose to coffee melanoidin formation is probably related to the formation of colored low molecular weight structures attached to the polysaccharides and protein or protein fragments.

The melanoidin fractions enriched with AGPs (Et75 and EtSn) obtained in the AGPCoffee model were the brownest, which is in agreement with Bekedam et al.[16] The influence of AGPs on coffee color might be related to the reactivity of the arabinose side chains, as they are predominantly lost during the roasting process[16,17,31,49,55] and can form furfural in high yield, which, together with sucrose, appears to be the major furfural precursor in roasted coffee.[61] The arabinose, combined with the protein moiety of the AGPs, can yield powerful colorants.[62] Nevertheless, even in the AGPCoffee model, the Et50 accounted for nearly 30% of the HMWM brown color, and in the original, control, and SucCoffee accounted for, on average, 47% of the HMWM brown color. The Et50 fraction is mainly composed by transformed galactomannans and galactomannan-enriched melanoidin populations,[17,18,63] and these results showed that the galactomannan-derived melanoidins accounted for a significant percentage of the brown color, as they also correspond to a significant percentage of the HMWM (on average 30% for the original coffee and coffee models). The amount of unknown material present in the HMWM of the AGPCoffee model was not significantly higher than the control coffee, although the amount of unknown material extracted from the original roasted coffee was 18% higher in relation to the control coffee, mainly due to the increase of the Et75 fraction. The contribution of Et75 to the total color of the HMWM was fairly low for the original, control, and SucCoffee (on average 10%). This fact is related to the low percentage of Et75 fraction in relation to the total HMWM material (on average 15%). The EtSn fraction is formed by AGP-derived melanoidins but also contains a significant amount of melanoidins with low carbohydrate content.[17,18] This fraction contains the majority of the color of the HMWM due to their abundance. Nevertheless, the EtSn fraction had a low MBI for the original and control coffee, due to the high amount of unknown material. The MBI of the EtSn fraction was higher for the SucCoffee and AGPCoffee, showing that both sucrose and AGP had a significant effect on this important melanoidin fraction.

One obvious and somewhat expected conclusion is that the green coffee soluble solids are important for the coffee melanoidin formation. The decrease in the hot water extractable components in the WECoffee decreased by 76% the amount of unknown material extracted from the roasted coffee when compared to the original coffee. Also, the $K_{mix\ 405nm}$ of the HMWM was the lowest of all the HMWM obtained, although it contained the highest amount of HMWM unknown material and, therefore, the lowest MBI. Although the WECoffee model contains mainly cell wall polysaccharides (69% of dry weight) and low amounts of water-soluble coffee components, its HMWM contains a large proportion of unknown material, probably resulting from the pyrolysis of cell wall polysaccharides. Nevertheless, there is still missing an explanation for the nature of the unknown material and for the origin of coffee melanoidins brown color. CGAs had been implicated in the brown color development of coffee melanoidins during coffee roasting.[17,18,23] Although condensed phenolic compounds corresponded to a considerable percentage of the HMWM unknown material (20%) and ethanol-precipitated fractions (3−35%) and can also probably contribute to the brown color, no significant correlation was observed for the amount of released condensed phenolic compounds from the ethanol-precipitated fractions and the MBI.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

AGP, arabinogalactan-protein; AGPCoffee, "in bean" model coffee made by direct incorporation of gum arabic; CGA, chlorogenic acids; CoQA, coumaroylquinic acids; CQA, caffeoylquinic acid; CGL, cinnamoyl-1,5-γ-quinolactones; diCQA, dicaffeoylquinic acid; Et50, high molecular weight material precipitated in 50% ethanol solution; Et75, high molecular weight material precipitated in 75% ethanol solution; EtSn, high molecular weight material soluble in 75% ethanol solution; FQA, feruloylquinic acids; HMWM, high molecular weight material; MBI, melanoidin browning index; MW, molecular weight; SucCoffee, "in bean" model coffee made by direct incorporation of sucrose; WECoffee, coffee shell made by exhaustive hot water extraction of green coffee.

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