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Effect of Roasting on the Antioxidant Activity of Coffee Brews

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Colombian Arabica coffee beans were roasted to give light, medium, and dark samples. Their aqueous extracts were analyzed by gel filtration chromatography, UV−visible spectrophotometry, capillary electrophoresis, and the $ABTS^{\bullet+}$ assay. A progressive decrease in antioxidant activity (associated mainly with chlorogenic acids in the green beans) with degree of roasting was observed with the simultaneous generation of high (HMM) and low molecular mass (LMM) compounds possessing antioxidant activity. Maximum antioxidant activity was observed for the medium-roasted coffee; the dark coffee had a lower antioxidant activity despite the increase in color. Analysis of the gel filtration chromatography fractions showed that the LMM fraction made a greater contribution to total antioxidant activity than the HMM components.

KEYWORDS: Antioxidant activity; coffee; roasting; $ABTS^{\bullet+}$ assay; capillary electrophoresis

INTRODUCTION

Whereas green coffee has a mild, green, bean-like aroma, the desirable aroma associated with coffee beverages develops during roasting. During roasting, the beans are heated to 200−250 °C, and the roasting time can range from 0.75 to 25 min, the optimum time being 1.5−6 min (*1*, *2*), depending on the degree of roasting required (light, medium, or dark). Many complex physical and chemical changes take place during roasting, including the obvious change in color from green to brown. The major compositional changes occurring are decreases in protein, amino acids, arabinogalactan, reducing sugars, trigonelline, chlorogenic acid, sucrose, and water and the formation of melanoidins (*2*). Many of these changes are due to the Maillard reaction.

Phenolic compounds are antioxidants in vitro and might reduce the risk of cardiovascular disease (*3*, *4*). The most prevalent phenolic compounds in foods are hydroxycinnamic acids (*5*, *6*), and the major component of this class is caffeic acid, which occurs in food mainly as esters called chlorogenic acid (CGA) (*3*). Coffee is the major source of CGA in the human diet, daily intake in coffee drinkers being 0.5−1 g, whereas coffee abstainers typically ingest <100 mg/day (*7*). Roasting markedly affects the composition of coffee (*2*), and we have previously used capillary electrophoresis (CE) to profile coffee brews prepared from beans subjected to different degrees of roasting (*8*). Although compounds with antioxidant properties (mainly CGA) are lost during roasting of coffee beans (*2*), the overall antioxidant properties of coffee brews can be maintained, or even enhanced, by the development of compounds possessing antioxidant activity, including Maillard reaction products (*9*−*11*). Maillard reaction products formed in both foods and model systems on heating are reported to possess various types of antioxidant activity and even pro-oxidant properties (*12*). However, links between levels of particular classes of compound in coffee and antioxidant activity have not been made. The antioxidant activity of fractions of the ethyl acetate extract of a dark-roasted coffee brew has been reported (*9*), but the effect of roasting on the antioxidant properties of the fractions was not investigated. Therefore, the aim of this study was to investigate the composition of extracts from coffee beans and to examine the contribution of their components to the overall antioxidant activity of coffee brews prepared from beans subjected to different degrees of roasting.

MATERIALS AND METHODS

Chemicals and Reagents. Chlorogenic acid hemihydrate (3-caffeoylquinic acid), sodium tetraborate decahydrate, and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Fluka (Gillingham, U.K.). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), catechol, caffeic acid, ferulic acid, vanillic acid, caffeine, and potassium persulfate were from Sigma (Gillingham, U.K.). Polyethylene glycol molecular mass markers (4120, 1470, and 1080 Da) were from Polymer Laboratories (Church Stretton, U.K.). High-purity water was produced in-house using a Purite (High Wycombe, U.K.) Labwater RO50 unit and was used throughout. HPLC glass-distilled dichloromethane was supplied by Rathburn Chemicals Ltd. (Walkerburn, U.K.), and HPLC grade ethanol was from BDH (Lutterworth, U.K.). Sephadex G-25 fine was from Pharmacia (Uppsala, Sweden).

Coffee Samples. Colombian Arabica coffee beans were roasted for 3 min at 225, 233, and 240 °C to give, respectively, light-, medium-, and dark-roasted samples. Green and roasted beans were provided by Kraft Foods (Banbury, U.K.).

Preparation of Coffee Extracts. Coffee beans (10 g) were ground to a powder in a Moulinex (Paris, France) coffee grinder model Super Junior "s" for 1 min. Hot water (75 °C, 300 mL) was added to ground

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Table 1. Effect of the Degree of Roasting on the Absorbance[a] of Coffee Extracts Diluted 1:100 in Water or Ethanol

	absorbance at 280 nm		absorbance at 420 nm	
sample	water[b]	ethanol[b]	water	ethanol[b]
green	4.46 (0.300)[1]	4.14 (0.294)[1]	0.23 (0.102)[1]	0.03 (0.013)[1]
light-roasted[c]	6.24 (0.266)[2]	4.66 (0.002)[2]	0.34 (0.036)[1]	0.13 (0.006)[2]
medium-raosted[d,e]	5.86 (0.033)[2]	4.07 (0.019)[1]	0.36 (0.008)[1]	0.15 (0.018)[2]
dark-raosted[c]	4.68 (0.702)[1]	3.35 (0.103)[3]	0.41(0.056)[1]	0.16 (0.003)[2]

[a] Mean, $n = 2$; relative standard deviation in parentheses. [b] Significant differences ($p < 0.05$) among aqueous and ethanolic dilutions of the different samples. Values followed by a different superscript number are significantly different. [c] Significant differences ($p < 0.05$) between aqueous and ethanolic dilutions of the same sample extract, measured at the same wavelength. [d] Significant differences ($p < 0.001$) between aqueous and ethanolic dilutions of the same extract, measured at 280 nm. [e] Significant differences ($p < 0.01$) between aqueous and ethanolic dilutions of the same extract, measured at 420 nm.

coffee (100 g) and stirred for 5 min. After filtration through Whatman no. 4 filter paper under vacuum, the extraction and filtration procedures were repeated twice using the same portion of ground coffee. The filtrates were combined, defatted with dichloromethane (2 × 200 mL), and concentrated by freeze-drying (*13*). The lyophilized coffees were stored in plastic containers and sealed in plastic bags containing silica gel. They were stored for <1 month at −20 °C, prior to analysis.

Gel Filtration Chromatography. Gel filtration chromatography was based on a previously described procedure (*13*), but on a smaller scale. Freeze-dried coffee (200 mg) was dissolved in water (2.4 mL), and the solution (25 μL) was applied to a column (20 cm × 1 cm i.d.) filled with Sephadex G-25 fine in water. Components were eluted with distilled water at a flow rate of 0.1 mL/min. Molecular mass ranges of fractions were estimated by means of polyethylene glycols, 3-caffeoylquinic acid, and caffeine, as calibration standards. Fractions eluting between 0.58 and 1.25 h (high molecular mass, HMM) and between 1.33 and 2.92 h (low molecular mass, LMM) were combined, freeze-dried, and diluted in water (500 μL) for analysis by capillary zone electrophoresis (CZE). Triplicate extracts of each coffee were fractionated and analyzed separately.

UV—Visible Spectrophotometry. The absorbance at 280 and 420 nm of appropriate dilutions (in water and ethanol) of freeze-dried extracts and the gel chromatography fractions was measured for duplicate samples.

CZE. The method was based on that of Ames et al. (*8*). Separations used a Hewlett-Packard (subsequently Agilent, Bracknell, U.K.) ^{3D}capillary electrophoresis instrument equipped with Chemstation software. The capillary was 48.5 cm long (40 cm to the detector) with an internal diameter of 50 μm and a ×3 bubble cell. Other conditions of analysis were as follows: buffer, 50 mM borate at pH 9.5; voltage, 20 kV; temperature of analysis, 25 °C; injection, 50 mbar for 5 s; electroosmotic flow (EOF) marker, benzyl alcohol. Electropherograms (e-grams) were monitored at 200 and 420 nm, and spectra were collected from 190 to 600 nm. The capillary was conditioned after each sample run by flushing for 3 min with 0.1 M NaOH and for 3 min with buffer. A standard calibration curve for CGA was constructed and used to quantify CGA in samples. Duplicate samples were analyzed.

Antioxidant Activity. The antioxidant activity of the freeze-dried extracts and the gel chromatography fractions was determined by using the $ABTS^{\bullet+}$ decolorization assay (*14*). $ABTS^{\bullet+}$ 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 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. Coffee extracts were diluted 1:100 in water or ethanol prior to analysis, and gel filtration chromatography fractions were analyzed directly. Samples (20 μL) were added to $ABTS^{\bullet+}$ 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. Appropriate solvent blanks were also run. Blanks of the samples were analyzed to evaluate the possible interference due to the natural color of the coffees. They were prepared by mixing the aqueous solution of the freeze-dried extract (8.3 μg/mL, 20 μL) with ethanol (2 mL) in place of $ABTS^{\bullet+}$ solution. All determinations were carried out at least in duplicate. 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.

Standard calibration curves were constructed by plotting percent inhibition values as a function of the concentration of Trolox or CGA. The antioxidant activity of the samples was calculated in terms of Trolox or CGA equivalents (micromolar), using the calibration curves.

Statistical Analysis. Statistical analysis was done using SPSS 10.0 for Windows program (SPSS Inc., 1999). Student's *t* test assuming equal variances was used to test for any significant 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, least significant difference (LSD) was applied to determine which values were different ($p < 0.05$).

RESULTS

UV—Visible Spectrophotometry. The absorbance data for coffee extracts are given in **Table 1**. Significant differences ($p < 0.05$) were found among the values at 280 nm for the aqueous solutions prepared from the different coffee samples. Higher values were detected for light and medium coffees compared to green coffee, suggesting the generation of new compounds. Further processing caused a decrease in absorbance at 280 nm, similar values being obtained for green and dark-roasted coffees. In comparison with the aqueous solutions, the absorbance values were lower for the samples diluted in ethanol, this difference being significant for light and medium coffees ($p < 0.05$ and $p < 0.001$, respectively). Significant differences ($p < 0.05$) were also detected among the 280 nm absorbance data for the ethanolic dilutions of different coffee brews. A precipitate was observed in each ethanol dilution and accounts for the lower absorbance values.

The roasted samples showed higher absorbance values at 420 nm than the extract prepared from green beans for the dilutions in both water and ethanol. Significantly lower absorbance values were obtained for the dilutions in ethanol of light-roasted ($p < 0.05$), medium-roasted ($p < 0.01$), and dark-roasted ($p < 0.05$) samples, compared to the aqueous dilutions. The absorbance of ethanol-diluted components increased with the degree of roasting until the medium-roasted sample, but no further increase occurred for the dark-roasted sample.

Gel Filtration Chromatography. Gel filtration chromatography profiles of the coffee extracts recorded at 280 and 420 nm are shown in **Figure 1**. Compounds absorbing at 280 nm eluted mainly over two time intervals for all samples. The first (0.58−1.25 h) and second (1.33−2.92 h) intervals corresponded to molecular mass ranges of >1470 and 350−1470 Da, respectively. The amount of material eluting at 1.33−2.92 h

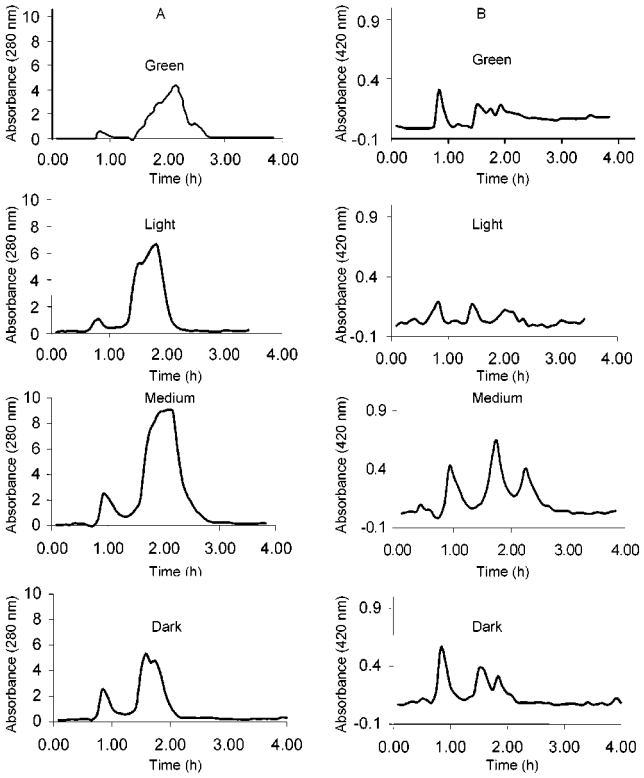

Figure 1. Gel filtration chromatography profiles monitored at (A) 280 nm and (B) 420 nm of the defatted coffee extracts.

increased with degree of roasting up to the medium-roasted coffee, followed by a decrease for the dark-roasted sample.

Three main peaks were observed in the profiles acquired at 420 nm. They corresponded to molecular mass ranges of ≥1470, 350−1470, and <350 Da. The profile changed with degree of roasting, but the overall strength of the chromatograms increased with roasting up to medium roast. The first peak, due to the components of highest molecular weight, was relatively larger in the dark-raosted than in the medium-roasted sample.

CZE. Figure 2 shows the e-grams acquired at 200 nm for the whole coffee extracts. The e-gram of the unroasted sample showed six peaks with a spectrum matching that of standard 3-caffeoylquinic acid. The main CGA peak, migrating at 8.46 min, decreased significantly ($p < 0.05$) with degree of roasting (by 18.9, 45.2, and 66.7%, respectively, for the light-, medium-, and dark-roasted samples). However, other peaks increased in area with heating, and some were identified by migration time and spectral matching. In light-roasted coffee, caffeic acid, ferulic acid, and vanillic acid were detected, the area of the latter compound increasing in the medium- and dark-roasted samples. Additionally, catechol was detected in dark-roasted coffee.

A broad "hump", migrating between 3.5 and 11.5 min, which was also detected on the e-grams with detection at 420 nm, increased in size with extent of roasting.

Fractions eluting within each of the main gel filtration chromatography groups of peaks of the green and medium-roasted coffees (detected at 280 nm) were combined and analyzed by CZE (**Figure 3**). The largest peak detected in the HMM fraction of the green sample could be due to proteins and peptides because they represent ~11% of the average content of unroasted coffee (*2*) and have nominal masses of >4 kDa (*15*, *16*) corresponding to the mass range of this fraction. This was smaller for medium-roasted coffee, but a hump, with spectral characteristics corresponding to melanoidins, migrated at the same time. In the LMM fraction, the

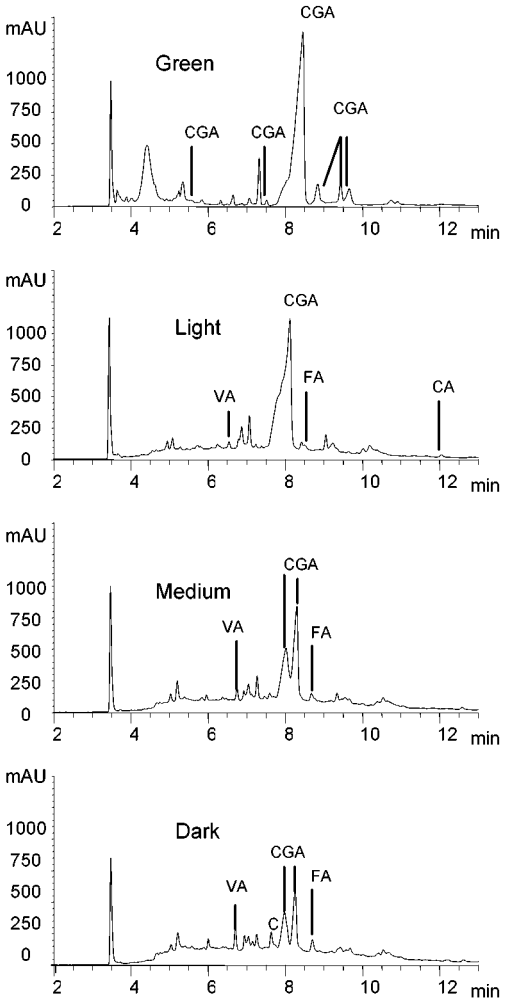

Figure 2. E-grams recorded at 200 nm of aqueous solutions of the defatted coffee extracts: CGA, chlorogenic acid; FA, ferulic acid; VA, vanillic acid; CA, caffeic acid; C, catechol.

largest peaks of the green coffee had spectra corresponding to that of CGA, whereas for medium-roasted coffee, the spectra of the main peaks matched those of CGA or its degradation products.

Antioxidant Activity. A CGA concentration of 1.21 ± 0.01 mM gave the same percent inhibition of absorbance of $ABTS^{\bullet+}$ at 734 nm as 1 mM Trolox (TEAC value). This figure is similar to TEAC values of CGA previously reported, that is, 1.14 ± 0.05 mM when determined by the persulfate ABTS decolorization assay and 1.24 ± 0.02 mM when determined by the myoglobin/ABTS decolorization assay (*17*).

Figure 4 shows that the radical scavenging activity of aqueous dilutions of the brews prepared from light- and medium-roasted coffee was greater than that for green coffee ($p < 0.05$) when either Trolox or CGA was the standard compound. The antioxidant activity of the ethanolic dilutions compared to aqueous dilutions was reduced by 17−33%. It appears that some components, making important contributions to the antioxidant activity of the aqueous dilutions, were not soluble in ethanol. This was particularly the case for dark-roasted coffee; the antioxidant activity of the ethanolic dilutions of dark-roasted coffee fall to below that for the green coffee.

Figure 5 shows the antioxidant activity (scavenging of $ABTS^{\bullet+}$) by the HMM and LMM fractions of the aqueous extracts of green and roasted coffee. The antioxidant activity is expressed as concentration of CGA (micromolar) having the

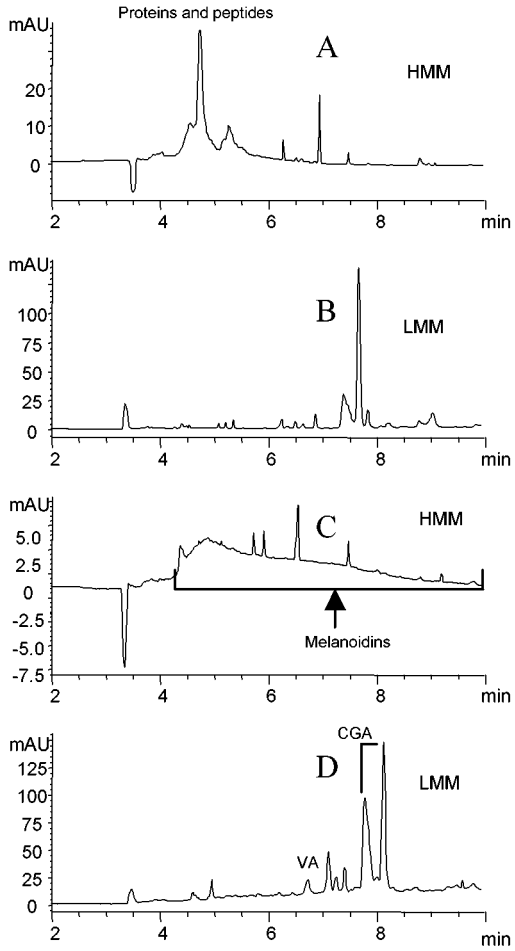

Figure 3. E-grams at 200 nm of gel chromatography peaks detected at 280 nm of coffee extracts (A, green coffee peak 1; B, green coffee peak 2; C, medium-roasted coffee peak 1; D, medium-roasted coffee peak 2) eluting as main groups of peaks by gel filtration chromatography: CGA, chlorogenic acid; VA, vanillic acid.

same antioxidant activity. The area under the curve is a measure of the antioxidant activity of the sample eluted from the column. Values increased with degree of roasting up to the medium-roasted sample but decreased for the dark-roasted sample. The LMM components showed higher antioxidant activity than the HMM fraction for all brews. The HMM material contributed no antioxidant activity to the green sample and only a small amount of activity to the light-roasted coffee.

DISCUSSION

Absorption values of the samples at 280 nm were measured because many components of green coffee, including proteins, peptides, free aromatic amino acids, trigonelline, caffeine, and CGA, absorb at this wavelength. In addition, several compounds absorbing at 280 nm were generated during roasting. The $ABTS^{\bullet+}$ method for assessing the antioxidant activity of food extracts usually employs ethanol as solvent (*14*), but not all coffee components are soluble in this solvent; therefore, absorption values of the aqueous coffee extracts diluted in ethanol were also measured. Absorption at 420 nm was used to monitor the development of brown color. The formation of water-soluble brown pigments increased with the degree of roasting, in line with previous results (*10*). Absorbance values obtained for aqueous dilutions of coffee extracts (**Table 1**) and the gel filtration chromatograms (**Figure 1**) confirm that, during roasting, simultaneously some compounds absorbing at 280 and

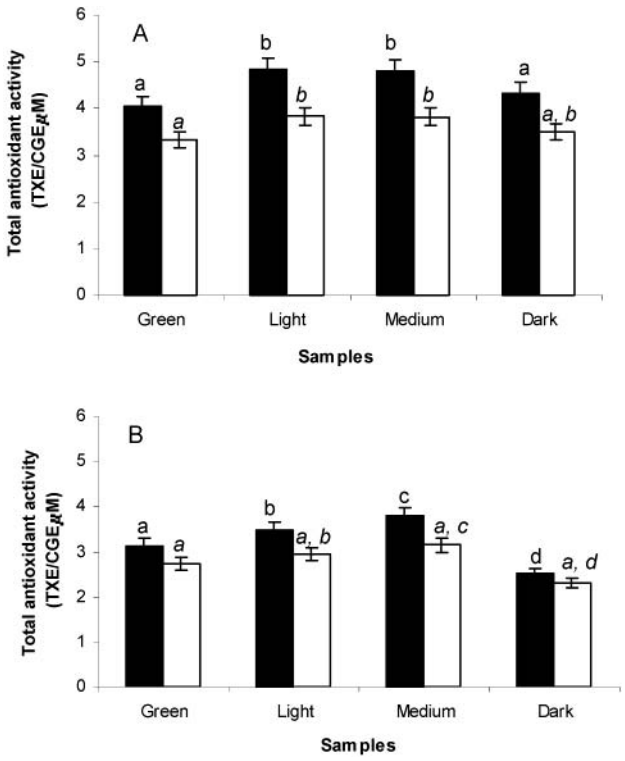

Figure 4. Scavenging of $ABTS^{\bullet+}$ at a fixed time point (5 min) for the different defatted extracts diluted in (A) water and (B) ethanol. Data are expressed in micromolar equivalents of Trolox (TXE, black bar) or CGA (CGE, white bar). Values are the means of triplicate analyses; error bars denote the relative standard deviation. Bars with different letters in normal style (Trolox) or italics (CGA) are significantly different ($p < 0.05$).

420 nm were degraded and others were formed. The chromatograms demonstrate further that both HMM and LMM compounds were modified during roasting.

CZE of the unfractionated coffee brews (**Figure 2**) resulted in distinctive profiles for each sample. In addition, a broad hump developed with progressive roasting and possessed spectra that were in agreement with those of melanoidins (*17*). The e-grams agreed with those previously described by us (*8*). CZE of the HMM and LMM fractions separated by gel chromatography resulted in better resolution of components, particularly those of LMM (**Figure 3**). CZE of the HMM fraction of green and medium-roasted coffees (**Figure 3A**,**C**) showed that material from both samples migrated over a similar time interval, indicating similar charge/mass ratios. It is suggested that proteins and peptides, responsible for this peak in green coffee, undergo the Maillard reaction during roasting and are thus transformed into melanoidins that contribute to the increased amount of HMM material in the medium-roasted sample. Although several peaks in the LMM fractions of both coffees are attributed to CGA-related compounds and these degradation products, several of the peaks remain unidentified (**Figures 2** and **3**).

Light and medium roasting increased total antioxidant activity (**Figure 4**), although the CGA content of these samples decreased by 18.9 and 45.2%, respectively, compared to the green extract (**Figure 2**). The observation that antioxidant activity does not increase linearly with degree of roasting agrees with previous findings (*9*, *10*). Relationships between degree of roasting and antioxidant activity vary according to the type of coffee (Arabica or Robusta), roasting conditions, extraction procedure, and antioxidant assay (*19*). Nicoli et al. (*10*) showed that the ability to scavenge either oxygen or peroxy radicals or to suppress lipid oxidation in the Rancimat test reached a

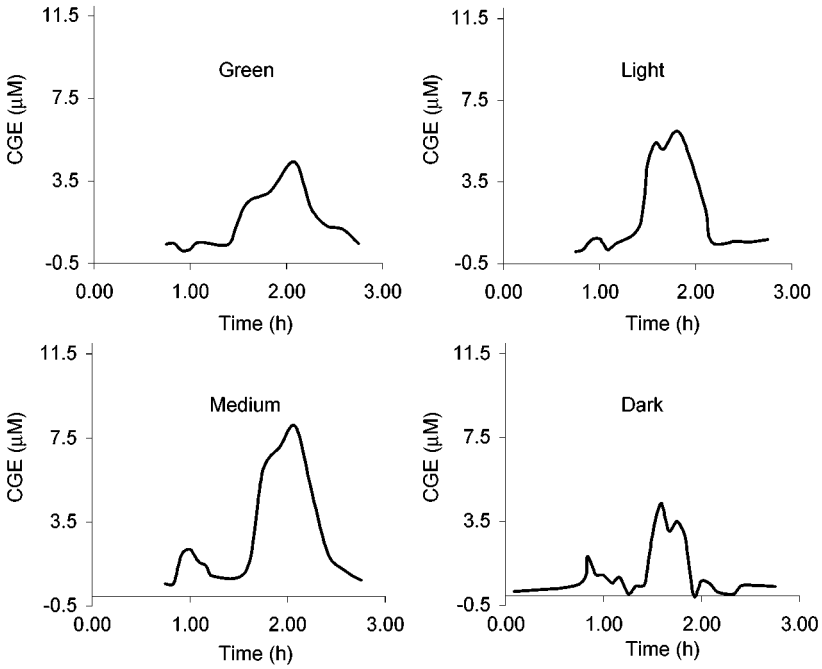

Figure 5. Scavenging of $ABTS^{\bullet+}$ by the HMM and LMM fractions of aqueous extracts of green and roasted coffees. Data are expressed in micromolar equivalents of CGA (CGE).

maximum for brews prepared from beans roasted at intermediate conditions, in line with the current study. In contrast, the ability of coffee brews to reduce ferric ions and to suppress oxidation in a β-carotene−linoleic acid model system generally increased with degree of roasting (*9*). Using an ex vivo assay, all of the roasted samples gave complete protection against rat liver cell microsome lipid peroxidation, compared to 24−62% protection for green bean extracts (*9*). In contrast, another ex vivo assay (*20*), involving measuring low-density lipoprotein (LDL) oxidation, has demonstrated a decrease in lag time (decrease in antioxidant capacity) with increase in extent of roasting. When the aim of a food or beverage manufacturer is to establish processing conditions giving maximum antioxidant activity in a product, it is essential that the type of antioxidant activity of interest be specified and an appropriate assay selected.

HMM and LMM compounds contributed to the antioxidant activity of the samples. Dark-roasting conditions caused partial degradation of some compounds contributing to antioxidant activity that were prominent in the medium-roasted sample (**Figures 1** and **5**), including CGA (**Figure 2**), and increased the concentration of the brown HMM compounds (melanoidins) (**Figure 1B**). The proportion of $ABTS^{\bullet+}$ scavenging activity due to HMM material in each extract increased with the degree of roasting, for light- and medium-roasted coffees, but no further increase was observed for the dark-roasted sample (**Figure 6**). In contrast with these results, absorbance at 420 nm increased with degree of roasting (**Table 1**). Thus, the development of color cannot be considered to be an index of overall antioxidant activity of coffee brews, as measured by the $ABTS^{\bullet+}$ assay. It has been suggested (*21*) that the formation of melanoidins with antioxidant capacity, expressed in terms of chain-breaking (peroxy radical scavenging) activity, occurs only at a particular phase of coffee roasting, and our evidence supports this claim. Nicoli et al. (*10*) also reported a loss of antioxidant capacity during the advanced phases of coffee roasting, in agreement with our results, and this was attributed to the transformation of phenolic compounds, as well as the formation of Maillard reaction products during roasting.

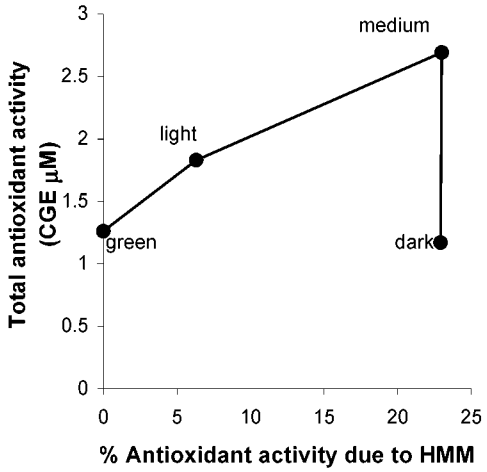

Figure 6. Influence of the degree of roasting on the relationship between total antioxidant activity and the proportion of antioxidant activity due to the HMM fraction.

In conclusion, this study has demonstrated that the LMM fraction of coffee brews possesses higher antioxidant activity than the HMM fraction over the range of roasting conditions examined. The concentration of colored, HMM compounds in the brews increased with degree of roasting, and the proportion of total antioxidant activity due to the HMM fraction increased with roasting for the light- and medium-roasted coffees, but not for the dark-roasted sample. Modification of the antioxidant activity of the LMM fraction also took place as a consequence of roasting. Future work should be directed toward understanding the contribution of individual components to the antioxidant activity of coffee brews and their mechanism(s) of action.

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