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Antioxidant Activity of Coffee Model Systems

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Coffee model systems prepared from combinations of chlorogenic acid (CGA), N^{α}-acetyl-L-arginine (A), sucrose (S), and cellulose (C) were roasted at 240 °C for 4 min prior to analysis by UV−visible spectrophotometry, capillary zone electrophoresis (CZE), and the ABTS radical cation decolorization assay. The A/CGA/S/C and A/S/C systems were also fractionated by gel filtration chromatography. Antioxidant activity of the systems showed a positive, nonlinear relationship with the amount of CGA remaining after roasting. Sucrose degradation was a major source of color in the heated systems. There was no relationship between antioxidant activity and color generation.

KEYWORDS: Coffee model systems; antioxidant activity; chlorogenic acid; ABTS radical cation assay; capillary electrophoresis

INTRODUCTION

Coffee beverages, prepared from roasted coffee beans, are widely consumed throughout the world for their physiological effects and attractive aroma and taste (*1*). Recent papers (*1*−*5*) demonstrate that antioxidant properties of roasted coffee may be attributed to Maillard reaction products formed during roasting in addition to certain phenolic compounds that are present in green coffee including chlorogenic acid (CGA), caffeic acid, ferulic acid, and *p*-coumaric acid. The contribution of volatile heterocyclic compounds to the antioxidant activity of brewed coffee has also been reported (*6*). Roasting markedly affects the composition of coffee phenolics (*7*, *8*), and products of chemical reactions occurring during roasting are the prevailing contributors to the antioxidant activity in coffee beverage (*1*).

Roasted coffee is a complex system, and the relative contribution of its different components to antioxidant activity still requires classification. Understanding in this area might be improved by studying appropriate model systems. CGA is the major phenolic compound in coffee, but only one investigation (*9*) has used it in a model food system ($a_w = 0.22-0.94$, heated at up to 70 °C) and color production rather than antioxidant activity was investigated in that study. In contrast, the antioxidant activity of many Maillard model systems has been studied (*10*, *11*). However, these have been either aqueous solutions heated at ∼100 °C or low-moisture systems incubated at close to room temperature. Some high-temperature/low-moisture Maillard model systems have been used to study color (*12*) or flavor (*13*, *14*), but antioxidant activity was not monitored.

Therefore, the aims of this investigation were to follow the fate of the constituents of model coffee systems during roasting and to examine these antioxidant properties before and after heating.

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MATERIALS AND METHODS

Chemicals and Reagents. Chlorogenic acid (3-caffeoylquinic acid) hemihydrate (CGA), sodium tetraborate decahydrate, 5-hydroxymethylfurfural (HMF), and sodium hydroxide solutions were obtained from Fluka (Gillingham, U.K.). Cellulose powder (C) (20 μm) was from Aldrich Chemical Co. (Gillingham, U.K.). N^{α}-Acetyl-L-arginine (A), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and sucrose (S) were obtained from Sigma (Gillingham, U.K.). D-(+)-Glucose and D-(−)-fructose were from Fischer Scientific, (Leicester, UK). Sodium chloride and sodium dihydrogen orthophosphate dihydrate, ammonia solution (25%), were of AnalaR grade from BDH (Lutterworth, U.K.). Disodium hydrogen orthophosphate (anhydrous) and hydrochloric acid (37%) were of GPR grade from BDH. Polyethylene glycol molecular weight markers (4120, 1470, and 1080 Da) were from Polymer Laboratories (Church Stretton, U.K.). Sephadex G-25 was from Pharmacia (Uppsala, Sweden). High-purity water was produced in-house using a Purite (High Wycombe, U.K.) Labwater RO50 unit and was used throughout.

Preparation of Model Systems. CGA, A, S, and C were used to prepare eight different coffee model systems, the compositions of which are described in **Table 1**. The weighed solids were mixed with 100 mM phosphate buffer (pH 5.8, 20 mL), frozen, and freeze-dried. Each mixture was transferred to a Petri dish and placed in a vacuum desiccator containing saturated sodium chloride solution at 20 °C until constant mass was reached (∼15% moisture). The procedure took ∼2 days, and the sample was stirred daily to ensure equilibration.

Equilibrated samples were divided into two portions. One was not heated. The other was spread in a thin layer on a glass microscope slide (76 × 26 × 1.0−1.2 mm), placed in an aluminum tray, and heated at 240 °C for 4 min in a preheated oven. Cooled samples and unheated samples were added to water (20 mL), stirred for 10 s, and allowed to stand for 10 min before filtering through Whatman no. 4 filter paper. The pH was measured immediately, and filtrates were stored at −20 °C for <7 days prior to other analyses.

Gel Filtration Chromatography. Filtrates of heated systems 1 and 3 were fractionated by gel filtration chromatography based on a published procedure (*15*). Filtrate (1 mL) was freeze-dried and dissolved in water (0.1 mL) to obtain solutions containing 4 mg of solids/50 μL.

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Table 1. Composition of the Model Systems

model system	N^{α}-acetyl-L-arginine (mg)	CGA[a] (mg)	sucrose (mg)	cellulose (mg)
1	30	50	80	720
2	30	50	–[b]	800
3	30	–	80	770
4	–	–	–	850
5	–	50	80	750
6	30	–	–	850
7	–	50	–	830
8	–	–	80	800

[a] CGA, chlorogenic acid. [b] The component was omitted from the model system.

Gel filtration was carried out on a Sephadex G-25 column (20 × 1.0 cm). Solution (50 μL) was applied onto the column and eluted with water at 0.1 mL/min. Fractions were collected every 5 min, 35 fractions being obtained. Molecular weight ranges of fractions were estimated by means of polyethylene glycol molecular weight markers and 3-caffeoylquinic acid, as calibration standards. The absorbance (at 280 and 405 nm) of each fraction was measured.

UV—Visible Spectrophotometry. Absorbance of triplicate filtrates was measured at 420 nm. No dilution was required for the unheated systems, whereas the heated systems were diluted 10-fold in water before measurement. For spectral determination (200−700 nm), filtrates from all systems were diluted 10-fold.

Capillary Zone Electrophoresis (CZE). Separations were carried out using a Hewlett-Packard (HP, subsequently Agilent, Bracknell, U.K.) ^{3D}capillary electrophoresis instrument equipped with HP ChemStation software. The fused-silica capillary was 48.5 cm long (40 cm to the detector), with an internal diamter of 50 μm and a ×3 bubble cell. The temperature was 25 °C, and injection was at 50 mbar for 5 s. Samples were prepared in triplicate, and two injections of each filtrate were analyzed. Components were quantified by reference to standard calibration curves.

CGA and N^{α}-Acetyl-L-arginine (A). Tetraborate buffer (50 mM, pH 9.5) was used. The voltage was 20 kV. Separations were monitored at 200 and 280 nm, and spectra were collected from 190 to 600 nm. Conditioning of the capillary before use involved a 30 min flush with 1 M NaOH followed by 20 min with 0.1 M NaOH and finally 15 min with water. Then the capillary was flushed with running buffer for 15 min. Between runs, the capillary was flushed with 0.1 M NaOH for 3 min and then running buffer for 3 min (*8*). A and HMF comigrated, and both absorbed at 200 nm, but only HMF (not A) absorbed at 280 nm. Therefore, the area due to A was quantified as follows:

$$\text{area of A at 200 nm} = \text{total area at 200 nm} - \frac{(\text{area of HMF at 280 nm}) \times \text{absorptivity at 200 nm}}{\text{absorptivity at 280 nm}}$$

$$\text{area of A at 200 nm} = \text{total area at 200 nm} - (\text{area of HMF at 280 nm})/3.75$$

Sucrose (S). The procedure was based on that of Bazzanella and Bachmann (*16*). The electrolyte was 6 mM copper(II) sulfate solution in 0.5 M ammonia solution. The pH was adjusted to pH 11.6 with 25% ammonia solution. The voltage was 25 kV, and separations were monitored at 245 nm. The capillary was conditioned each day prior to use with water (2 min), 0.1 M HCl (10 min), water (2 min), 0.1 M NaOH (10 min), water (2 min), and copper(II) electrolyte (20 min). Then the capillary was flushed with run buffer for 15 min. After every run, the capillary was flushed with 0.1 M NaOH for 3 min and electrolyte for 3 min. Fresh buffer was used for every run. After use, the capillary was rinsed with 0.5 M ammonia solution for 5 min followed by 5 min with water and finally dried with air for 5 min.

Antioxidant Activity. The antioxidant activity of the samples was determined by the $ABTS^{\bullet+}$ decolorization assay (*17*). $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in 10 mL of water) and keeping the mixture in the dark at room temperature for 12−16 h before use. The radical is stable in this form for more than 2 days stored in the dark at room temperature. The aqueous $ABTS^{\bullet+}$ solution was diluted with ethanol (1:100) to an absorbance of 0.7 (±0.02) at 734 nm in a 1-cm cuvette at 30 °C. Filtrates were diluted 10-fold in water and vortex-mixed for 10 s prior to analysis. Gel chromatography fractions were analyzed directly. Samples (20 μL) were added to the $ABTS^{\bullet+}$ solution (2 mL). After mixing, the absorbance was measured at 734 nm after exactly 1 min and then every minute for 7 min. Readings taken after 5 min of incubation were used to calculate percent inhibition values. A solvent blank was also run. Absorbance values were corrected for the solvent as follows:

$$\Delta A_{\text{sample}} = (A_{t=0(\text{sample})} - A_{t=5(\text{sample})})/ A_{t=0(\text{sample})} - (A_{t=0(\text{solvent})} - A_{t=5(\text{solvent})})/A_{t=0(\text{solvent})}$$

Percent inhibition values were obtained by multiplying ΔA_{sample} values by 100.

A standard calibration curve was constructed by plotting percentage inhibition against concentration of CGA. Antioxidant capacities of the samples were calculated in CGA equivalents using this curve. Triplicate determinations were made for triplicate samples and standards.

Statistical Analysis. Statistical analysis was done using SPSS 10.0 for Windows program (SPSS Inc., Chicago, IL). Student's *t* test was used to ascertain any statistical difference between two groups of means. One-way analysis of variance (ANOVA) was used to look for differences between means of more than two groups. Where ANOVA indicated differences, the least significant difference (LSD) test ($p < 0.05$) was applied to determine which values were different.

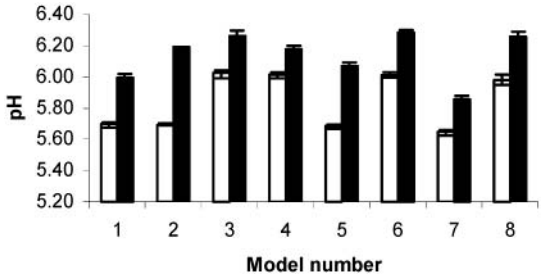

Figure 1. Changes in pH of aqueous extracts of the model systems on heating: (white bars) unheated models; (black bars) heated models. See **Table 1** for system composition. Values are the means of triplicate analyses. Error bars show the relative standard deviation. Differences between the same system before and after heating were significant ($p < 0.01$).

RESULTS AND DISCUSSION

CGA and sucrose were selected as reactants because they are the most abundant phenolic compound and sugar, respectively, in green coffee (*18*). Arginine is completely degraded under coffee roasting conditions (*18*−*20*). Because most arginine is present in coffee as peptides or proteins, N^{α}-acetyl-L-arginine was chosen as the reactant. In addition, cellulose was used to keep the concentrations of the precursors similar to those in coffee beans. pH and moisture content have important effects on the Maillard reaction, so these parameters were adjusted to represent the values in green beans, that is, pH 5.8 and 15% moisture (*18*, *21*, *22*).

pH. After heating, the pH of the filtrates of all systems increased significantly ($p < 0.01$) (**Figure 1**). Compared to the other systems, those containing CGA had a lower pH before heating, and their more pronounced pH increase may be attributed to CGA degradation. The highest increase in pH (from 5.7 to 6.4) was detected for system 2 (A/CGA/C). This compares to a pH increase from 5.63 to 5.83 for system 7 (CGA/C). The data in **Figure 1** suggest that the Maillard reaction and caramelization products played an important role in the increase in pH of the heated systems. The findings agree with those

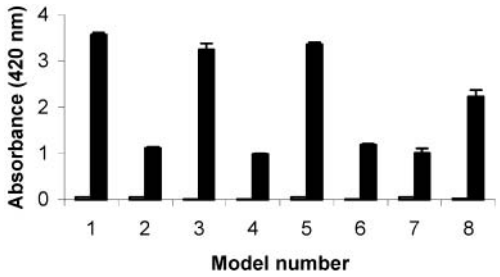

Figure 2. Changes in absorbance at 420 nm of aqueous extracts of the model systems on heating: (white bars) unheated models; (black bars) heated models. See **Table 1** for system composition. Values are the means of triplicate analyses. Error bars show the relative standard deviation. Differences between the same system before and after heating were significant ($p < 0.01$).

reported by Ko (*22*), who found that the pH values of roasted coffee increased with increasing roast time (range = 1−14 min) and decreasing temperature (range = 220−270 °C).

UV—Visible Spectrophotometry. Absorption at 420 nm was used to monitor the formation of brown pigments during roasting. The absorbance of the filtrates from all of the models increased significantly ($p < 0.001$) after roasting (**Figure 2**), the highest values being obtained for system 1 (A/CGA/S/C), followed by systems 5, 3, and 8. These were the S-containing systems, and the data imply that, during heating, sugar caramelization was a more important source of color than the Maillard reaction.

Comparisons of models 3 (A/S/C) and 8 (S/C) and of models 3 and 5 (CGA/S/C) demonstrate the role of S as a major source of color generation, although the addition of A to S and C (model 3) did significantly increase absorbance, presumably due to reactions between A and S-degradation products. Comparison of models 1, 3, and 5 establishes that the addition of CGA and A to S and C (model 1) resulted in little additional color generation compared to models 3 (A/CGA/C) and 5 (CGA/S/C), and the presence of A had a relatively small effect (models 1 and 5).

UV−vis spectra of the models before and after heating are shown in **Figure 3**. Unheated systems containing CGA (models 1, 2, 5, and 7) possessed spectra similar to that of pure CGA. The other unheated systems possessed featureless spectra.

On roasting, the spectra changed, due to the degradation of CGA and the generation of new compounds. The spectra of models 2 (A/CGA/C) and 7 (CGA/C) resembled that of CGA, but they were less intense, owing to the partial degradation of CGA during roasting and the generation of breakdown products of CGA, such as caffeic acid, the spectrum of which is almost identical to that of CGA. Compared to model 2, the spectra of models 1 (A/CGA/S/C) and 5 (CGA/S/C) after heating showed a different wavelength of maximum absorption (280 nm) as well as a decrease in intensity. The spectra of heated models from which CGA has been excluded (3, 4, 6, and 8) each showed a peak with a λ_{max} at 280 nm. The increase in absorption at 280 nm may be attributed to the formation of HMF, a major sugar degradation product (*23*) that can form from S and C.

CZE. The profiles of the filtrates of the models were monitored by CZE and provided further information about their compositions. CGA decreased in every model containing it (**Table 2**), the lowest decrease (46%) occurring in model 7 (CGA/C). The presence of A in model 2 (A/CGA/C) caused a greater loss of CGA, as compared with model 7 (CGA/C), than the presence of S in model 5 (CGA/S/C). The presence of both A and S in model 1 (A/CGA/S/C) caused a nonsignificantly ($p > 0.001$) lower loss of CGA compared with model 2, possibly due to competing reactions between S and A or their degradation products.

Figure 4 shows the electropherogram (e-gram) in borate buffer of model 1 after heating. Seven new peaks, with spectral characteristics identical to those of CGA, were detected due to compounds formed on roasting. They could be due to CGA-related compounds such as isomers (*20*). In addition, a peak was observed with a spectrum and migration time that matched those of standard caffeic acid. All of these peaks were detected in the e-grams of all the CGA-containing models.

A broad band, migrating between 4 and 18 min was observed in the e-grams of all of the roasted systems. It absorbed at 420 nm, possessed spectral characteristics similar to those of melanoidins (*24*), and is likely to be due to colored macromolecular material formed on caramelization and in the Maillard reaction.

Losses of A ranged from 41 to 82% (**Table 2**), losses being lowest (41%) for model 6 (A/C). Decreases in A were significantly higher ($p < 0.001$) on incorporation of either CGA (model 2) or S (model 3) in the model. The *o*-quinone forms of CGA and its degradation products, for example, caffeic acid, would be expected to react with the guanidino group of A, in a manner analogous to the reaction between glyoxal and N^{α}-t-BOC-arginine, which gives an imidazoline (*25*). Reactions between CGA and the side chains of lysine and tryptophan residues of lysozyme have recently been reported (*26*). Addition of both CGA and S (model 1) increased loss of A still further. Loss of A was significantly greater in model 1 than in model 2, providing further evidence for reactions between S and A or their degradation products in model 1.

S decreased by 87−90% in every model containing it, and there was no significant difference ($p > 0.05$) among the systems (**Table 2**). Thus, breakdown of S occurred at the same rate in every model, regardless of its composition, providing additional evidence that sugar caramelization was a reaction of major importance in these systems. Comparison of the data in **Table 2** with the 420 nm absorbance data in **Figure 2** provides further evidence that S degradation was the major source of color in these systems.

Antioxidant Activity. Normally, the antioxidant activity of samples obtained from the ABTS method is expressed in terms of Trolox equivalents (*27*−*29*), but CGA was used as a standard for this study instead of Trolox because it was a component of several of the model systems and is known to possess antioxidant activity (*30*). **Figure 5** shows that the antioxidant activity of the CGA-containing models (1, 2, 5, and 7) did not differ significantly ($p > 0.05$) before roasting. The antioxidant activity of models 5 (CGA/S/C) and 7 (CGA/C) did not change significantly on heating, whereas the antioxidant activity of the systems containing A and CGA (1 and 2) were significantly lowered ($p < 0.05$). There is a positive, but nonlinear, relationship between loss of antioxidant activity (**Figure 5**) and loss of CGA (**Table 2**).

Statistical analysis of the antioxidant activity of the CGA-containing systems after roasting showed no significant differences ($p > 0.05$) between models 1 (A/CGA/S/C) and 2 (A/CGA/C) or between models 1 and 5 (CGA/S/C). However, the antioxidant activity of system 1 was significantly lower ($p < 0.05$) than that of model 7 (CGA/C). Also, model 2, with the lowest antioxidant activity of the CGA-containing systems, had significantly lower activity compared to both systems 5 and 7.

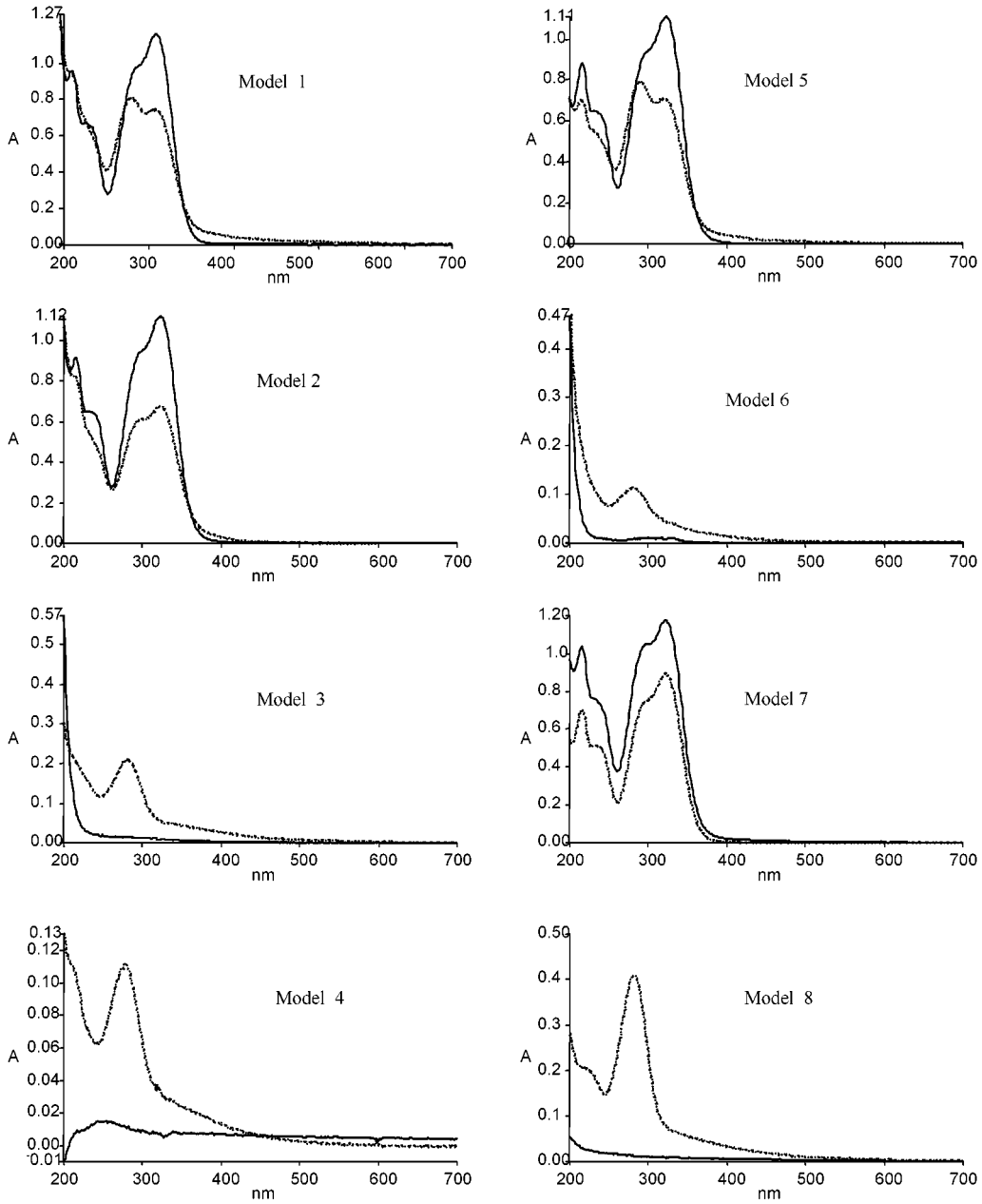

Figure 3. UV–vis spectra of model systems: (solid line) unheated models; (dashed line) heated models. See **Table 1** for system composition.

Table 2. Decrease (Percent) in N^{α}-Acetyl-L-arginine, Chlorogenic Acid (CGA), and Sucrose on Heating of the Model Systems[a,b]

model system	N^{α}-acetyl-L-arginine	CGA	sucrose
1	82.2 ± 3.3[c]	75.2 ± 2.5[b,c]	90.1 ± 1.5
2	67.7 ± 4.4b	85.7 ± 1.8c	–
3	74.9 ± 5.6bc	–	90.4 ± 3.4
4	–[c]	–	–
5	–	66.7 ± 11.1b	88.5 ± 1.7
6	40.7 ± 9.6a	–	–
7	–	46.2 ± 2.3a	–
8	–	–	87.6 ± 2.5

[a] Values are the means (± relative standard deviation) of triplicate determinations. [b] Means followed by different letters within a column were significantly different ($p < 0.05$). [c] The component was omitted from the model system.

It appears that the incorporation of A into the systems results in reduced antioxidant activity (**Figure 5**) as well as greater loss of CGA (**Table 2**).

It has been reported (*11*) that partially oxidized polyphenols can exhibit elevated antioxidant activity compared with the unoxidized counterparts, but this appears not to be the case for the CGA-containing systems, as assessed by the $ABTS^{\bullet+}$ assay, in the current study. Under the conditions employed in this study, CGA degradation may be more important than partial oxidation, and certain CGA degradation products, for example, caffeic acid (identified in the CGA-containing systems), are reported to have antioxidant activities similar to that of CGA (*28*), possibly accounting for this finding.

Models 3 (A/S/C), 4 (C), 6 (A/C), and 8 (S/C) possessed no antioxidant activity before heating but some activity developed on roasting. The exclusion of CGA from systems resulted in significantly less antioxidant activity after roasting. Melanoidins, the final products of the Maillard reaction, possess radical scavenging activity (*10*, *31*−*33*). Their activity, and that of related compounds formed by caramelization, in models 3, 4, 6, and 8 was small, compared to the effect of CGA or its degradation products in models 1, 2, 5 and 7. HMF and other

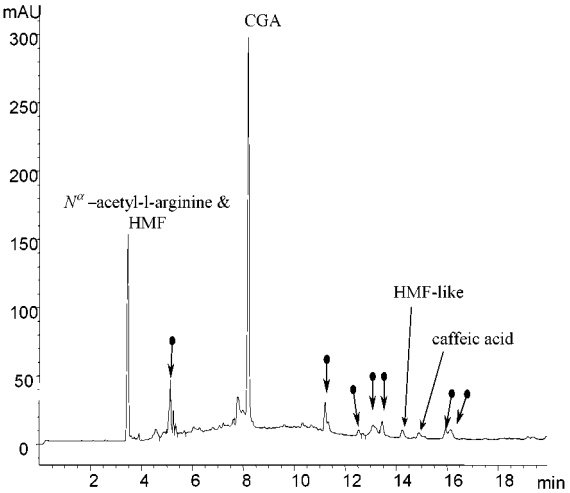

Figure 4. E-gram at 200 nm of model 1 (A/CGA/S/C) obtained using borate buffer: CGA, chlorogenic acid; ●→, peak with a spectrum matching that of standard CGA; HMF, 5-hydroxymethylfurfural; HMF-like, peak with a spectrum matching that of standard HMF.

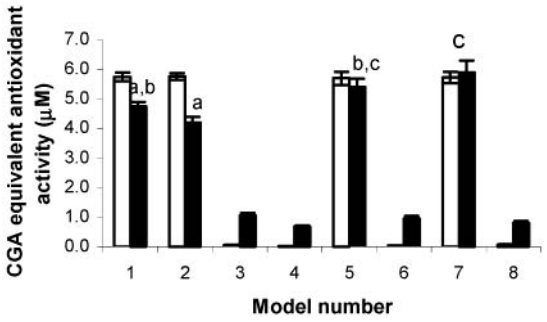

Figure 5. CGA-equivalent antioxidant activity (micromolar) of unheated (white bars) and heated (black bars) models. See **Table 1** for model composition. Values are the means of triplicate analyses. Error bars show the relative standard deviation. Bars with the same letters indicate no significant difference ($p > 0.05$).

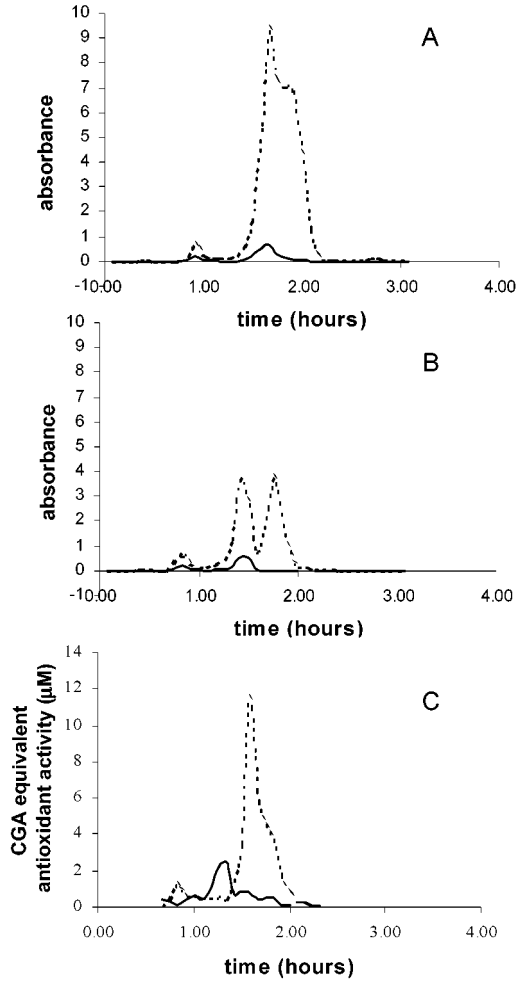

Figure 6. Gel filtration chromatography profiles at 280 nm (dashed line) and 405 nm (solid line) of heated models 1 (A) and 3 (B) and antioxidant activity (model 1, dashed line; model 3, solid line) (C).

volatile heterocyclic products of the Maillard reaction or caramelization may also contribute to the antioxidant properties of the models (*6*), but the levels at which these compounds occur is likely to make their contribution small.

Gel Filtration Chromatography. Filtrates of heated models 1 and 3 were fractionated by gel filtration chromatography prior to analysis of their antioxidant activity. Model 1 (A/CGA/S/C) was the most complex, and model 3 (A/S/C) was the same mixture without CGA.

Figure 6 (parts A and B) shows that, at 405 nm, two peaks were detected, which were absent from the unheated models and that are due to products of reactions such as caramelization and the Maillard reaction. Three peaks were detected in both models at 280 nm, the first two corresponding to the peaks detected at 405 nm in each system, respectively, and the third peak was a shoulder on the second peak in model 1. For both models, peak 1 eluted over 0.67−1.17 h, corresponding to the molecular mass range ~1470−4000 Da. The molecular mass range of peak 2 was ~35−1470 Da, corresponding to elution times of 1.33−1.75 and 1.33−1.58 h for systems 1 and 3, respectively. Peak 3 of models 1 (1.75-2.25 h) and 3 (1.58−2.25 h) had a molecular weight range of <350 Da.

UV−visible spectrophotometry of fractions of peak 1 of model 1 possessed spectra typical of those of melanoidins (*24*). Fractions corresponding to the center of peak 2 possessed spectra that matched that of CGA, whereas the spectra of fractions collected on the up and down slopes of peak 2 corresponded to aromatic compounds generated by thermal degradation of the reaction precursors. Spectra of the fractions comprising the first part of peak 3 matched that of standard HMF. Fractions of the three peaks collected from model 3 had nearly the same spectra as those from model 1 except that the spectrum of CGA was absent.

According to the antioxidant activity data for the fractions of models 1 and 3 (**Figure 6C**), model 1 possessed more antioxidant activity. The components of model 1 that contributed most to antioxidant activity eluted between 1.38 and 2.25 h, corresponding to peak 2 (**Figure 6A**), which contained CGA. Components of peaks 1 and 3 of model 1 also possessed some antioxidant activity (**Figure 6A**,**C**). In contrast, fractions of model 3 possessed only weak antioxidant activity, most being contributed by unidentified components of peak 2 (**Figure 6B**,**C**). The components of peak 1 of model 3 appeared to possess virtually no antioxidant activity.

In conclusion, data from this study demonstrate the predominant contribution to antioxidant activity made by CGA in these coffee model systems. Color generation was strongly associated with sucrose degradation. There was no relationship between antioxidant activity and color generation. Further work should aim to characterize individual reaction products and to determine their contribution to the total antioxidant activity of each system.

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