JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Roasting Effects on Formation Mechanisms of Coffee Brew Melanoidins

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The effect of the roasting degree on coffee brew melanoidin properties and formation mechanisms was studied. Coffee brew fractions differing in molecular weight (Mw) were isolated from green and light-, medium-, and dark-roasted coffee beans. Isolated fractions were characterized for their melanoidin, nitrogen, protein, phenolic groups, chlorogenic acid, quinic acid, caffeic acid, and sugar content. It was found that the melanoidin level in all fractions correlated with both the nitrogen and the protein content. The melanoidin level also correlated with the phenolic groups' level and ester-linked quinic acid level. It was concluded that proteins and chlorogenic acids should be primarily involved in melanoidin formation. Initial roasting, from green to light-roasted beans, especially led to the formation of intermediate Mw (IMw) melanoidins when compared to high Mw (HMw) melanoidins. Indications were found that this IMw melanoidin formation is mainly due to Maillard reactions and chlorogenic acid incorporation reactions between chlorogenic acids, sucrose, and amino acids/protein fragments. Additionally, it was found that prolonged roasting predominantly led to formation melanoidins with a high Mw. Furthermore, arabinogalactans seem to be relatively more involved in melanoidin formation than galactomannans. It was hypothesized that chromophores may be formed or attached through the arabinose moiety of arabinogalactan proteins (AGP). Finally, it could be concluded that galactomannans are continuously incorporated in AGP-melanoidins upon roasting.

KEYWORDS: Coffee; brew; melanoidins; degree of roast; formation mechanisms

INTRODUCTION

Coffee is a popular beverage that is consumed worldwide by many people every day. The total coffee consumption is estimated to be almost 7 billion kilograms of coffee beans annually (*1*). The roasting process of coffee beans leads to the formation of the characteristic coffee aroma and the dark-colored compounds. These flavor compounds are formed mainly as the result of the Maillard reaction (*2*) that takes place between carbohydrates or degraded carbohydrates (*3*) and proteins (*4*). These dark-colored compounds are referred to as melanoidins, and they make up to 25% of the dry matter in coffee brew (*5*). The chemical structure of coffee melanoidins is extremely complex and is still largely unknown. This complexity is due to the fact that many green coffee bean components might play a role in melanoidin formation (*6*). For example, it was shown that next to common Maillard reactions, incorporation of chlorogenic acid plays an important role in coffee brew melanoidin formation as well (*7*). Because complete knowledge on melanoidin structures is lacking, melanoidins are generally defined as brown, nitrogenous macromolecular compounds that absorb light at 405 nm (*5, 8–11*). The introduction of the parameter $K_{mix\ 405nm}$, which is the specific extinction coefficient at 405 nm, allowed quantification of the melanoidin level in a coffee fraction (*11*).

Melanoidins are not only of interest because of their contribution to the color of coffee brew but also for their flavor-binding properties (*9, 12–16*), antioxidative capacity (*5, 10, 17, 18*), metal-chelating properties (*18, 19*), and reactivity in coffee brew (i.e., aging of coffee) (*20*). It is also therefore that a renewed interest in melanoidin structure can be observed in literature. Recent research has focused on the involvement of chlorogenic acids (*7, 21*), galactomannans (*21*), and arabinogalactan proteins (AGPs) (*22*) in melanoidin formation as well as on digestibility (*23, 24*), ionic charge properties (*22*), molecular size properties (*25*), and acidifying properties (*7*) of melanoidins. Even though this renewed interest in coffee melanoidins led to an improved understanding of melanoidin structures, the mechanisms involved in coffee melanoidin formation are not or at most poorly

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10.1021/jf800999a CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/05/2008

understood. The most logical approach to obtain insight in melanoidin formation mechanisms is probably the comparison of coffees prepared from beans with varying degrees of roast. Light-roasted coffees should contain relatively more intermediate melanoidins, whereas dark-roasted coffees should contain more melanoidins that have evolved toward the final melanoidin structures. In literature, information can be found on the effects of the degree of roasting on coffee composition and coffee properties (*10, 12, 17, 26–28*). In some studies, this comparison was even performed to obtain information on the melanoidin structure (*21*). However, no attempts to understand underlying melanoidin formation mechanisms were reported.

The objective of this study was to investigate the effect of roasting degree on melanoidin development in coffee brews. To this end, coffee brews were prepared from beans with varying degrees of roast, then melanoidin populations differing in molecular size were isolated, and melanoidin chemical and chromatographic characteristics were investigated.

MATERIALS AND METHODS

Materials. Green Colombian coffee beans (*Coffea arabica*) were roasted to three degrees of roast by a local factory, and this factory provided both the green and the roasted beans. The degree of roast, which is the total weight loss upon roasting, of the light-, medium-, and dark-roasted beans was 14.7, 16.4, and 19.2% (w/w), respectively, and was 6.1, 8.0, and 11.1% (w/w) on a dry matter basis, respectively. The colors of the light-, medium-, and dark-roasted beans were 60, 50, and 40, respectively, according to the color test Neuhaus (CTN). The roasting degree was indicated as "green", "light", medium", and "dark" for green coffee and light-, medium-, and dark-roasted coffee fractions, respectively. Chemicals were bought from Sigma Aldrich (Sigma Chemical Corp., St. Louis, MO) in the highest purity available.

Preparation of Coffee Brew. Green (frozen with liquid nitrogen) and roasted coffee beans were ground using a Retsch ZM200 mill equipped with a 0.4 mm sieve and operating at maximum speed (18000 rpm). Coffee brew was prepared at 90 °C for 15 min as described previously (*11*). For characterization purposes, part of the brew was lyophilized, yielding "brew". The major part of the brew was used for further isolation.

Isolation of High Molecular Weight (HMw) Coffee Brew Material. HMw material was obtained from brew by diafiltration (cutoff of 3 kDa) as described previously (*11*). The retentate and dialysate were lyophilized, yielding a HMw fraction ("HMw") and a fraction with a lower Mw ("DF-dialysate"), respectively.

Isolation of Intermediate (IMw) and Low Mw (LMw) Coffee Brew Material. IMw and LMw materials were obtained from defatted DF-dialysate sample by membrane dialysis. Aqueous DF-dialysate solutions (0.5 L, 100 g/L) were dialyzed using a Visking size 9 dialysis membrane with a cutoff of 12−14 kDa (Medicell International Ltd., London, United Kingdom) for 3 days at 4 °C against 5 L of demineralized water with four water renewals. The first two dialysate fractions were pooled and lyophilized, yielding the LMw fraction ("LMw"), whereas the last three dialysate fractions were discarded. The retentate was lyophilized yielding the IMw fraction ("IMw").

AGP Isolation. AGP was isolated from HMw coffee material in duplicate using the Yariv reagent as was described previously (*22*). The dialyzed AGP-containing fraction was lyophilized, yielding "AGP".

Defatting of Coffee Samples. Lyophilized coffee brew samples were defatted by Soxhlet extraction using a Soxtherm, which was connected to a Multistat system (Gerhardt, Königswinter, Germany), as described previously (*22*). The solvent used for extraction was dichloromethane.

Determination of the Unbound and Total Caffeic Acid (CA) and the 5-Caffeoylquinic Acid (5-CQA) Content. For determination of the unbound CA and 5-CQA contents, an aqueous sample solution was centrifuged, and the supernatant was subjected to analysis. For determination of the total CA content, samples were saponified in the presence of ascorbic acid and ethylenediaminetetraacetic acid prior to analysis as described previously (*7*). Aqueous and saponified samples were analyzed by reversed-phase chromatography using a 150 mm × 4.6 mm i.d. XTerra MS C18 3.5 μm column in combination with a 20 mm × 3.9 mm i.d. XTerra MS C18 3.5 μm guard column (Waters, Milford, MA) using the procedure described previously (*7*). Aqueous 5-CQA and CA solutions were used as reference for the 5-CQA and unbound CA contents, respectively. Saponified CA solutions were used as reference for the total CA contents. Experiments were performed at least in duplicate. The average coefficient of variation was 1%.

Table 1. Yields of the Isolated Coffee Brew Fractions

	brew (%, w/w)	HMw (%, w/w)	IMw (%, w/w)	LMw (%, w/w)	recovery (%, w/w)	AGP[a] (%, w/w)
green	18.9	17.6	5.2	62	85	0.72
light	15.9	14.8	19.5	54	88	0.93
medium	16.0	16.7	22.5	51	90	0.96
dark	16.0	17.2	24.7	46	88	0.69

[a] On green bean basis. [b] On brew dry matter basis. [c] Combined HMw, IMw, and LMw yields.

Determination of the Unbound and Total Quinic Acid (QA) Content. For the determination of the unbound QA content, an aqueous sample solution was centrifuged, and the supernatant was subjected to analysis. For the determination of the total QA content, samples were saponified prior to analysis as described previously (*7*). Aqueous and saponified samples were analyzed by ion-moderated partitioning chromatography using a 300 mm × 7.8 mm i.d. Aminex HPX 87H column equipped with a cation H^+ guard column filled with AG 50W-X4 (Bio-Rad, Hercules, CA) using the procedure described previously (*7*). Aqueous and saponified QA solutions were used as reference for the unbound and total QA contents, respectively. Experiments were performed at least in duplicate. The average coefficient of variation was 3%.

Analysis of Nitrogen Content. The nitrogen content of various samples was estimated according to the Dumas method using an NA2100 nitrogen and protein analyzer (Carlo Erba Instruments, Milan, Italy) according to the manufacturer's instructions. Methionine was used as a standard, and the average coefficient of variation was 2%.

Sugar Analysis. The neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (*29*) using inositol as an internal standard. Briefly, samples were prehydrolyzed with 72% (w/w) H_2SO_4 for 1 h at 30 °C followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C, and the constituent sugars released were analyzed as their alditol acetates. The uronide content was determined by the automated colorimetric *m*-hydroxydiphenyl method (*30, 31*).

Total Phenolic Groups Level. The total phenolic groups content of the coffee samples was determined with the Folin−Ciocalteau assay as described previously (*11*). The used reference compound was 5-CQA, and the average coefficient of variation was 2%.

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed using liquid phase hydrolysis in 6 M HCl at 110 °C for 24 h. Amino acid analyses were performed using a Hewlett-Packard Aminoquant 1090 M using an automated two-step precolumn derivatization with two different reagents, *o*-phthalaldehyde for primary and 9-fluorenylmethylchloroformate for secondary amino acids (*32*).

Specific Extinction Coefficient of Coffee Material at 280, 325, and 405 nm. The absorption of aqueous coffee fractions (1 g/L) was determined at 280, 325, and 405 nm using a Hitachi U-3000 spectrophotometer (Hitachi, Tokyo, Japan). The solutions were diluted in case the absorption was higher than 1.3. The specific extinction coefficients K_{mix} (L/g/cm) at different wavelengths and the K_{mix} ratios were calculated as previously described by Bekedam et al. (*11*). The average coefficient of variation was 1% of the K_{mix} values.

RESULTS AND DISCUSSION

Effect of Roasting on Isolation Yields of Coffee Brew Fractions. It is shown in **Table 1** that all brews that were prepared from roasted beans had a yield of 16%. It is known that the extractability of some coffee bean compounds, for

Table 2. Melanoidin Levels ($K_{mix\ 405nm}$) in Various Coffee Fractions[a]

	brew (L/g/cm)	HMw (L/g/cm)	IMw (L/g/cm)	LMw (L/g/cm)	AGP (L/g/cm)
green	0.14	0.25	0.24	0.05	ND
light	0.57	0.74	1.05	0.31	0.73
medium	0.67	1.11	1.08	0.36	1.07
dark	0.71	1.66	1.03	0.32	1.59

[a] On the basis of the fraction. ND, not determined.

example, galactomannans, might increase upon roasting (*3*) and that the extractability of other coffee bean compounds like amino acid containing components might decrease upon roasting (*33*). However, the amount of material that becomes soluble and the amount that becomes insoluble are similar for the different degrees of roast.

These constant extraction yields allowed the comparison of HMw, IMw, and LMw yields when expressed on the basis of the corresponding brew as well (**Table 1**). The recovery of the HMw, IMw, and LMw isolation procedure from the brews was on average 88%, and the limited loss was due to discarding of part of the dialysate after membrane dialysis. Roasting of green to light-roasted beans led to a decrease in HMw yield from 18 to 15% (**Table 1**). This decrease might be due to protein denaturation and aggregation during roasting but might also be due to polysaccharide and protein degradation. Upon prolonged roasting from light- to dark-roasted beans, the yield of both the HMw and the IMw fractions increased. This increase might be due to chemical reactions, like condensation reactions, that lead to transformation of LMw compounds into IMw and HMw compounds, likely melanoidins (*34*). Additionally, it could also be that specific HMw coffee bean compounds, like galactomannans, are more readily extracted after prolonged roasting (*3*). In green brew, the IMw fraction was only 5%. The size of this IMw fraction increased rapidly to 20% in light roast and finally up to 25% in dark roast brew. This steep increase in yield of IMw material implies degradation of macromolecules to IMw molecules upon roasting. Alternatively, LMw components might transform into to IMw molecules as well.

Roasting Effects on Melanoidin Levels in Various Brew Fractions. The melanoidin level is represented by the $K_{mix\ 405nm}$ value (*11*), and the melanoidin level of coffee fractions is shown in **Table 2**. The $K_{mix\ 405nm}$ value for green coffee was expected to be zero since no roasting products are present. However, a value of 0.1 for green brew was obtained, which was due to turbidity. Roasting led to a marked increase of melanoidins in the brews (**Table 2**). These results confirm that the formation of water-soluble melanoidins occurs continuously upon roasting of coffee beans.

When looking to the HMw, IMw, and LMw fractions of the coffee brews (**Table 2**), it stands out that the melanoidin level in the IMw and LMw fractions remained more or less constant, whereas the melanoidin level in the HMw fractions increased upon prolonged roasting. This suggests that IMw material consists of highly reactive compounds that participate in chemical reactions during the first moments of roasting. Oppositely, HMw material should then consist of somewhat less reactive compounds that react slower but more continuously throughout the roasting process, leading to a continuous formation of HMw melanoidins during roasting. It could also be that IMw melanoidins might act as intermediate melanoidin structures, which evolve toward HMw melanoidins upon prolonged roasting.

It should be realized though that the yields of the fractions varied upon roasting (**Table 1**), which could mask the contribution of a fraction to the whole brew. Therefore, the $K_{mix\ 405nm}$ value was also expressed on the basis of the brews (**Figure 1**). This allowed comparison of fractions with different molecular weights and comparison of fractions with different degrees of roast. These results show that, in absolute terms, coffee brew melanoidins accumulate in both the IMw and HMw material upon prolonged roasting. In light-roasted brew, the contribution of IMw melanoidins is larger than the contribution of HMw melanoidins. However, the contribution of HMw melanoidins increases more rapidly than the contribution of IMw melanoidins upon prolonged roasting. As a result, the contribution of HMw melanoidins is larger of IMw melanoidins. Investigation of these fractions for their chemical composition should provide more insight in melanoidin formation mechanisms.

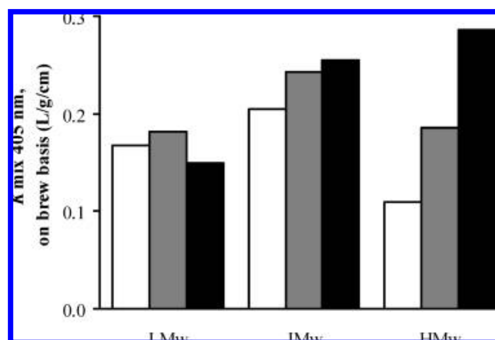

Figure 1. Melanoidin levels of HMw, IMw, and LMw material from light- (white bars), medium- (gray bars), and dark (black bars)-roasted coffees expressed on the basis of the corresponding brews.

Table 3. Nitrogen Content and Total Amino Acid Content of Various Coffee Fractions[a]

	brew (%, w/w)	HMw (%, w/w)	IMw (%, w/w)	LMw (%, w/w)	AGP (%, w/w)
nitrogen					
green	4.3	11.3	7.4	2.3	1.2
light	3.0	1.2	2.7	3.6	0.8
medium	2.9	1.4	2.6	3.7	1.0
dark	3.0	1.7	2.4	3.8	1.5
total amino acids					
green	16.9	68.6	40.9	1.8	6.4
light	6.8	5.5	12.9	3.4	2.2
medium	6.0	5.7	11.9	3.9	2.9
dark	6.7	7.0	10.8	5.0	4.8

[a] On the basis of the fraction's dry matter.

Nitrogen and Total Amino Acid Contents in Coffee Brew Fractions. Previously, it was shown that the nitrogen level was closely related to the melanoidin level (*11*). The nitrogen and total amino acid levels of various coffee fractions are shown in **Table 3**. The roasted brews all had nitrogen levels of about 3%, and the nitrogen level did not show correlation with the melanoidin level that increased upon prolonged roasting (**Table 2**). This is due to the presence of other nitrogenous LMw compounds like caffeine and trigonelline. Therefore, the nitrogen level in brew and LMw does not provide structural information on melanoidins. Nitrogen levels of 11.3% in green HMw and 7.4% in green IMw indicated the presence of 14% proteins in green bean brew when using a nitrogen-to-protein factor of 5.5 (**Table 3**) (*11*). Amino acid analysis revealed that green HMw contains 69% proteins in HMw and IMw contains 41% proteins (**Table 3**). Amino acids and/or protein fragments that are incorporated in HMw and IMw melanoidin material were for clarity reasons still denominated as proteins.

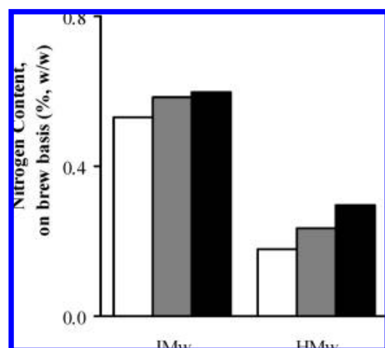

Figure 2. Nitrogen contents of HMw and IMw material from light- (white bars), medium- (gray bars), and dark (black bars)-roasted coffees expressed on the basis of the corresponding brews.

Roasting of green to light roast beans led to a lower nitrogen and protein level in HMw material (**Table 3**). It was calculated that the total amount of proteins was 18 times lower for light-roasted than for green bean HMw material. The calculated total amount of protein/amino acids remained constant in IMw and LMw material upon roasting from green to light-roasted beans. In total, 66% of the proteinous material in green coffee brew was not recovered in light-roasted brew. This enormous loss may be due to participation of amino acids in Maillard-like reactions and due to protein denaturation leading to limited protein extractability. Comparison of nitrogen and protein losses revealed that 25% of the total amino acid loss in HMw should be due to participation of amino acids in Maillard-like reactions and that 75% should be due to limited solubility of denatured proteins.

When light coffee was further roasted to dark coffee, the nitrogen and protein levels increased in HMw material but decreased in IMw material (**Table 3**). The contribution of each Mw fraction to the brew was calculated. These calculations revealed that the amount of amino acids/proteins increased by a factor 1.3 for LMw, 1.1 for IMw, and 1.5 for HMw material when roasting from light to dark roast. Thus, the amount of amino acids/proteins increases for all molecular sizes upon roasting, which indicates that prolonged roasting results in solubilization of denatured proteins. This improved extractability might be caused by chemical modifications of the protein, like chlorogenic acid incorporation, resulting in more proteins in HMw coffee material. Additionally, degradation of proteins into smaller fragments should also occur because the total amount of proteinous material increased in the lower Mw fractions as well.

With respect to melanoidins, it can be seen that the melanoidin level (**Figure 1** and **Table 2**), the nitrogen content, and protein content (**Figure 2** and **Table 3**) all significantly increased in HMw material upon prolonged roasting. The content of these compounds also increased, although less pronounced, in IMw material upon prolonged roasting (**Figures 1** and **2**). These findings indicate once again that the nitrogen and the melanoidin levels are closely related and that nitrogenous compounds should be directly involved in melanoidin formation. It is remarkable though that the nitrogen to melanoidin ratio was higher for all roasted IMw fractions than for the HMw fractions. A possible explanation for this could be that IMw material contains relatively many protein-based melanoidin structures (**Table 3**), while HMw material contains more carbohydrate-like melanoidin structures as will be discussed below.

Phenolic Groups and the 5-CQA Levels in Various Brew Fractions. The level of phenolic groups in brews with varying degree of roast was quite constant, which indicated that coffee bean phenolics are quite unreactive (**Table 4**). However, the level of 5-CQA, the most abundant chlorogenic acid and most abundant phenolic compound in green beans, decreased drastically from 16 to less than 2% (**Table 4**). Additionally, it was found that the phenolic groups level in the HMw fractions, which were all free from unbound 5-CQA, increased gradually from 3% in green coffee HMw up to 18% in dark-roasted HMw. These findings were in line with literature (*35*) and showed that coffee bean phenolics are quite reactive. These results also indicated that incorporation of phenolic compounds in HMw coffee material is a reaction that occurs continuously upon prolonged roasting.

Table 4. Phenolic Groups and 5-CQA Level of Various Coffee Fractions[a]

	brew (%, w/w)	HMw (%, w/w)	IMw (%, w/w)	LMw (%, w/w)	AGP (%, w/w)
phenolic groups[b]					
green	24	3	21	24	2
light	24	9	21	24	6
medium	23	13	19	23	10
dark	21	18	18	19	14
5-CQA					
green	15.7	0.0	9.7	18.2	0.0
light	4.2	0.0	0.8	5.8	0.0
medium	2.9	0.0	0.4	4.3	0.0
dark	1.5	0.0	0.2	2.3	0.0

[a] On the basis of the fraction's dry matter. [b] As chlorogenic acid equivalents.

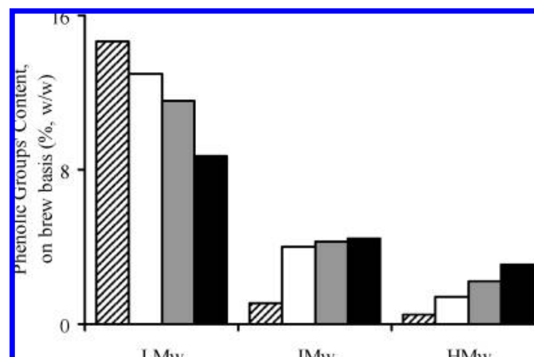

Figure 3. Melanoidin levels of HMw, IMw, and LMw material from green coffee (striped bars) and light- (white bars), medium- (gray bars), and dark (black bars)-roasted coffees expressed on the basis of the corresponding brews.

Expression of the phenolic groups' level on the basis of the brews instead of the fractions itself eliminates the effect of fraction sizes, which then shows the total amount of phenolics present in each fraction relative to the brew (**Figure 3**). Now, it is even more evident that the amount of phenolic groups in HMw material steadily increased upon roasting (**Figure 3**). Additionally, it was found that the pronounced increase in IMw yield concealed that the total quantity of phenolic groups in IMw material increased upon roasting as well (**Figure 3**). Thus, the total amount of phenolic groups increased in both the HMw and The IMw material upon roasting. As a result, the total quantity of phenolics in LMw material decreased upon roasting (**Figure 3**). It was previously shown that chlorogenic acids are incorporated in melanoidins upon roasting (*7*). The results presented in **Figure 3** indicate that roasting leads to a continuous transfer of green bean LMw phenolics, that is, chlorogenic acids, into IMw and especially HMw coffee material, likely melanoidins.

Comparing results for HMw and IMw fractions, it was found that the ratio between the amount of phenolic groups and the

Table 5. Unbound and Total Quinic and CA Levels of Various Coffee Fractions[a]

		brew (%, w/w)		HMw (%, w/w)		IMw (%, w/w)		LMw (%, w/w)		AGP (%, w/w)
	QA	CA	QA	CA	QA	CA	QA	CA	QA	CA
unbound										
green	ND	0.1	0.0	0.1	0.1	0.0	ND	0.1	0.0	0.0
light	3.4	0.1	0.0	0.0	0.0	0.0	5.7	0.1	0.0	0.0
medium	3.0	0.1	0.0	0.0	0.0	0.0	5.2	0.1	0.0	0.0
dark	2.6	0.0	0.0	0.0	0.0	0.0	4.7	0.1	0.0	0.0
total										
green	ND	10.7	0.1	0.1	7.3	8.1	ND	12.7	0.0	0.0
light	12.7	6.0	1.5	0.5	5.2	2.0	20.4	7.7	0.8	0.2
medium	11.2	4.7	1.8	0.6	4.4	1.4	17.2	6.4	1.0	0.2
dark	9.7	3.0	2.1	0.5	3.5	1.1	15.9	4.1	1.3	0.2

[a] On the basis of the fraction's dry matter. ND, not determined

melanoidin level (**Tables 2** and **5**) was higher for all IMw fractions than for HMw. Similar observations were made for the ratio between the nitrogen and the melanoidin levels. Therefore, it might be hypothesized that the phenolics are preferably linked to nitrogenous structures.

Roasting Effects on Unbound QA and CA in Various Brew Fractions. The brews contained quite some unbound QA and almost no unbound CA (**Table 5**), and these unbound CA and QA were recovered in the LMw fractions. The unbound 5-CQA, QA, and CA levels for medium-roasted coffee fractions were similar to the levels obtained previously (*7*). The CA and QA levels in coffee brews decreased upon roasting (**Table 5**), indicating that both components are reactive under roasting conditions. The HMw and IMw fractions were free from unbound 5-CQA, QA, and CA; therefore, it can be stated that all QA and CA measured after saponification should be ester-linked to HMw and IMw material.

Roasting Effects on Chlorogenic Acid Incorporation in Various Brew Fractions. The total QA and CA levels in the coffee fractions are shown in **Table 5**. The roasted coffees had high total QA levels. The QA level in the medium brew was similar to the level previously reported (*7*). The total QA level decreased from 12.7% in light-roasted brew to 9.7% in dark-roasted brew. This loss of 24% total QA can be due to incorporation of QA into unextractable coffee bean material. Alternatively, this loss might be also due to chemical degradation of QA or incorporation via bonds other than ester linkages. The total CA level in the coffee brews decreased more rapidly upon roasting than the total QA level, which can be ascribed to a higher susceptibility of CA to oxidative changes upon roasting (*36*).

The green coffee HMw material contained 0.1% total QA and 0.1% total CA levels, which showed that green coffee HMw material contained negligible amounts of ester-linked QA and CA (**Table 5**). The ester-linked QA and CA content in HMw material initially increased steeply to 1.5 and 0.5% in light roast HMw, respectively (**Table 5**). Subsequently, the ester-linked QA content increased gradually upon further roasting up to 2.1% in dark-roasted HMw, whereas the ester-linked CA contents remained rather stable at ~0.5% upon further roasting (**Table 5**). These results indicate that the previously proven incorporation of chlorogenic acids (*7*) is a process that occurs continuously upon roasting into HMw material. Both the ester-linked QA and the phenolic groups' levels increased, while the ester-linked CA level was lower and rather stable upon prolonged roasting (**Table 4** and **5**). Therefore, these results further strengthen the proposed incorporation of chlorogenic acids in melanoidins via CA through a nonester linkage (*7*).

Table 6. Sugar Composition (Mol %) and Content of Various Coffee Fractions

	Rha	Ara	Man	Gal	Glc	uronic acid	total sugar (%, w/w)[a]
brew green	1	7	13	11	62	6	24
brew light	2	16	30	35	7	10	26
brew medium	2	15	35	34	5	9	28
brew dark	1	11	43	33	4	8	30
HMw green	5	25	18	42	2	8	26
HMw light	3	14	33	43	1	6	78
HMw medium	2	11	40	40	1	6	75
HMw dark	2	9	46	37	1	5	73
IMw green	1	5	34	11	42	7	14
IMw light	2	14	41	33	3	7	50
IMw medium	2	13	45	32	2	6	53
IMw dark	1	9	55	28	2	5	60
LMw green	0	1	12	4	78	5	24
LMw light	2	20	11	25	26	16	9
LMw medium	3	24	9	31	16	17	8
LMw dark	2	21	9	37	14	17	8
AGP green	3	31	1	56	3	6	60
AGP light	1	16	3	72	2	6	70
AGP medium	1	13	7	71	2	6	68
AGP dark	1	11	11	68	3	6	61

[a] On the basis of the fraction's dry matter.

Roasting Effects on the Sugar Content and Composition of Various Brew Fractions. The sugar content in coffee brews increased slightly upon roasting (**Table 6**), indicating an improved extractability of polysaccharides upon roasting. The two main polysaccharides populations in coffee brews are galactomannans and arabinogalactans; the latter are predominantly present in AGPs in green beans (*22, 37*). The pronounced increase of mannose shows that especially galactomannans become better extractable upon prolonged roasting (**Table 6**). Arabinose, from arabinogalactans, is known to be susceptible to degradation upon roasting (*3*), and this was seen in **Table 6** as well.

The sugar content in the carbohydrate-rich HMw fractions slightly decreased from 78% in light-roasted HMw to 73% in dark-roasted HMw. This should be due to sugar degradation and/or to the additional protein and chlorogenic acid moieties present in HMw material upon prolonged roasting (**Tables 35**). It was calculated that the total amount of polysaccharides in HMw material slightly increased (+8%) when beans were roasted from light to dark roast. This was due to solubilization of galactomannan (+50% mannose), while a large part of the arabinose (−30%) was degraded. The IMw fractions contained less polysaccharides than the HMw fractions. However, it was calculated that the total amount of carbohydrates in IMw material increased significantly (+50%) upon roasting from light- to dark-roasted beans. This was again mainly due to an increase in galactomannan (+100% mannose), while the loss of arabinose was only 3%. Obviously, many macromolecular coffee bean galactomannans were degraded into IMw galactomannans upon roasting. The Gal/Ara ratio increased for both HMw and IMw fractions upon roasting (**Table 6**), indicating that part of the arabinose in both HMw and IMw had been degraded during roasting. With the combination of the latter with the fact that the amount of arabinose in HMw decreased much more than arabinose in IMw, it can be concluded that HMw arabinogalactans are probably degraded into IMw molecules upon roasting. Thus, both arabinogalactan and galactomannan seem to be degraded into lower Mw molecules upon roasting.

Insight in how sugars are involved in melanoidin formation might be obtained by combining melanoidin data with sugar

composition and content. For HMw material, the melanoidin level (+160%) increased much more than the galactomannan level (+50% mannose) upon prolonged roasting. For IMw material, the melanoidin level increased less (+24%) than the galactomannan level (+100%) upon prolonged roasting. Nunes and Coimbra identified brown-colored structures at the reducing end of galactomannans (*27*), showing that galactomannans may be involved in melanoidin formation. However, no information was given on the contribution of these structures to the overall color of coffee, which is likely rather limited since each galactomannan molecule has only one reducing group. Furthermore, results presented in this study show that the increase in galactomannan content can not be correlated to the increase in melanoidin. Therefore, the results imply that galactomannans are not the main polysaccharide involved in melanoidin formation. With respect to arabinose, it can be suggested that arabinose, from AGPs, is involved in melanoidin formation as the HMw fractions showed the largest increase in melanoidins (+160%) and showed the largest decrease in arabinose (−30%). The IMw fractions only showed a slight increase in melanoidins (+24%) and only a slight decrease in arabinose (−3%). This inverse correlation between arabinose content and melanoidin level could suggest that chromophores might be formed from or attached to the arabinose moiety from AGPs upon roasting.

Roasting Effects on AGPs in Coffee Melanoidins. It was previously shown that AGPs become part of melanoidin structures upon roasting, yielding the AGP-melanoidins (*22*). The Yariv reagent was used to isolate AGPs from HMw coffee material to be able to investigate the changes in AGP-melanoidin properties upon roasting (**Table 1**). The initial increase in AGP yield from 0.72 to 0.93% (**Table 1**) is likely due to disruption of the cell wall leading to an improved AGP extractability. The AGP yield subsequently decreased upon prolonged roasting, which might be ascribed to modifications of the AGP molecules (*7, 22*), leading to a lowered affinity to precipitate with the Yariv reagent.

The AGP-melanoidins had quite some similar properties and showed quite some similar changes upon roasting as the whole HMw fractions did. First, the melanoidin level increased upon prolonged roasting as was observed for HMw as well (**Table 2**). Because the AGP fractions represent quite a large part of the HMw fractions, it can be concluded that AGP-melanoidins contribute significantly to the increase in melanoidin level of HMw material. Second, also the nitrogen and the protein content increased upon roasting as was observed for HMw material (**Table 3**). The nitrogen in green bean AGP was quite similar to the 1.88% reported by Redgwell et al. (*37*). The increase in nitrogen and protein content upon roasting indicated a higher level of proteinous material in AGP-melanoidins, which could be due to degradation of arabinogalactan by which the relative nitrogen content increases. Alternatively, this could also be due to incorporation of additional proteinous material into AGP-melanoidin. Third, the AGP fraction also did not contain unbound 5-CQA, QA, or CA (**Table 4** and **5**), while the phenolic groups' level increased upon roasting (**Table 4**). Fourth, the last observation was that the ester-linked QA level increased while the ester-linked CA level remained constant upon prolonged roasting (**Table 5**). From these results, it can be concluded that chlorogenic acids are incorporated in the AGP-melanoidin structures upon roasting. This incorporation probably occurs at the proteinous moiety of AGP-melanoidin structures or at the arabinose molecules, which are degraded upon roasting.

However, the sugar compositions of the AGP and HMw fractions were dissimilar. The AGP fractions mainly consisted of arabinogalactan, which was clear from the high arabinose and galactose contents. The galactose to arabinose was 1.8 in green bean AGP and increased upon roasting to 6.2 in dark-roasted AGP-melanoidins (**Table 6**). This indicated that arabinose in AGP must be quite reactive. Combining the gradual decrease in arabinose and gradual increase in melanoidin level also suggests that arabinose is likely involved in melanoidin formation. The sugar composition also revealed that mannose increased from 1 to 11 mol % in AGP-melanoidins upon roasting (**Table 6**). As galactomannan is the only source of mannose in coffee beans, it can be decisively concluded from these results that galactomannan must be incorporated into AGP-melanoidins upon roasting, as was previously suggested (*22*).

General Discussion. A striking melanoidin-related observation was that roasting from green to light roast especially led to the formation of melanoidins with an IMw as compared to melanoidins with a HMw. This suggests that especially light roast conditions (time, temperature) were suitable to yield IMw melanoidins. Another striking observation was that prolonged roasting especially led to melanoidin formation in HMw material, indicating that components present in, or migrating to, the HMw fraction are good precursors for melanoidin formation on the longer run.

It is hypothesized that initial melanoidin formation involves both (i) Maillard reactions between sucrose and amino acids/protein fragments and (ii) CGA-incorporation reactions between CGAs and amino acids/protein fragments that lead to LMw and IMw melanoidins (**Figure 1**). This hypothesis is strengthened by the facts that (i) sucrose is rapidly degraded upon roasting (*38*), (ii) covalent bonds between CGA and coffee bean proteins are formed (*39*), and (iii) low Mw melanoidins contain incorporated CGAs and intact glucose, the latter most probably originating from sucrose (*25*). It was already mentioned that galactomannans, present in quite large quantities in IMw material, are probably not involved in reactions that dominate melanoidin formation. Altogether, this proposed hypothesis can explain why the majority of the initially formed melanoidins have a LMw to IMw.

The observation that prolonged roasting especially led to formation of melanoidins in HMw material might be explained by two reaction mechanisms. First, denatured and aggregated coffee bean proteins are resolubilized upon prolonged roasting due to chemical modifications. Part of this protein has likely been modified by Maillard reactions and/or by CGA incorporation by which these proteins become soluble and brown, leading to an increased melanoidin level in HMw material upon prolonged roasting. Additionally, the fact that the protein level in AGP-melanoidins more than doubled upon prolonged roasting (**Table 3**) indicated that nonAGP proteinous material may have been incorporated in AGP-melanoidins. Otherwise, half the amount of arabinogalactans had to be split off from the AGP molecules to achieve doubling of the protein content. Whichever of the two above-mentioned reactions prevails, the increase in proteinous material alone can not fully account for the increase in HMw melanoidins. This is due to the fact that even for dark-roasted brew, the protein content remains higher in IMw material than in HMw material, while the melanoidin level is

significantly higher in HMw material. Proteins alone can therefore not be responsible for the increase in melanoidin level.

The other explanation for HMw melanoidin formation involves the arabinose from AGP. It was observed that the arabinose content in the HMw and AGP fractions gradually decreased upon roasting (**Table 6**), while the melanoidin level increased upon roasting (**Table 2**). It might well be that Maillard reaction-like or CGA-incorporation-like reactions occur at or are bound to these degraded arabinose molecules resulting in a gradual increase in brown color, QA content, and phenolic groups' levels upon roasting. Too many modifications on these AGP-melanoidins lead to a lower affinity of the AGP to precipitate with the Yariv reagent, explaining the presence of arabinogalactan in the AGP-free moiety from HMw (*22*). Degradation of AGP is a continuous process that occurs too upon roasting as an increase of arabinogalactan in the IMw fractions was observed (**Table 6**).

Summarizing, it can be concluded that this study led to new insights in mechanisms involved in coffee brew melanoidin formation. However, it can not be decisively concluded which reaction mechanisms lead in coffee brew melanoidin formation. The authors of this study are of the opinion that chlorogenic acid incorporation plays a far more important role, especially the effect that it has on solubility, in coffee melanoidin formation than expected till now. The authors therefore propose to investigate melanoidins prepared by relatively complex model systems, containing carbohydrates (sucrose, arabinogalactans, and/or galactomannans) and proteinous material (amino acids and/or proteins). These model reactions should be conducted in the presence or absence of chlorogenic acid moieties (5-CQA, QA, and/or CA) with the aim to investigate the effect that chlorogenic acids have on coffee brew melanoidin formation.

ACKNOWLEDGMENT

This research was financially supported by the Wageningen University Graduate School VLAG.

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Received for review March 31, 2008. Revised manuscript received June 11, 2008. Accepted June 14, 2008.

JF800999A