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ARTICLE

Conceptual Study on Maillardized Dietary Fiber in Coffee

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There is a methodological and conceptual overlap between coffee melanoidins and dietary fiber. Green Uganda coffee beans were roasted in a range from 8.1 to 21.6% of weight loss to evaluate melanoidins and dietary fiber. Samples were characterized by color, moisture, solubility, water activity, carbohydrates, polyphenols, protein, soluble dietary fiber (SDF), and melanoidins content. Hydroxymethylfurfural and chlorogenic acids were also measured as chemical markers of the extent of roasting. Melanoidins rapidly increased from 5.6 (light roasting) to 29.1 mg/100 mg soluble dry matter (dark roasting). A melanoidins-like structure was already present in green coffee that might overestimate up to 21.0% of the melanoidins content as determined by colorimetric methods. However, its contribution is variable and very likely depends on the method of drying applied to green coffee. SDF content (mg/100 mg soluble dry matter) gradually increased from 39.4 in green coffee to 64.9 at severe roasting conditions due to incorporation of neoformed colored structures and polyphenols. Then, SDF progressively turns to a maillardized structure, which increased from 11.0 to 45.0% according to the roasting conditions. It is concluded that the content of coffee melanoidins includes a substantial part of dietary fiber and also that coffee dietary fiber includes melanoidins. A conceptual discussion on a new definition of coffee melanoidins as a type of maillardized dietary fiber is conducted.

KEYWORDS: Fiber; melanoidins; coffee; Maillard reaction; maillardized dietary fiber

INTRODUCTION

Melanoidins are polymeric colored structures formed in the last stages of the Maillard reaction (MR) and are widely distributed in a large number of thermally processed foods. For decades, melanoidins have been considered to be antinutritional food constituents, but there is an increasing evidence of their beneficial properties such as antioxidant capacity (*1*, *2*), desmutagenic activity (*3*), anticariogenic activity (*4*), and induction of chemopreventive enzymes (*5*), although most of the mechanisms of action remain unclear. In addition, melanoidins are fermented in the colon and may act as dietary fiber by modulating bacterial population with a significant influence on human gastrointestinal health (*6*−*9*) and, more specifically, are able to promote *Bifidobacteria* growth (*10*).

Coffee melanoidins have been the objective of many investigations because it is well-known that coffee brew is one of the main sources of melanoidins intake in the diet, accounting for up to 25.0% of the total solids (*2*). However, the chemical structure of melanoidins is complex and remains largely unknown because many green coffee constituents could take part in it. During roasting of coffee green beans, polysaccharides, galactomannan-like and arabinogalactan-like carbohydrates (*11*−*13*), proteins (*14*), and phenolic compounds, mainly hydroxycinnamates (*2*, *15*−*17*), contribute to the formation of coffee melanoidins. Phenolic compounds can also be noncovalently linked to coffee melanoidins and act as carriers of low molecular weight (LMW) substances (*18*).

Coffee brew also contains a significant amount of soluble dietary fiber (SDF), higher than other common beverages. This SDF is made up of indigestible polysaccharides with an appreciable amount of associated polyphenolic antioxidants. In this context, significant contributions of coffee brew to the intake of antioxidants and SDF in the diet have been reported (*19*−*21*).

Coffee melanoidins content has been traditionally calculated gravimetrically by difference with the rest of the major constituents of roasted coffee beans and more recently by spectrophotometic methods. However, SDF might be included in both gravimetric and spectrophotometric determinations. Similarly, analytical methods for dietary fiber determine SDF as indigestible polysaccharides with associated polyphenols and proteins; thus, it can be expected that the SDF content in roasted products also includes Maillard reaction compounds (*22*).

Although melanoidins cannot be strictly considered as dietary fiber because they are formed upon thermal processing, there exists a methodological and conceptual overlap between both coffee constituents. Nowadays, this is an open question that has not been answered yet. This paper aims to settle a critical evaluation of coffee melanoidins content in roasted coffee as opposed to SDF and vice versa. The concept of maillardized dietary fiber is discussed with the aim to take a step forward in the state of the art of melanoidins research.

MATERIALS AND METHODS

Chemicals. Pepsin (2000 FIP/U) and gallic acid were obtained from Merck (Darmstadt, Germany). Bovine serum albumin (BSA), α-amylase (A-3176, 23 U/mg), pancreatin (P1750, 4× U.S.P.), 5-hydroxymethylfurfural

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(HMF), and 5-caffeoylquinic acid (5-CQA) were obtained from Sigma-Aldrich (Madrid, Spain), and α-amyloglucosidase (14 IU/mg) was obtained from Roche (Mannheim, Germany). 3,5-Dinitrosalicylic acid, Folin−Ciocalteu reagent, hydrochloric acid (37%), sodium carbonate, sodium hydroxide, and sulfuric acid (98%) were obtained from Panreac Química S.A. (Barcelona, Spain). All reagents used were of analytical grade. The water used was double-distilled (18.2 mequiv X/cm) using a Milli-Q System (Millipore Ibérica, Madrid, Spain).

Procedure. Unwashed Ugandan Drugar coffee beans (*Coffea arabica*) were supplied by a local coffee company. Green coffee was roasted in a set of three convection home roasters (Heathware Precision Coffee Roaster, Wheeling, IL) at a fixed temperature but different roasting times. A temperature of 200 °C was reached after 60 s with a further slow increase to a maximum temperature of 240 °C. The degree of roast was quantified by the percentage of dry weight loss of green coffee beans, on a dry basis. Coffee was naturally brought to ambient temperature (20 ± 2 °C), packed in plastic pouches, and stored in vacuum conditions. Prior to analysis samples were ground in a coffee mill (Super Junior "S", Moulinex, Spain).

Assessment of Extent Coffee Roasting. Color analyses were carried out using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head according to the method of Andueza et al. (*23*). Moisture content was determined by a gravimetric method as described in AOAC 925.10 (*24*). Samples (500 mg) were weighed accurately into a test tube and dried until constant weight in an oven (14 h, 103 ± 2 °C). The experiment was carried out in duplicate. Solubility was determined gravimetrically as weight difference of the water-soluble fraction after two consecutive extractions of 1 g of coffee with 25 mL of water at 50 °C. The residue was dried and weighed, and the difference from the starting sample content was identified as the soluble fraction. A portable LabMaster-a_w (Novasina, Lachen, Switzerland) instrument was used for determination of the water activity (a_w). The value was recorded at equilibrium when a_w values of any two readings were <0.001 at constant temperature (25 °C). A commercial humidity standard of an unsaturated salt solution of a specific molality representing a known a_w was used for calibration. CGAs content in coffee was determined as described by Fujioka and Shibamoto (*25*) as the sum of caffeoylquinic acids, feruoylquinic acids, and dicaffeoylquinic acids. 5-CQA was used as external standard for calibration. HMF was determined by reversed-phase liquid chromatography (RP-HPLC) as described by Arribas-Lorenzo and Morales (*26*).

Obtention of Reference Coffee Melanoidin (RCM). RCM was prepared following the recommendation of COST-919 group for coffee melanoidin analysis (*27*) and as described elsewhere (*18*). Roasted coffee beans (*C. arabica*) were provided by a local factory with a medium roasting degree accounting for a weight loss of 15.0% (w/w) on a dry matter basis in relation to green coffee weight. Ground coffee (100 g) was stirred in 300 mL of distilled water at 75 °C for 5 min. The solution was filtered, and an aliquot of filtrate was defatted with dichloromethane (2 × 200 mL). Coffee extract was subjected to ultrafiltration (Amicon ultrafiltration cell model 8400, Amicon, Beverly, MA) with a 10 kDa nominal molecular mass cutoff membrane. The retentate corresponding to melanoidins was filled to 200 mL with water and ultrafiltered again at least three times, and then the high molecular weight (HMW) fraction (approximately 50 mL) was freeze-dried and stored. The absence of LMW compounds was basically confirmed by high-performance gel permeation chromatography (HPGPC) using a TSK-GEL 3000SW column (60 cm × 7.5 mm i.d., TosoHaas, Germany). Void column volume was calculated with a standard solution of 1 mg/mL blue dextran (2000 kDa) and set at 7.4 min.

RCM was submitted to acidic hydrolysis according to Goñi et al. (*22*), for further characterization. Briefly, samples were treated with 12 M sulfuric acid at 60 °C for 60 min, subsequently diluted to 1 M with water, and incubated at 100 °C for 90 min. RCM dissolved in water was used as control. In addition, RCM was submitted to enzymatic hydrolysis following the same procedure used for SDF determination. Total carbohydrates (HC), phenols (PP), and proteins (PR), and melanoidins contents were determined in RCM solution, acid hydrolysates, and SDF.

Determination of Coffee Melanoidins Content by Spectrophotometry. Melanoidins were obtained after ultrafiltration (10 kDa cutoff) of green and roasted coffee extracts as described by Delgado-Andrade and Morales (*18*). Briefly, 100 mg of ground and defatted coffees were extracted in 5 mL of hot water (50−60 °C), vigorously shaken at room temperature for 20 min, and centrifuged at 5000*g* for 10 min at 4 °C. The process was repeated twice, and the coffee extracts were pooled. Extracts were then subjected to ultrafiltration using a Microcon YM-10 regenerated cellulose 10 kDa (Millipore, Bedford, MA) at 12000*g* for 10 min. Retentates containing the HMW fraction, corresponding to coffee melanoidins, were recovered by inverting the Microcon device and centrifuged at 1000*g* for 10 min. Finally, the retentates were dissolved in 0.45 mL of distilled water and measured spectrophotometrically at 405 nm as described by Bekedam et al. (*17*) but adapted to a microplate reader (Synergy-HT, BioTEK Instruments, Winooski, VT). Linearity was settled between 0.1 to 0.8 unit of absorbance. Coffee melanoidins were quantified using the external standard method within the range of 0.1−1 mg/mL with RCM (mg/mL = $0.6961 \times Abs_{405} + 0.0096$; $r^2 = 0.9995$). Sample reporting levels outside the calibration range were additionally diluted 10-fold. Melanoidins content was expressed as milligrams per 100 g of soluble dry matter.

SDF Determination. The SDF of coffee samples and RCM were measured on the basis of the procedure described by Saura-Calixto et al. (*28*) with some modifications. This method combines enzymatic treatments and separation of digestible compounds by dialysis using physiological conditions (temperature and pH). Samples (500 mg for green and roasted coffees and 50 mg for RCM in triplicate) were weighed into 50 mL centrifuge tubes and dissolved in 15 mL of 0.1 M HCl−KCl buffer, pH 1.5, and the pH was adjusted to 1.5 with 2 N HCl. Samples were incubated with the addition of 0.2 mL of pepsin (300 mg/mL in HCl−KCl buffer) at 40 °C for 40 min with continuous agitation. Next was added 3.5 mL of 0.1 M phosphate buffer, pH 7.5, and the pH was adjusted to 7.5 with 2 N NaOH. Samples were incubated with the addition of 1 mL of pancreatin (5 mg/mL in phosphate buffer) at 37 °C for 6 h with continuous agitation. Then was added 9 mL of 0.1 M Tris−maleate buffer, pH 6.9, and the pH was adjusted to 6.9 with 2 N HCl. Samples were incubated with the addition of 1 mL of α-amylase (120 mg/mL in Tris−maleate buffer) at 40 °C for 3 h. Next, 10 mL of 0.2 M sodium acetate buffer, pH 4.75, was added, and the pH was adjusted to 4.75 with 2 N acetic acid. Samples were incubated with the addition of 0.4 mL of amyloglucosidase at 60 °C for 45 min with continuous agitation. After the enzymatic treatments, samples were transferred into dialysis membranes (12−14 kDa MW cutoff, Visking dialysis tubing; Medicell International Ltd., London, U.K.) and dialyzed against water for 48 h at 37 °C (water flow = 7 L/h). Finally, dialysates (17 mL) were hydrolyzed in 1 M sulfuric acid at 100 °C for 90 min. Hydrolyzed samples (0.5 mL) were alkalinized by adding 0.25 mL of 3.9 M NaOH and 1 mL of dinitrosalicylic acid (*29*). The mixture was colorimetrically measured at 530 nm.

Chemical Characterization of Green and Roasted Coffee Extracts, Melanoidins, and SDF. Green and roasted coffees extracts, their respective SDF, and melanoidins were chemically characterized for their content in proteins (PR), phenolic (PP), and carbohydrates (HC) as well as for their visible scan. Green and roasted coffee extracts were obtained as follows: 500 mg was subjected to an extraction following the SDF procedure described above but without enzyme treatment. The soluble fractions obtained were used for the chemical characterization. Total HC content was determined according to the method of Englyst and Cummings (*29*), soluble PR was measured by the Bradford assay (*30*) using BSA as a standard, and total PP was determined according to the Folin−Ciocalteu method (*31*) using gallic acid as standard. The results were expressed as gallic acid equivalents. Visible spectra (350−800 nm) of green and roasted coffee extracts and RCM were recorded with a BioTek PowerWave XS spectrophotometer (BioTek Instruments, Inc.).

Statistical Analysis. Results are expressed as mean values ± standard deviations. Means were compared by one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Roasting. Three roasting degrees were carried out to obtain representative roasted coffee samples. Samples were named LR, MR, and DR for light, medium, and dark roasting, respectively. For each treatment, two sublevels of roasting (I and II) were obtained. LR-I and DR-II represented under- and over-roasting conditions, respectively. Roasting degree was calculated by

Table 1. Physicochemical Properties of Green and Roasted Coffee Samples

	coffee samples						
	green	LR-I	LR-II	MR-I	MR-II	DR-I	DR-II
roasting time at 240 °C (min)	0	3.7	5.3	5.5	6.0	9.5	11.6
weight loss (%)		8.1	11.1	13.2	14.7	18.2	21.6
L^{*a}		42.9 ± 1.4	29.8 ± 0.7	24.8 ± 0.3	21.4 ± 0.1	17.1 ± 0.5	15.7 ± 0.3
a^{*a}		13.7 ± 0.1	14.0 ± 0.2	12.3 ± 0.2	10.4 ± 0.1	6.9 ± 0.2	4.9 ± 0.1
b^{*a}		29.5 ± 0.6	21.2 ± 0.3	15.3 ± 0.6	11.3 ± 0.5	5.7 ± 0.2	3.5 ± 0.1
solubility (g/100 g)	37.5	25.9	26.6	28.5	28.9	27.0	25.2
moisture (g/100 g)	9.0	5.6	4.8	4.4	4.7	4.3	4.7
water activity		0.30	0.17	0.12	0.13	0.12	0.12
HMF (mg/kg of product)	nd[b]	460.4 ± 8.4	656.0 ± 1.7	285.6 ± 0.5	145.0 ± 0.8	30.8 ± 0.4	11.4 ± 0.3
CGAs (mg/g of product)	30.0 ± 0.1	21.2 ± 0.0	16.0 ± 0.1	10.0 ± 0.1	5.5 ± 0.1	1.0 ± 0.0	0.4 ± 0.0

[a] *L**, lightness variable (0, black; 100, white); chromatic coordinates: *a**, red (0, no pigment; 50, maximum red), *b**, yellow (0, no pigment; 50, maximum yellow). [b] nd, not detected.

Table 2. Chemical Composition of Coffee Extracts and SDF of Ground Green and Roasted Coffees[a]

	coffee extracts				SDF solution			
sample	HC[b]	PP	PR	melanoidins	polysaccharides	PP	PR	melanoidins
green	66.5 ± 5.2	17.8 ± 1.9	2.1 ± 0.5	3.7 ± 0.3	28.7 ± 0.9	5.2 ± 0.1	5.5 ± 0.5	18.3 ± 2.4
LR-I	74.2 ± 3.5	24.4 ± 0.8	3.2 ± 0.3	5.6 ± 0.5	37.4 ± 0.9	6.9 ± 0.1	6.9 ± 0.6	35.1 ± 1.2
LR-II	69.0 ± 2.7	21.0 ± 0.5	3.0 ± 0.4	17.7 ± 0.4	45.3 ± 0.8	8.4 ± 0.2	10.7 ± 0.9	65.9 ± 5.0
MR-I	58.6 ± 1.0	17.1 ± 0.5	2.3 ± 0.4	21.2 ± 0.3	44.9 ± 0.6	8.2 ± 0.1	10.2 ± 0.2	69.4 ± 0.7
MR-II	54.1 ± 0.9	15.0 ± 0.2	3.4 ± 0.7	30.3 ± 0.8	48.6 ± 0.8	8.6 ± 0.1	10.0 ± 0.3	79.4 ± 3.2
DR-I	51.3 ± 2.2	13.1 ± 0.7	3.4 ± 0.4	29.0 ± 0.6	46.5 ± 1.1	8.2 ± 0.3	8.8 ± 0.5	67.5 ± 1.9
DR-II	46.7 ± 1.3	11.8 ± 0.2	2.3 ± 0.5	29.1 ± 2.2	47.1 ± 1.0	8.4 ± 0.4	9.4 ± 1.6	63.9 ± 6.6

[a] Carbohydrates (HC), polyphenols (PP), and protein (PR) (mg/100 mg of soluble dry matter). [b] HC = SDF (polysaccharides) plus sucrose and other LMW carbohydrates.

weight loss as difference between the weight of coffee beans before and after roasting and expressed per 100 g of coffee (**Table 1**). Extent of the roasting was further characterized by CIE-*Lab* color, solubility, moisture, and water activity as described in **Table 1**.

Solubility of samples increased gradually with roasting degree, but at more severe roasting conditions a decrease was observed (**Table 1**). It is known that the solubility of coffee carbohydrates (i.e., galactomannans) might increase during roasting (*32*) and that higher complexity of polymers will coffee it more insoluble (*33*). The insoluble fraction of roasted beans was not considered in this study because it was not present in the coffee brew, it is not edible, and subsequently it has no potential physiological effect as claimed for melanoidins and fiber.

During roasting several physical and chemical changes take place in coffee beans, which implies the degradation/interaction among natural constituents toward the formation of new structures (*34*). Maillard and Strecker reactions, caramelization, pyrolysis, and degradations of proteins, polysaccharides, trigonelline, and CGAs make major contributions to the changes occurred during roasting, such as color and aroma (*14*). The extent of the roasting process was chemically evaluated by monitoring the levels of HMF and CGA (**Table 1**). HMF is a well-known heat-induced marker of both caramelization and MR. As expected, HMF was rapidly formed in the first stages of roasting to reach a maximum at 656.0 mg/kg, and thereafter it decreased. At more severe roasting conditions the yield of evaporation and losses due to HMF reactivity are enhanced, and subsequently the final amount of HMF in the roasted bean is decreased (*35*).

Respectively, phenolic compounds are present predominantly as a family of esters formed between certain hydroxycinnamic acids and quinic acid, collectively known as chlorogenic acids (CGAs) (*14*). Due to their thermal instability when submitted to roasting conditions, CGAs are degraded into phenol derivatives and participate in the formation of melanoidins (*11*, *36*). The disappearance of CGAs was related to the degree of roasting and ranged from 29.5 to 98.6% (**Table 1**).

Carbohydrates, proteins, polyphenols, and melanoidins were measured in green and roasted coffee extracts and their respective SDF fraction (**Table 2**). Coffee extracts were obtained by using the same procedure for SDF determination but without enzyme treatment for better comparison of results. Data are expressed as soluble dry matter to avoid the effect of variability of sample solubility because aqueous extraction was necessary for all measurements. All in all, it is necessary to put these results in context with the definitions of melanoidins and SDF.

Melanoidin Approach. Genesis of coffee melanoidins has been linked to a variety of chemical components present in green coffee beans: sucrose (after inversion), polysaccharides (galactomannans and arabinogalactans), amino acids, proteins (11S storage and cell wall proteins), CGAs, and newly formed substances during roasting. All of these precursors might increase the chemical complexity and heterogeneity of coffee melanoidins (*11*). Moreover, it was questionable whether the soluble HMW fraction of coffee extracts can be differentiated by "proteins", "polysaccharides", and "melanoidins", because polysaccharides and proteins are stated to be integral components of coffee melanoidin complexes (*17*).

HPGPC was applied to separate soluble HMW polymers, and its response was recorded spectrophotometrically at 405 nm and by refractive index as described somewhere (*33*). Reference coffee melanoidins showed two major populations of melanoidins with similar retention times, 11.0 and 11.8 min, respectively. Both populations gave a similar response at 405 nm and by refractive index detection (r = 0.9988, from 0.250 to 10 mg/mL), indicating that all eluting mass was colored.

The chromatographic method was compared with the direct spectrophotometric method. Response (area value) of chromatographic peaks corresponding to the HMW fraction showed a high correlation with the direct spectrophotometric method after

membrane ultrafiltration. A good linearity was obtained (r^2 = 0.9996; $y = 1.0002x - 0.003$), and there were no significant differences between techniques. Results confirm that direct application of the spectrophotometric method gives a reliable content of the soluble HMW fraction. In the literature, spectrophotometric methods have already been proposed for the determination of melanoidins, which are based on the intrinsic color character of these polymers (*37*). Spectrophotometric measurement combines an ultrafiltration step (10 kDa cutoff) and is cost-effective and less time-consuming than HPGPC analysis.

A spectrophotometric method was applied to evaluate melanoidins content in coffee extracts. Melanoidins content increased from 5.6 to 29.1 mg/100 mg of soluble dry matter from the light (LR-I) to the most severe roasting conditions (DR-II), respectively (**Table 2**). The highest content was obtained at moderate roasting conditions (MR-II) and did not further increase. This is likely due to the formation of more insoluble structures that cannot be further extracted in water and will not be present in the beverage. These results are in concordance with levels of SDF obtained and discussed later.

However, it is noteworthy that melanoidin-like structures were also detected in green coffee (3.7 mg/100 mg), which is conceptually wrong (**Table 2**). Green coffee should not contain melanoidins because they are polycondensation brown end products of the MR and thermal treatment is needed for their formation. This observation encouraged us to get more insight into the composition of coffee melanoidins and melanoidin-like structures in green coffee.

Melanoidins are brown soluble anionic polymer compounds with an apparent molecular mass of > 10 kDa, which absorb in the range between 400 and 450 nm (*2, 18, 38*). **Figure 1** depicts the VIS scan of the reference coffee melanoidin and soluble HMW fraction of green coffee and MR-II coffee. RCM and the HMW

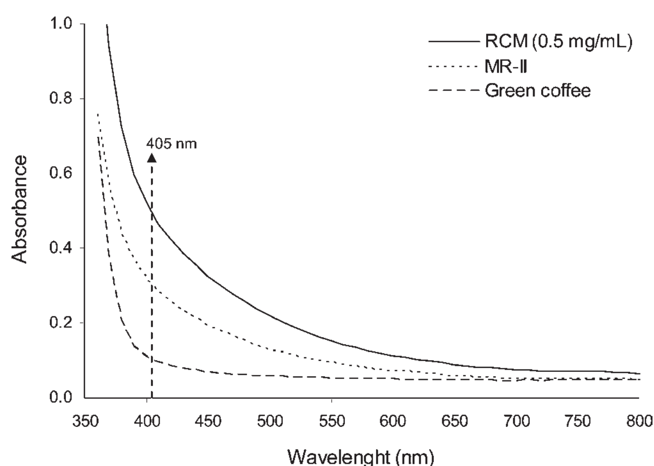

Figure 1. Visible spectra from reference coffee melanoidins, melanoidin from medium-roasted (II) coffee extract, and melanoidin-like structure in green coffee extract.

fraction of coffee extracts showed similar profiles with a significant absorption in the visible range. However, the soluble HMW fraction from green coffee extracts showed a residual but measurable absorbance at 405 nm, indicating the presence of natural structures that could interfere in the colorimetric measurement of coffee melanoidins. It is well-known that postharvest processing has pronounced effects on the chemical composition of coffee seeds, especially in water-soluble components such as sugars, caffeine, trigonelline, and CGAs (*34*). Although all methods aim at removing the fruit flesh of coffee cherry, in the dry method, the whole cherry (bean, mucilage, and pulp) is dried under the sun or in a mechanical dryer, followed by mechanical removal of the dried outer parts (*39*).

According to the **Table 2**, melanoidin-like structures in green coffee extracts accounted for 66, 21, 18, 12, 13, and 13% of total melanoidins content in samples LR-I, LR-II, MR-I, MR-II, DR-I, and DR-II, respectively. Therefore, melanoidins content in roasted coffee might be overestimated by colorimetric procedures. For roasting treatments applied to commercial samples, overestimation was expected to be in the range from 13 to 21%. Then, it was necessary to subtract the residual absorbance of colored structures in green coffee to melanoidins content in roasted coffee samples. Unfortunately, this was not always possible for unknown samples and a compromise could be to apply an average of 17% for commercial roasting processes. In addition, it is expected that residual value will vary according to the origin of the green coffee and more likely the method of drying applied.

To get more insight into this fact, six green coffees (Arabica variety) with different origins (Brazil, Colombia, Costa Rica, Guatemala, and Vietnam) were analyzed to estimate the contribution of melanoidins-like structures to the spectrophotometric measurement. An average content of 9.1 mg of melanoidins/100 g of soluble dry matter ± 2.7 (from 4.2 to 14.5) was obtained. It was concluded that the contribution of melanoidins-like structures in green beans to the spectrophotometric method was variable and likely depends on the coffee bean composition and method of drying. According to the hypothesis for the structure of melanoidins suggested by the literature, a common internal skeleton or core is established basically from carbohydrates. This complex primary structure could be already built in the green coffee and branched with proteins, polyphenols (oxidized or not), or even Maillard reaction products during postharvest. Chemical characterization of the pale yellow-green melanoidins-like structure in green coffee gave a content of 5.7 mg of carbohydrates, 1.49 mg of polyphenols, and 6.36 mg of proteins per 100 mg of soluble dry matter (**Table 3**).

To gain more knowledge on the structure the HMW fraction, carbohydrate, protein, and polyphenol contents were analyzed in the reference coffee melanoidin (**Table 3**). RCM was constituted by 48% HC, 12% PP, and 8% PR; about 32% of the weight was referred to unknown substances. If compared with the composition of melanoidin-like structures in green beans, HC and PP contents extensively increased but minor changes were found for

Table 3. Chemical Composition of Reference Coffee Melanoidin under Different Treatments and Melanoidin-like Structure in Green Coffee[a]

	HC	PP	PR	melanoidins[b] (%)
reference coffee melanoidin				
water solution	48.4 ± 0.5	12.4 ± 0.1	8.2 ± 0.1	98.7
enzymatic treatment and dialysis (SDF)	35.1 ± 1.4	6.7 ± 0.2	9.2 ± 0.6	92.2
acidic hydrolysis				25.5
melanoidin-like structure in green coffee				
water solution	5.7 ± 0.36	1.49 ± 0.08	6.36 ± 0.08	27.4

[a] Data expressed as mg/100 mg. [b] Melanoidins recovered by spectrophotometric method (405 nm).

protein content (**Table 3**). According to the proposal of structure for coffee melanoidins (*17*), unknown substances are formed, which might be LMW MR products and sugar degradation products. The HMW fraction of green coffee was also analyzed, and only 27.4% of the soluble dry mass was identified as melanoidins (**Table 3**). This value is in line with that obtained after acidic hydrolysis of the reference coffee melanoidin, which suggested a common primary structure that remains after roasting of coffee.

The reference coffee melanoidin was enzymatically treated and subsequently dialyzed to obtain SDF; nearly 20% of HC and PP contents was lost from the polymeric structure. However, only 6% of the melanoidins was lost as compared with the untreated sample (**Table 3**). It can be concluded that the response of melanoidin at 405 nm is mainly due to the unknown constituents that remains linked to the core structure. Compounds released from reference coffee melanoidin after enzymatic digestion were carbohydrates, proteins, and polyphenols but not the newly formed colored structures. Subsequent dialysis did not decrease significantly the absorbance of melanoidins at 405 nm.

Sulfuric acid hydrolysis of the reference coffee melanoidin induced a dramatic loss of response at 405 nm, and just 25% of the initial amount of RCM was found in the hydrolysates. This value is expected to represent the core of the coffee melanoidins, which remain after the removal of polysaccharides, proteins, and polyphenols by this strong acidic treatment. This observation can be applied for a more robust spectrophotometric quantitation of melanoidins because the contribution of melanoidins-like structure can be extrapolated after acid hydrolysis of the melanoidins from roasted coffee beans whether or not original green coffee is available. However, further studies are needed to confirm that remaining HMW material after acid treatment is unmodified.

Fiber Approach. Dietary fiber is the nondigestible part of vegetable foods and beverages and plays an important role in nutrition and health. Conceptually, melanoidins have been included in the very unspecific box of fiber for decades. This concept is partly correct because melanoidins and fiber share some physical−chemical and physiological properties as a prebiotic effect. Indeed, melanoidins could be redefined as a maillardized fiber but with a much more complex structure because proteins, polyphenols, and LMW MR products are expected to be linked to the primary carbohydrate skeleton (*18*).

Dietary fiber coffee brew contained a significantly high amount of SDF as indigestible polysaccharides and associated phenolics and proteins as pointed out by Diaz-Rubio and Saura-Calixto (*21*). Our data show that the sum of SDF (polysaccharides), PR, and PP contents (or total dietary fiber matrix) increased from 39.4% in green beans to 64.9% in DR-II samples, although the highest increment was reached at moderate roasting conditions (**Table 2**). Fiber is an original constituent of green coffee that might not increase, but our data pointed out an apparent increment of the fiber content. This effect is explained by a gradual degradation of insoluble structures in green beans during roasting but mainly by the incorporation of newly formed substances to the soluble structure of the fiber matrix as explained before. Minor changes are observed at more severe roasting conditions very likely due to the formation of more complex structures that are less water-soluble.

In parallel, the color of the SDF as determined at 405 nm increased rapidly, suggesting the presence of new colored residues linked to the structure. Bekedam et al. (*36*) hypothesized that melanoidins formation involves both MR between sucrose and amino acids/protein fragments and CGA incorporation reactions between CGAs and amino acids/protein fragments via the caffeic moiety through nonester linkages. In our approach about 60% of the SDF was maillardized under roasting. In addition, a basal level of maillardized portion of the SDF in the green samples was also detected. Correcting with the values in green coffee as an approximate blank, quantitation of melanoidins structures in the SDF was of the same order of magnitude as the melanoidins alone. Sucrose, polysaccharides (galactomannans and arabinogalactans), proteins, and phenols are potentially the sources for the formation of melanoidin skeleton in green coffee beans.

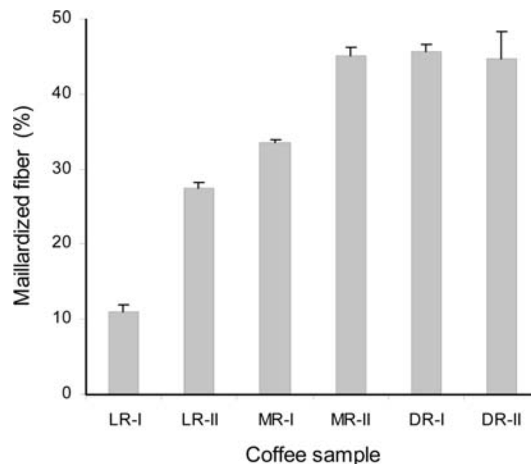

Figure 2. Estimation of the proportion of SDF that turns to maillardized fiber (melanoidins) due to roasting process. Identification of coffee samples is described under Materials and Methods.

Maillardized Dietary Approach. Coffee is one of the most popular beverages around the world, constituting a significant portion of daily intake of antioxidants and fiber in many Western countries (*21*). Phenolics and melanoidins are the two main groups of antioxidants found in coffee brews. Our results showed an overestimation of melanoidin content in roasted coffee because soluble indigestible fiber is also included as present in green coffee. Nowadays, this task is very challenging because there has been no defined chemical structure, although some inherent properties for melanoidins such as color and high molecular weight are used for its calculation.

In this context, it is plausible to redefine the melanoidins as a type of soluble maillardized fiber. It is very complex to structurally separate fiber from melanoidins and vice versa because carbohydrates are integral components of coffee melanoidins, as also supported by Nunes and Coimbra's (*11*) investigations. Our results agreed that a primary skeleton of melanoidins is constituted by carbohydrates, including dietary fiber, polyphenols, and proteins. Later and according to the extent of roasting conditions, the complexity of this primary structure increased and new LMW compounds were linked to the melanoidin skeleton backbone. Then, SDF progressively turned to a melanoidinic structure by increasing the number of colored residues. In other words, a portion of initial SDF is maillardized to nearly 45% (w/w) at more severe roasting conditions as depicted in **Figure 2**. At this point, the beneficial effects of maillardized fiber on gastrointestinal health have to be evaluated when combined with the antioxidant activity of melanoidins and the low transit of these polymers.

In summary, our results indicate that the quantification of coffee melanoidins includes the SDF fraction of coffee and also the common analytical methods to determine dietary fiber in coffee include a significant (major) part of melanoidins associated with the fiber matrix. A maillardized dietary fiber approach considering both structures as unique might be useful for a better understanding of the formation and nutritional/health implications

of coffee melanoidins and subsequently expanding knowledge in the field.

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