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Effect of in Vitro Enzymatic Digestion on Antioxidant Activity of Coffee Melanoidins and Fractions

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Traditionally antioxidant activity of melanoidins has only been evaluated in food for implication in shelf life but gastrointestinal digestion is necessary to study their potential bioactivity. In addition, the biological fate of melanoidins has been stressed during the past decade since they did not behave as inert substances. In the present paper a soluble coffee melanoidin isolated from brewed coffee after ultrafiltration with a 10 kDa cutoff membrane was treated ionically and enzymatically collecting the respective high and low molecular weight fractions. Antioxidant activity of these fractions was evaluated with five well-described assays (DPPH, ABTS, ORAC, HOSC, and FRAP) that were previously setup in a plate reader based automatized analysis. Low molecular weight compounds released from melanoidin after gastrointestinal digestion exerted the highest antioxidant activity, even higher than compounds bound ionically to melanoidins. Gastrointestinal digestion is able to modify coffee melanoidins to some extent, as hypothesized from their absolute antioxidant activities. Two options are plausible: by modifying/releasing the ionically bound compounds and/or by genesis of new more active structures from the melanoidin skeleton after enzymatic treatment.

KEYWORDS: Coffee; Maillard reaction; melanoidin; gastrointestinal digestion; antioxidant activity; DPPH; ABTS; FRAP; ORAC; HOSC

INTRODUCTION

Coffee (the second most traded commodity after petroleum) is one of the most popular beverages in the world where 75% of the soft drinks consumed regularly are coffee (*1*). During the roasting of coffee beans, flavors and colored compounds are formed as a result of pyrolysis (*2*) and the Maillard reaction (*3*) that takes place between carbohydrates or degraded polysaccharides (*4*) and amino acids or proteins (*5*). Brown-colored compounds are formed in the final stage, and these compounds are referred to as melanoidins. Coffee infusions are one of the main sources of melanoidin intake in human nutrition, accounting for up to 25% of the total solids (*6*). Coffee melanoidins are brown anionic polymeric material that contains nitrogen (*7*). The chemical structure of melanoidins is complex and still remains largely unknown (*8–12*) since many constituents of the green beans that are newly formed during roasting could play a role in its formation (*13*). However, there is increasing evidence that polysaccharides, galactomannan-like and arabinogalactan-like carbohydrates (*12, 14, 15*), proteins (*16, 17*), and phenolic compounds (*18–23*) are constituents of coffee melanoidins. In addition, it was demonstrated that phenolic compounds can also be noncovalently linked to coffee melanoidins and melanoidins could acts as carriers of low molecular weight substances (*24*).

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Melanoidins have been studied in recent years because of their technological, nutritional, biological, and health implications such as reactivity in brewed coffee (i.e., aging of coffee) (*25*), flavor binding properties (*26, 27*), antioxidative capacity (*6, 24, 28*), metal-chelating properties (*29*), and antimicrobial (*30*) and in vitro antihypertensive activity (*31*). In line with their antioxidant activity, some physiological effects of coffee melanoidins after gastrointestinal digestion have been stated such as the ability to protect human hepatoma HepG2 cells against oxidative assault by modulating reduced glutathione concentration, malondialdehyde production, and antioxidant enzyme activity, although the mechanisms of action still remain unknown (*32*).

As stated above, coffee melanoidins present antioxidant activity which could be attributed in part to the low molecular weight compounds noncovalently linked to them, such as Maillard reaction products and phenolic compounds (*12, 23, 24*). But, the potential biological activity of melanoidins and related fractions should be evaluated after simulating the gastrointestinal digestion. In this sense this research is aimed to investigate the antioxidant properties of coffee melanoidins and the effect of a simulated gastrointestinal digestion on these properties. The specific antioxidant activity associated to noncovalently bound to melanoidin compounds is assayed by different approaches to offer robustness to conclusions. These findings will give more insight into the knowledge of the real contribution of noncovalently bound to melanoidin compounds to the overall antioxidant effect of brewed coffee

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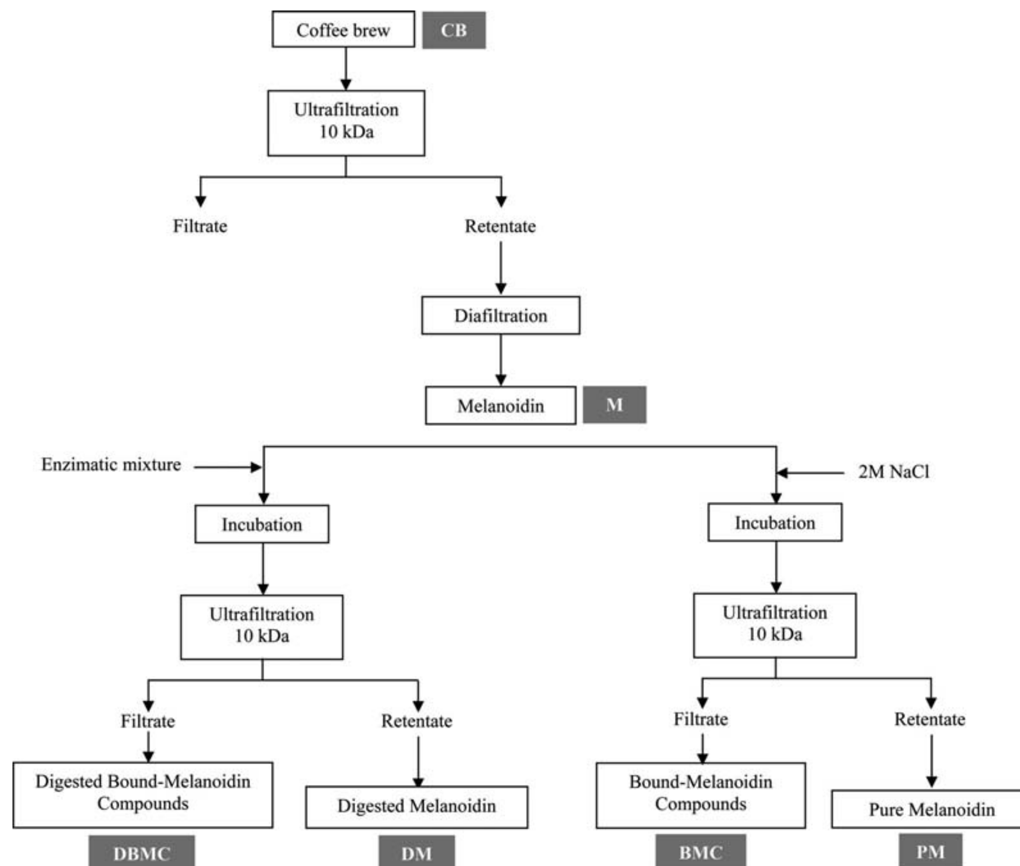

Figure 1. Flow-chart diagram for obtaining different melanoidin fractions.

and subsequently their possible health implications by suppressing radical mediated reactions in vivo.

MATERIAL AND METHODS

Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), fluorescein disodium, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), pepsin (800–2500 U/mg of protein), pancreatin (4xUSP), bile salts, and potassium persulfate were purchased from Sigma (St. Louis, MO). 2,2′-Azobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) were from Fluka Chemicals (Madrid, Spain). Iron(III) chloride, sodium phosphate monobasic, sodium bicarbonate, hydrogen peroxide, sodium chloride, chloroform, and hydrochloric acid were purchased from Panreac (Madrid, Spain).

Preparation of Brewed Coffee. Soluble coffee (blend of 80% Arabica and 20% Robusta; 18.9% roasting loss) was obtained directly from the producer. Samples were ground, packed under vacuum, and frozen. Then, 1 g of ground coffee was resuspended in 100 mL of hot water (50–60 °C) for 3 min under continuous stirring. The coffee brew (CB sample) obtained was then filtered (Whatman filter paper no. 40, ashless, Whatman, U.K.), freeze-dried (Mod. Lioalpha, Telstar, ES), and stored at 4 °C until analysis.

Preparation of Melanoidins, Pure Melanoidin and Digested Melanoidin Extracts from Brewed Coffee. Melanoidins were obtained after ultrafiltration of filtered coffee brew as previously described by Delgado-Andrade and Morales (*24*). A scheme of the process is depicted in **Figure 1**. Briefly, an aliquot of the coffee brew was subjected to ultrafiltration, using an Amicon ultrafiltration cell, model 8400 (Amicon, Beverly, MA), equipped with a 10000 Da nominal molecular mass cutoff membrane (Ultracel YM-10, regenerated cellulose, Amicon-Millipore, DE). The retentate was filled up to 200 mL with water and washed again. This washing procedure (diafiltration) was repeated at least three times. The soluble high molecular weight fraction corresponding to melanoidins (M sample) was freeze-dried and stored in a desiccator at 4 °C until analysis.

Obtaining of pure melanoidins (PM sample) was performed as previously reported . A solution containing 20 mg mL^{-1} melanoidin (to obtain a representative amount of product) was prepared in 2 M NaCl. NaCl was used to release compounds ionically bound to the melanoidin (BMC). After 18 h of incubation, solutions were again ultrafiltered (Microcon YM-10, regenerated cellulose 10000 Da, Bedford, MA) at 14000*g* for 50 min. Retentates, containing the soluble PM fraction, were resuspended in water and then freeze-dried and stored in a desiccator at 4 °C until analysis. Filtrates containing noncovalently bound to melanoidin compounds (BMC sample) were freeze-dried and conserved at 4 °C until antioxidant analysis.

Simulated gastrointestinal digestion of coffee melanoidins was carried out by following the method described by Ames et al. (*33*). Briefly, 0.5 g of coffee melanoidin was diluted with distilled water (4 mL). After freshly prepared pepsin was added at a concentration of 3.2 g in 20 mL of 0.1 M HCl, the pH was adjusted to 2.0 using 6 M HCl, and the mixture was incubated at 37 °C for 3 h in a shaking water bath. After the gastric digestion step, the pH of samples was adjusted to 7.0 with 0.5 M $NaHCO_3$ and 1.25 mL of freshly prepared pancreatinin–bile mixture (0.4 g of pancreatinin and 2.5 g of bile salts in 100 mL of 0.1 M $NaHCO_3$) before incubating 1 h at 37 °C in a shaking water bath. At the end of the gastrointestinal digestion, the sample was heated in a boiling bath for 4 min in order to inactivate the enzymes. Finally, the digested solution was ultrafiltered (Microcon YM-10, Amicon-Millipore, DE) at 14000*g* for 50 min. The retentate represents the digested melanoidin (DM sample) fraction. Filtrate contains the low molecular weight compounds released after gastrointestinal digestion of melanoidin (DBMC sample). Samples were resuspended in water and then freeze-dried and stored in a desiccator at 4 °C until analysis.

Antioxidant Assays. *DPPH Assay.* The antiradical activity of different samples was estimated according to the procedure reported by Delgado-Andrade et al. (*28*). A 200 μL aliquot of sample (CB, M, PM, BMC, DM, or D-BMC) was added to 1 mL of methanolic solution of $DPPH^{\bullet}$ (74 $mg \cdot L^{-1}$). A daily prepared solution of $DPPH^{\bullet}$

gave a final absorption at 520 nm of 1.8 AU. The mixture was shaken for 1 h at room temperature, and then absorption was measured at 520 nm. Temperature in the measurement chamber was set at 30 °C. The antiradical activity of the sample was expressed as the disappearance of the initial purple color; then the higher the disappearance, the greater the antiradical activity. Aqueous solutions of trolox at various concentrations were used for calibration (0.15–1.15 mM). The results are expressed as micromole equivalents of trolox per gram of sample.

$ABTS^{+\bullet}$ Assay. The antioxidant capacity was estimated in terms of radical scavenging activity, following the procedure described by Delgado-Andrade et al. (*28*). Briefly, $ABTS^{+\bullet}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{+\bullet}$ solution (stable for 2 days) was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. After addition of 10 μL of sample (CB, M, PM, BMC, DM, or DBMC) or trolox standard to 4 mL of diluted $ABTS^{+\bullet}$ solution, an absorbance reading was taken at 20 min using a Shimadzu UV–visible 1601 spectrophotometer (Duisburg, Germany). Calibration was performed, as described previously, with a trolox stock solution. Results were expressed as micromole equivalents of trolox per gram of sample.

FRAP Assay. The ferric reducing ability of each standard solution was estimated according to the procedure applied by our research group to coffee melanoidins (*28*). Briefly, 900 μL of FRAP reagent, prepared freshly and warmed at 37 °C, was mixed with 90 μL of distilled water and 30 μL of a test sample (CB, M, PM, BMC, DM, or DBMC) or water as appropriate reagent blank. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot H_20$ and 25 mL of 0.3 M acetate buffer, pH 3.6. Readings at the absorption maximum (595 nm) were taken every 15 s using a Shimadzu UV–visible 1601 spectrophotometer equipped with a thermostated automatic sample positioner. Temperature was maintained at 37 °C, and the reaction was monitored up to 30 min. Trolox stock solutions were used to perform the calibration curves. Results were also expressed as micromole equivalents of trolox per gram of sample.

ORAC Assay. The antiradical activity against AAPH was estimated according to the procedure slightly modified from that reported by Dávalos et al. (*34*). The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. Melanoidins (previously diluted 1/350) or trolox (20 μL) and fluorescein (120 μL; 70 nM, final concentration) solutions were placed in each well of a black 96-well polystyrene microplate (Biogen Científica, Madrid, Spain). The mixture was preincubated for 15 min at 37 °C on the microplate reader (BioTek Instruments, VT). AAPH solution (60 μL; 12 mM, final concentration) was added rapidly using the plate reader syringe, the microplate was shaken for 15 s, and the fluorescence was recorded every minute for 90 min at 485 and 528 nm excitation and emission wavelengths, respectively. A blank (fluorescein + AAPH) using phosphate buffer instead of the sample solution and eight calibration solutions using Trolox (1–8 μM, final concentration) as antioxidant were also carried out in each assay. All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample.

Raw data were processed by the microplate reader, and the area under the curve (AUC) was calculated according to the equation

$$AUC = F_0/F_0 + F_0/F_1 + F_0/F_2 + ... + F_0/F_n$$

with F_0 the fluorescence intensity at time 0, F_1 the fluorescence intensity at time 1 min, and so forth. Finally, the net AUC was calculated by subtracting the AUC of the blank sample to the AUC of each sample. ORAC values were expressed as trolox equivalents by using the standard curve calculated for each assay, and final results were expressed in micromole equivalents of trolox per gram of sample.

HOSC Assay. The hydroxyl radical scavenging capacity of melanoidins and melanoidin fractions was estimated according to the procedure recently developed by Moore et al. (*35*). The assay reactions were carried out in black 96-well polystyrene plates from Biogen Científica (Madrid, Spain) and analyzed using a Biotek plate reader at an excitation wavelength of 485 nm, emission wavelength of 528 nm, and 0.1 s read time for each well with each plate read once per minute for 3 h. The reaction mixtures contained 170 μL of a 93 nM fluorescein solution prepared in 75 mM sodium phosphate buffer (pH 7.4), 30 μL of blank or sample or trolox, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$, added in that order. Trolox concentrations of 20, 40, 60, 80, and 100 μM were used as standards. HOSC values were calculated using the regression equation for area under the curve (AUC) and net AUC values were calculated in the same way as that for the ORAC assay except for the assay period (180 min instead of 90 min). HOSC values were expressed as trolox equivalents by using the standard curve calculated for each assay, and final results were expressed in micromole equivalents of trolox per gram of sample.

Statistical Treatment. All of the analyses were performed at least in triplicate. The Statgraphics v5.1 statistical procedures were performed at a significance level of 95%.

RESULTS AND DISCUSSION

Coffee brew (CB), coffee melanoidin (M), and derived fractions (PM, BMC, DM, DBMC) were analyzed by five different well-described methodologies (DPPH, ABTS, FRAP, HOSC, ORAC) for assessing the antioxidant activity (antiradical activity and ferric reducing capacity). Initially, results are expressed as micromole equivalents of trolox per gram of sample for CB and M samples giving information on their net activity. But, results for melanoidin-derived fractions are expressed in two different ways, namly, the relative and absolute approach. The relative approach explains the contribution of each fraction to the overall antioxidant activity of their parent coffee melanoidin. Then, antioxidant activity of pure melanoidins (PM), noncovalently bound to melanoidin compounds (BMC), digested melanoidins (DM), and low molecular weight compounds released after enzymatic digestion of melanoidins (DBMC) are expressed as micromole equivalents of trolox per PM, BMC, DM, or D-BMC released from 1 g of melanoidin. These results are summarized in the **Table 1** within the column called relative. In contrast, the absolute approach compares directly the net antioxidant activity of chemical structures of each melanoidin fraction regardless to their concentration in the media and results are expressed per gram of sample. Then, the absolute approach takes into consideration the abundance of each melanoidin fraction. In this sense, in order to obtain the absolute results of the melanoidin fractions, the mean recovery obtained from their parent melanoidin was applied (10.6, 14.2, 85.8, and 89.4% for DBMC, BMC, PM, and DM, respectively). These results are summarized in the column called absolute in **Table 1**. Melanoidin represents 38.4% of the coffee brew and results are in line with previous results from our group (*24, 28*) and others in the field (*6, 7, 12*).

Different blanks were performed in order to check the absence of interaction between the procedures employed to release low-molecular weight compounds from melanoidins and the antioxidant assays. First melanoidins were resuspended in water, incubated for 18 h, and subsequently ultrafiltered as stated previously for the NaCl incubation assay. No antioxidant activity was found in the filtrate whereas the retentate one was similar to that of melanoidins not incubated. This proved that the compounds recovered in the BMC fraction are released only due to the high ionic strength treatment and not simply due to the higher time in an aqueous solution. In addition it was assayed with two different blanks in order to prove the absence of antioxidant activity due to the enzymatic digestion. On the one hand PM and BMC were heated in the same way as DM and DBMC. Because of a similar antioxidant activity was obtained for heated and unheated PM and BMC, it could be stated that

Table 1. Antioxidant Activities of Brewed Coffee, Melanoidin, and Melanoidin Fractions As Determined by DPPH, ABTS, FRAP, ORAC, and the HOSC Methods

	TEAC									
	DPPH		ABTS		FRAP		ORAC		HOSC	
sample[a]	relative[c]	absolute[b]	relative[c]	absolute[b]	relative[c]	absolute[b]	relative[c]	absolute[b]	relative[c]	absolute[b]
CB		505 ± 6		1272 ± 16		481 ± 9		3534 ± 74		914 ± 28
M		342 ± 4		877 ± 12		479 ± 11		2440 ± 56		744 ± 16
PM	105 ± 3	123 ± 4	289 ± 7	337 ± 6	106 ± 4	124 ± 6	648 ± 29	755 ± 24	353 ± 11	411 ± 7
BMC	303 ± 6	2136 ± 13	929 ± 13	6540 ± 18	366 ± 7	2580 ± 29	1249 ± 36	8795 ± 89	657 ± 19	4624 ± 44
DM	68 ± 2	76 ± 3	268 ± 6	300 ± 9	80 ± 3	90 ± 2	713 ± 16	797 ± 31	365 ± 4	407 ± 12
DBMC	316 ± 4	2978 ± 11	1026 ± 16	9684 ± 27	374 ± 6	3524 ± 24	1301 ± 41	12276 ± 101	669 ± 9	6307 ± 51

[a] CB (coffee brew), M (melanoidin), PM (pure melanoidin), BMC (noncovalently bound to melanoidin compounds), DM (digested melanoidin), DBMC (noncovalently bound to melanoidins compounds after digestion). Values are mean ± standard deviation. [b] Data expressed as micromole equivalents of trolox/gram of sample. [c] Data expressed as micromole equivalents of trolox/fraction released per gram of melanoidin.

no antioxidant substances are formed during this step. On the other hand a mixture of enzymes and the other components used during digestion was assayed for antioxidant activity after heating without the presence of melanoidins. Negligible activity was obtained, proving that enzymes do interference with the antioxidant activity of DM or DBMC.

Absolute Approach. TEAC (trolox equivalent antioxidant capacity) values obtained for CB and M samples depend on the method applied although overall antioxidant activity of CB was 1.32-fold higher than M at the same concentration. DPPH, ABTS, ORAC, and HOSC methods analyze the radical scavenging activity whereas FRAP estimates the ferric reducing ability of samples. In addition, ABTS, ORAC, and HOSC are developed in aqueous media whereas DPPH is performed in a methanolic one. DPPH assay showed the lowest response compared to ABTS, HOSC, and ORAC. Del Castillo et al. (*36*) analyzing coffee brews from several roasting processes, reported higher responses by the ABTS test in aqueous dilution versus ethanolic dilution. They attributed the difference to the fact that some components, making an important contribution to the antioxidant activity of the aqueous dilutions, were not soluble in ethanol. Probably the fact that the DPPH method was developed in methanolic media was responsible of the lower response found. In the case of ORAC, the higher antioxidant activity is related to the better capacity of quenching AAPH radicals, whereas in the case of HOSC, the lower results indicate a small capacity of quenching hydroxyl radicals. In the case of the ABTS method, similar results were obtained by Cämmerer and Kroh (*11*) in instant coffees whereas for the FRAP methods the results are in line with those reported by Sánchez-González et al. (*37*) and Saura-Calixto and Goñi (*38*) in instant and soluble coffees, respectively.

As depicted in **Table 1** the absolute antioxidant activity of PM or DM fractions is close to that expressed also in a relative way due to the high recovery of PM and DM (85.8 and 89.4%, respectively) from coffee melanoidin. However, greater differences in the BMC and DBMC activity are found because of the low recovery of these fractions (10.6 and 14.2%, respectively). At this stage, it is important to notice that PM and BMC are just present in the food whereas DM and DBMC fractions are only present after digestion. Therefore, antioxidant activity of BMC could be exerted in the food and in vivo as well, whereas DBMC fractions are potentially bioactive just in vivo. **Figure 2** illustrates the comparison of the net antioxidant activity of different fractions as measured by different procedures regardless to their abundance in the coffee. Antioxidant activity was normalized to the antioxidant activity of coffee melanoidin to avoid bias by using different assays and to allow comparison as well. PM and DM showed similar antioxidant activity but

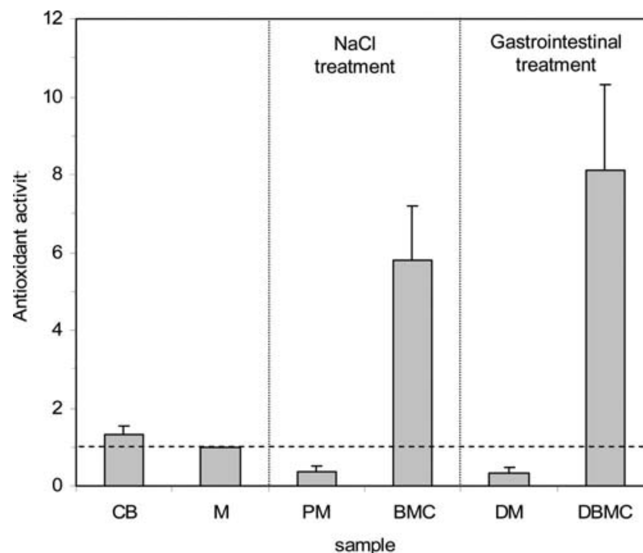

Figure 2. Average antioxidant activity of coffee brew (CB), coffee melanoidin (M), pure melanoidin (PM), noncovalently bound to melanoidin compounds (BMC), digested melanoidin (DM), and noncovalently bound to melanoidins compounds after digestion (DBMC) as measured by five different antioxidant procedures. Values are normalized to M (response = 1) for each antioxidant assay for comparison. Horizontal dotted line indicates relative value of coffee melanoidin of 1.

lower than that for coffee melanoidin. Results were highly consistent among the different methods applied showing that PM and DM were not greatly influenced by the method applied. However, it is noteworthy that the antioxidant activity of the low molecular weight fractions (BMC and DBMC) was up to 8 times higher than that of their parent coffee melanoidin, indicating the strong activity of these compounds when isolated. In these samples, higher differences were found among the method applied where ORAC exerted a significantly lower activity for both fractions as compared with the melanoidin fraction. DMBC gives a higher antioxidant activity than BMC and differences become significant if results from ORAC are skipped. It is plausible to suggest that chemical structures of the substances included in BMC and DBMC are not necessarily similar in their antioxidant activity. Gastrointestinal digestion of coffee melanoidins promotes the formation of powerful antioxidant substances as measured by five different antioxidant procedures. The high antioxidant activity (antiradical and ferric reducing activity) of the DBMC fraction is of great importance because these low molecular weight compounds could be absorbed in vivo whereas a mechanism of action for melanoidins is difficult to establish and is still under study.

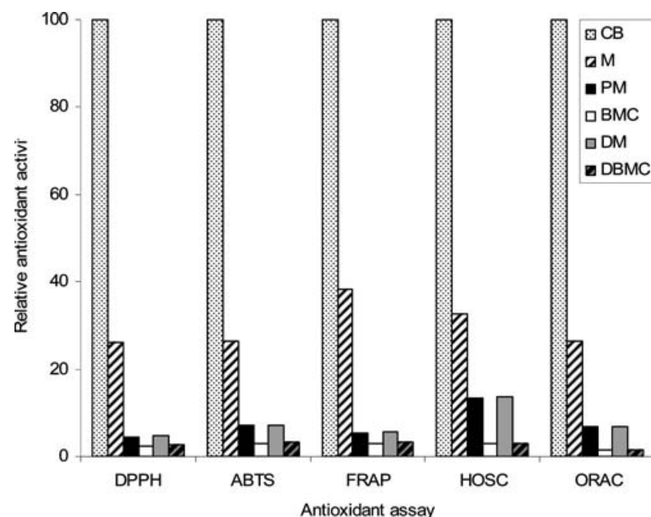

Figure 3. Contribution of melanoidins (M), pure melanoidin (PM), digested melanoidin (DM), and noncovalently bound to melanoidin compounds released after digestion (DBMC) fractions to the total antioxidant activity of coffee brew (CB) as determined by different approaches. The 100% of activity is assigned for coffee brew.

Relative Approach. Antioxidant activity of brewed coffee, coffee melanoidin, and its derived fractions were studied according to their relative concentration in the coffee brew. As shown in **Table 1** (relative column) for the DPPH assay, the relative antioxidant activity of the PM decreased to a great extent whereas the activity of the BMC fraction was similar to that of melanoidin. Similar results were found for the rest of the antioxidant assays. In the case of ABTS, the activity of BMC was also higher than that of their parent melanoidin. The difference among the antioxidant activity of M and PM confirms the contribution of low molecular weight compounds to the antioxidant properties of melanoidins from coffee brews regardless the method used.

The application of enzymatic digestion gives two additional fractions, digested melanoidin (DM) and noncovalently bound to melanoidin compounds released after digestion (DBMC). DM and DBMC fractions showed similar antioxidant activity to that reported for PM and BMC for all methods (**Table 1**, relative column). This is an important finding because DM is a melanoidin that reflects in a more realistic way the antioxidant activity of coffee melanoidin into the gastrointestinal tract whereas PM is not present as itself. In addition, incubation with 2 M NaCl could be useful as a fast way to reproduce the effect of an enzymatic digestion over the release of low molecular weight compounds with antioxidant activity from the melanoidin since differences between relative antioxidant activity between DBMC and BMC fraction were not found. But enzymatic digestion of melanoidin is able to generate low molecular weight structures which possesses significantly more antioxidant activity than that released after ionic treatment.

For DM and DBMC fractions, similar results to that reported for DDPH were obtained for the rest of the antioxidant assays (**Table 1**, relative column). Within the antiradical assays, DBMC showed the highest efficiency against AAPH radicals (ORAC assay) whereas the lowest one was for the DPPH radical. It must be pointed out that both DM and DBMC exerted strong antioxidant activity against the hydroxyl radicals (HOSC assay) which are important for quenching physiologically produced radicals (not like ABTS, DPPH, or AAPH which are artificial stable radicals).

Figure 3 shows percentages of contribution of M, DM, and DBMC to the overall antioxidant activity of the coffee brew (assigned 100% of activity) by the different methods assayed. Data have been calculated from percentage of melanoidins present in the coffee brew and the recovery of each melanoidin fraction. Melanoidins account for 26–38% of the overall antioxidant activity of the coffee brew (depending on the antioxidant method applied). Antioxidant activity of DM is significantly lower ($P < 0.05$) than M, representing 5–14% of the total CB antioxidant activity. DBMC are substances with high antioxidant activity, but their low amount explains the low contribution (2–3%) to the overall antioxidant activity of the CB.

In summary, results support those previously obtained by our research group (*28*) concerning the important contribution of noncovalently bound to melanoidin compounds to the overall antioxidant activity of coffee brew. These findings are in accordance with Del Castillo et al. (*36*) who demonstrated that the low molecular weight fraction of coffee brews possesses higher antioxidant activity than the high molecular weight fraction. Moreover, our present work demonstrates that DBMCs are liberated from the melanoidin by a simulated gastrointestinal enzymatic digestion and they exerted efficient antioxidant properties as compared with the BMC fraction. Their low molecular weight could allow a rapid absorption and potential bioactivity. The DBMC fraction has a remarkable high antioxidant activity as determined by different assays. Since antioxidant activity of the BMC fraction is lower, it is hypothesized that gastrointestinal digestion affects to some extent the melanoidin fraction by enhancing the antioxidant capacity of the low molecular weight compounds ionically bounded to the structure or by releasing new structures from the melanoidin skeleton. However, it is still unknown what kind of chemical changes are responsible for such modification. These chemical changes have not been reported in the literature since similar recoveries for PM and DM melanoidins were also recorded in this study indicating no degradation. However, some structural modification after gastrointestinal digestion is necessary to explain the higher antioxidant activity of the low molecular weight fraction. On the other hand, the battery of antioxidant methods used on brewed coffee, melanoidins, and different melanoidins fractions reported similar results for antiradical activity, although ORAC gives lower net values. HOSC is a very promising procedure for further investigation on melanoidin and melanoidins fractions and can be easily automated. Future investigations are being carrying out to identify structurally the most active compounds at the DBMC fraction.

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