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Antiradical Activity, Phenolics Profile, and Hydroxymethylfurfural in Espresso Coffee: Influence of Technological Factors

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The influence of technological factors (decaffeination, brew volume, coffee species, and roast degree) on antiradical activity and phenolics content of espresso coffee is described. The screenings of phenolics profile and other compounds (caffeine and trigonelline), as well as the quantification of hydroxymethylfurfural, were performed by LC-DAD-ESI-MS. Significantly lower ($p < 0.05$) scavenging activities and phenolics contents were found in decaffeinated espressos when compared with regular ones (32 vs 38% and 324 vs 410 mg/30 mL cup, respectively). A long espresso (70 mL) offers more than twice the phenolics amount of a short one (20 mL). Robusta brews showed higher ($p < 0.05$) antiradical activity and phenolic contents than arabica ones, for all roast degrees (light, medium, and dark). No significant differences ($p > 0.05$) were observed for scavenging activities of differently roasted robusta brews, whereas an increase in medium-dark brews was observed for arabica samples. Total phenolics in robusta espressos decreased ($p < 0.05$) with the increase of roast degree, but no significant differences ($p > 0.05$) were found between arabica espressos from different roasts. By LC-DAD-ESI-MS, 23 hydroxycinnamic derivatives were found, including chlorogenic acids, lactones, and cinnamoyl−amino acid conjugates. The amount of each compound was differently affected by species and roast. Robusta brews presented superior levels of caffeine and chlorogenic acids, whereas arabica ones contained more trigonelline. Hydroxymethylfurfural contents in the brew (30 mL) varied from 2.60 to 0.84 mg for light- and dark-roasted arabicas and from 1.29 to 0.68 mg for light- and dark-roasted robustas, respectively.

KEYWORDS: Coffee species; roast; antioxidant; polyphenols; chlorogenic acids; hydroxymethylfurfural; LC-DAD-ESI-MS

INTRODUCTION

Under normal metabolism, the levels of oxidants and antioxidants in humans are balanced, an important factor for sustaining optimal physiology. Reactive oxygen species are usually well controlled by antioxidant enzymes (e.g., superoxide dismutase and catalase), thiol-containing peptides (e.g., glutathione), metabolites (e.g., uric acid), and dietary antioxidants (e.g., vitamin C, vitamin E, and polyphenols). Overproduction of oxidants and/or reduction of antioxidant defenses might cause an imbalance in redox state, leading to oxidative damage on critical sites of tissues and cells (*1*).

The expectation of supplementing the body's defenses against various oxidant challenges increased the attention on natural products. The complex composition of plants, which includes a mixture of different antioxidants with complementary mechanisms of action, seems to provide a greater protection against free radical injury than any single compound, which highlights the importance of the synergic action of the redox network (*2*).

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The antioxidant properties of some beverages drew a lot of attention, especially due to the convenience of their consumption and the excellence in efficacy reported by some authors (*1*). From this perspective, coffee has been a focus of researchers' attention, especially because several studies compared its antioxidant activity with that of other beverages (wine, tea, beer, juices, etc.) and revealed that coffee has an important contribution to the dietary antioxidants intake of some populations (*3*). Indeed, moderate coffee consumption has been recently associated with a decrease in the development risk of several pathologies, namely, degenerative diseases (Parkinson's and Alzheimer's), cirrhosis, asthma, and type II diabetes (*4*).

Current literature suggests that the main compounds responsible for the antioxidant activity in roasted coffee are several phenolic compounds naturally present in green coffee beans, such as chlorogenic acids (CGA) (*5*), and Maillard reaction products formed in the roasting process, namely, melanoidins (*6*).

CGA are the main components of the phenolic fraction of green coffee beans, reaching levels of up to 14% (dry matter basis). The main groups that constitute CGA include caffeoylquinic acids,

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dicaffeoylquinic acids, feruloylquinic acids, diferuloylquinic acids, *p*-coumaroylquinic acids, and diesters of caffeic and ferulic acids with quinic acid (*5*, *7*). During roasting, CGA and their thermal degradation products seem to be also involved in the formation of coffee brew melanoidins, along with other compounds, such as polysaccharides (galactomannans and arabinogalactans) and proteins (*8*, *9*).

The antioxidant activity of coffee has been assessed using different detection systems, which makes it difficult to compare and interpret results, especially because activity obtained with one test might not be correlated with the results of another (*10*−*15*).

From this perspective, this study aimed to ascertain the influence of several technological factors (decaffeination, brew volume, coffee species, and roast degree) on the antioxidant activity of espresso coffee (EC) using always the same assay (the DPPH$^{\bullet}$ test). Total phenolic contents of the beverages were also evaluated by spectrophotometry. In addition, the screening of the antioxidant profile (chlorogenic acids and derivatives, trigonelline, and caffeine) and the quantification of hydroxymethylfurfural in EC, as influenced by roast degree and coffee species, were carried out by LC-DAD-ESI-MS. We also present a database with MS data and UV spectra of the analyzed compounds and, as far as we know, the presence of *p*-coumaroyltryptophan in arabica coffees is here reported for the first time.

MATERIALS AND METHODS

Chemical and Reagents. 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH$^{\bullet}$) (95%), the standard chlorogenic acid (composed by 5-caffeoylquinic acid), and hydroxymethylfurfural were obtained from Aldrich (Madrid, Spain). Folin−Ciocalteu's reagent and sodium carbonate decahydrate were purchased from Panreac (Barcelona, Spain). Methanol of reagent grade was from Scharlau Chemie S.A. (Sentmenat, Spain). HPLC-UV-IR grade methanol and acetic acid were obtained from Panreac.

Coffee Samples. Different commercial blends of caffeinated (n = 5) and decaffeinated (n = 5) (roasted beans) coffees as well as coffee pods (n = 5) were obtained in local supermarkets. Green coffee beans of *Coffea arabica* (n = 5) and *Coffea canephora* var. *robusta* (n = 5), from different geographical origins, were kindly supplied by an industrial importer and roaster of coffee (BICAFÉ). The arabica samples were from Honduras (n = 2, A and B), Brazil (n = 2, A and B), and Kenya (n = 1). The robusta ones were from Ivory Coast (n = 3, A, B, and C) and Vietnam (n = 2, A and B). All of the green samples were individually roasted at 210 °C in a Probat Pré 1Z 2000 (Probat-Werke), being subjected to three different lengths of exposure (8−11 min) to achieve three final roasting degrees (light, medium, and dark), not exceeding the range of commercial roasts usually practiced in Portugal. The roasting degree was determined by photometric analysis with infrared radiation using a Colorimeter Colorette 3, from Probat-Werke, and also by the organic roast loss (ORL) evaluation in dry weight. Sample moisture to calculate ORL was determined by drying at 103 ± 2 °C until constant weight.

Sample Preparation. Coffee beans were ground to pass through a 0.75 mm sieve. ECs (6.5 g/30 mL) were percolated by deionized water in an HL3854 Espresso Professional (Philips, The Netherlands). ECs (30 mL) from coffee pods (commercial prepacked doses of coffee powder covered with a paper layer) were prepared in the same espresso machine by changing the filter chamber to one adapted to pods.

To evaluate the influence of percolation volume on free radical scavenging activity and total polyphenols content, a commercial coffee sample was used to prepare ECs with different volumes (from 20 to 70 mL) using always 6.5 g of coffee powder.

Dilutions of ECs, independent of their final volume, were performed for the different analyses, namely, 1:100 in deionized water for DPPH$^{\bullet}$ scavenging assays, 1:400 for total phenolics determination, and 1:10 to LC-DAD-ESI-MS analyses.

All brews were prepared in triplicate, and analyses were performed in duplicate.

Analytical Methodologies. *DPPH$^{\bullet}$ Scavenging Assay*. The hydrogen-donating ability of coffee extracts was evaluated using the DPPH$^{\bullet}$ scavenging assay of Brand-Williams et al. (*16*), with minor modifications. A 6.1×10^{-5} M DPPH$^{\bullet}$ methanolic solution was prepared immediately before use. The solution was adjusted with methanol to an absorbance of 0.63 at 515 nm in a 1 cm cuvette at 25 °C (Jasco V-530 UV−/vis spectrophotometer, Tokyo, Japan). The reaction was started by adding 20 μL of a diluted EC (1:100) to 980 μL of DPPH$^{\bullet}$ solution. The bleaching of DPPH$^{\bullet}$ was followed at 515 nm every 5 min for 40 min. The DPPH$^{\bullet}$ scavenging effect of the sample extracts was calculated according to the following formula:

$$\text{scavenging effect (\%)} = [(\text{absorbance}_{t=0\text{min}} - \text{absorbance}_{t=40\text{min}})/\text{absorbance}_{t=0\text{min}}] \times 100$$

Determination of Total Phenolics (TP). The TP content was spectrophotometrically determined according to the Folin−Ciocalteu procedure (*17*) with minor modifications. Briefly, 500 μL of a diluted EC (1:400) was mixed with 2.5 mL of the Folin−Ciocalteu reagent (1:10) and 2 mL of a $Na_2CO_3 \cdot 10H_2O$ solution (7.5% m/v). The mixture was incubated at 45 °C, during 15 min, and after 30 min at room temperature, absorbance readings at 765 nm were performed, against a reagent blank, on a Jasco V-530 UV−vis spectrophotometer. A calibration curve for the standard chlorogenic acid was used to obtain a correlation between sample absorbance and standard concentration (range = 0−100 μg/mL, $r > 0.9997$). The TP concentration was expressed as milligrams of chlorogenic acid equivalents (CGAE) per cup of EC.

HPLC-DAD-ESI-MS Analysis. The chromatographic analysis was carried out in a Jasco HPLC integrated system, equipped with an LG-1580-04 quaternary gradient unit, a PU-980 pump, a UV-1575 UV−vis detector, and a Rheodyne model 7725 loading sample injector with a 20 μL loop (Rheodyne, Berkeley, CA).

The compounds were analyzed as described by Rubilar et al. (*18*) with some modifications. Chromatographic separation was achieved on a Kromasil 100 C18 (25 × 0.4 cm i.d., 5 μm) column from Teknokroma (Barcelona, Spain), operating at 30 °C. The solvent system used was a gradient of HPLC grade methanol (eluent A) and Milli-Q water acidulated with acetic acid (0.5%) (eluent B): 0 min, 5% A; 5 min, 50% A; 45 min, 60% A; 55 min, 5% A, performed at a constant flow rate of 0.7 mL/min. Chromatograms were registered at 280 and 320 nm.

The HPLC system was interfaced to an HP series 1100 mass selective detector equipped with an atmospheric pressure ionization electrospray (API-ES) chamber, with a single quadrupole as a mass analyzer. The conditions were as follows: nitrogen as the drying gas at 13 L/min and 350 °C, nebulizer pressure at 2.76 bar, and fragmentor voltage at 60 × 10^3 V. The chromatograms were recorded under full-scan conditions in the positive ionization mode of operation (m/z range 100−2800).

Data were analyzed using HP ChemStation software (Hewlett-Packard Co., Amsterdam, The Netherlands).

Statistical Analysis. Data are reported as mean ± standard deviation. Statistical analyses were performed using the statistical package SPSS v 15.0 (SPSS for Windows; SPSS Inc., Chicago, IL). Student's t tests were used to discriminate between any two groups under consideration, one-way ANOVA was used to compare two or more groups, and post hoc Dunnett's test was performed for simultaneous paired comparisons. Simple linear regression analysis was used to evaluate the relationship between compound amount and brew volume.

RESULTS AND DISCUSSION

Commercial Samples: Regular, Pods, and Decaffeinated. Scavenging activities (%) against the stable free radical DPPH$^{\bullet}$ and TP contents (expressed in mg of CGAE per cup of brew) are presented in **Table 1** for regular ECs, decaffeinated ECs, and ECs prepared from pods. The sample composition was practically unknown because all brews were prepared from commercial mixtures of arabica and robusta, and the exact proportion of both species was rarely labeled.

According to Student's t tests, results for regular ECs and ECs prepared from pods (both caffeinated) (**Table 1**) were not statistically different ($p > 0.05$). However, significantly lower ($p < 0.05$) values for both parameters were observed in decaffeinated ECs,

Table 1. DPPH• Scavenging Activities and Total Phenolics of ECs from Commercial Samples[a]

sample	scavenging activity (%)	total phenolics[b]
regular[c]		
1	38 ± 2	453 ± 18
2	40 ± 4	339 ± 35
3	38 ± 4	391 ± 40
4	39 ± 4	426 ± 18
5	33 ± 3	438 ± 20
total mean	38 ± 4	410 ± 48
pods[c]		
1	34 ± 4	441 ± 51
2	44 ± 3	451 ± 47
3	35 ± 2	387 ± 45
4	34 ± 1	352 ± 22
5	31 ± 2	374 ± 40
total mean	36 ± 5	400 ± 55
decaffeinated		
1	26 ± 1	266 ± 19
2	30 ± 3	268 ± 31
3	35 ± 3	319 ± 38
4	31 ± 2	379 ± 27
5	36 ± 3	387 ± 43
total mean	32 ± 3	324 ± 60

[a] Data are reported as mean ± standard deviation. [b] Total phenolics expressed in mg of chlorogenic acid equivalents (mg CGAE) per cup of EC (30 mL). [c] Caffeinated samples.

showing that the decaffeination procedure might be responsible for a slight decrease in scavenging properties of coffee brew, as well as some loss of phenolics compounds.

Although antioxidant properties have been described by other authors (*19*) for caffeine, namely, by inhibition of lipid peroxidation induced by reactive oxygen species (hydroxyl radical (•OH), peroxyl radical (ROO•), and singlet oxygen ($^1O_2$)), caffeine contents cannot be responsible for the differences reported for DPPH• scavenging results in **Table 1**. Indeed, according to other researchers (*20*), when caffeine was tested alone against DPPH•, no scavenging activity was observed, due to the absence of hydrogen-donating groups in its molecular structure. On the other hand, Nebesny et al. (*21*) reported that the antioxidant efficiency and activity in DPPH• scavenging of coffee extracts are predominantly dependent on the polyphenols concentration, and, according to this, if decaffeinated ECs contained mean lower amounts of total phenolics, lower DPPH• scavenging activities were expected.

Results published in the literature concerning caffeinated and decaffeinated coffees are variable according to the methodology used and should be considered as complementary information. Pellegrini et al. (*3*), using three different assays, namely, Trolox equivalent antioxidant capacity (TEAC), total radical trapping antioxidant parameter (TRAP), and ferric reducing-antioxidant power (FRAP), found 25−30% lower total antioxidant capacity in decaffeinated coffee beverages when compared to regular ones, considering the probable involvement of caffeine absence in this difference. Parras et al. (*22*) found lower activities in the hydroxyl radical scavenging and TEAC assays for decaffeinated coffees from Colombia and Brazil when compared with caffeinated coffees from the same origins. On the other hand, decaffeinated coffees were more effective as scavengers of lipoperoxyl radical and hydrogen peroxide than their equivalents with caffeine (*22*).

Influence of Coffee Brew Volume. Each consumer has his own preferences concerning EC volume. In Portugal, the brew can vary from 20 mL ("short" EC) to 70 mL ("long" EC), ranging from a more concentrated brew (20−30 mL) to a less concentrated one (70 mL), due to a consequent increase in the water/coffee ratio.

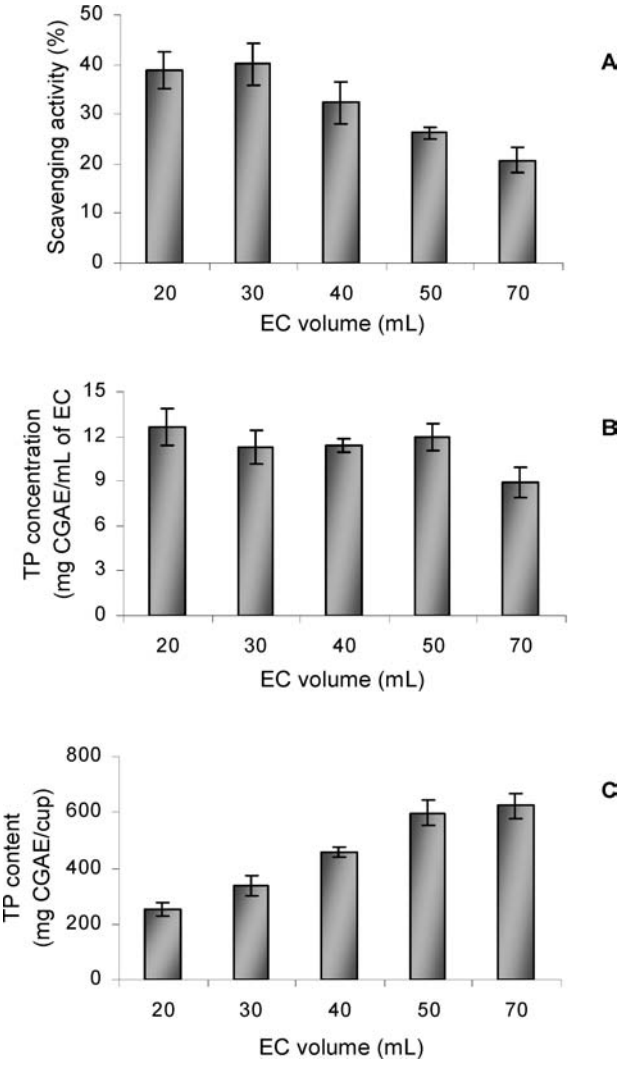

Figure 1. DPPH• scavenging activity (%) of a diluted (1:100) EC aliquot (20 μL) (**A**), TP concentration in mg CGAE/mL of EC (**B**), and TP content per total volume of EC (mg CGAE/cup) (**C**).

To study the influence of brew length in the DPPH• scavenging activity, total phenolics concentration (mg CGAE/mL of EC), and total phenolics content per cup of EC (mg CGAE/cup), ECs of different volumes were prepared using the same coffee blend amount. Results are described in **Figure 1**.

The DPPH• scavenging activity (**Figure 1A**) was higher for aliquots of shorter ECs (20−30 mL, ∼40%). When the brew volume was increased, the antiradical activity decreased, with aliquots of the longest ECs presenting approximately half of the value (∼20%). On this basis, it seems that the compounds responsible for DPPH• scavenging activity are mainly extracted in the beginning of the percolation process (until 20−30 mL) and, afterward, are diluted.

With regard to TP concentration (mg CGAE/mL) (**Figure 1B**), it was possible to observe that although it was significantly lower ($p < 0.05$) in the longest ECs when compared with short ones, the behavior profile of extraction was not similar to that observed for DPPH• assay (**Figure 1A**). Indeed, no significant differences ($p > 0.05$) in the TP concentration were found when the EC volume was varied from 20 to 50 mL, the TP extraction rate being constant until 50 mL and decreasing afterward. The differences observed between panels **A** and **B** of **Figure 1** show that the brews' antiradical activity is not dependent only on TP content. Otherwise, scavenging activities would follow the TP concentration in a proportional way and graphic profiles would be similar.

Table 2. DPPH• Scavenging Activities and Total Phenolics of ECs Prepared from Arabica and Robusta Coffees Subjected to Different Roast Degrees (Light, Medium, and Dark)[a]

sample	roast degree	color	ORL[b] (%)	scavenging activity (%)	total phenolics[c]
arabica					
Honduras (A)	light	175	8	33 ± 5	388 ± 33
	medium	117	9	35 ± 1	371 ± 35
	dark	94	14	34 ± 5	331 ± 16
Honduras (B)	light	161	6	37 ± 2	391 ± 30
	medium	122	7	39 ± 3	423 ± 45
	dark	92	12	39 ± 3	393 ± 39
Brazil (A)	light	172	8	27 ± 2	378 ± 36
	medium	126	9	30 ± 4	380 ± 35
	dark	97	11	35 ± 5	376 ± 48
Brazil (B)	light	181	6	30 ± 2	228 ± 12
	medium	117	10	35 ± 3	208 ± 11
	dark	91	12	34 ± 1	189 ± 11
Kenya	light	180	9	32 ± 3	420 ± 38
	medium	144	11	34 ± 3	435 ± 33
	dark	96	12	36 ± 4	386 ± 38
robusta					
Ivory Coast (A)	light	172	5	52 ± 5	541 ± 30
	medium	139	8	49 ± 3	447 ± 41
	dark	93	12	52 ± 2	402 ± 20
Ivory Coast (B)	light	154	7	40 ± 4	490 ± 34
	medium	112	11	44 ± 6	409 ± 21
	dark	89	13	42 ± 3	424 ± 26
Ivory Coast (C)	light	162	7	38 ± 4	468 ± 17
	medium	129	9	41 ± 4	429 ± 40
	dark	94	11	42 ± 5	449 ± 52
Vietnam (A)	light	170	5	36 ± 3	475 ± 32
	medium	135	8	41 ± 1	485 ± 32
	dark	98	11	40 ± 4	382 ± 45
Vietnam (B)	light	181	6	35 ± 4	439 ± 53
	medium	127	8	36 ± 5	417 ± 48
	dark	92	12	41 ± 3	356 ± 46

[a] Data are reported as mean ± standard deviation. [b] ORL, organic roast loss. [c] Total phenolics are expressed in mg of chlorogenic acid equivalents (CGAE) per cup of EC (30 mL).

In relation to the TP uptake through the brew, a long EC will contribute a higher content (~620 mg CGAE/70 mL) than a short one (~250 mg CGAE/20 mL). During the percolation, the TP amount increased with volume, following a high correlation ($r > 0.994$) until 50 mL, and stabilized after that (**Figure 1C**).

Influence of Coffee Species and Roast Degree. To ascertain the influence of coffee species and roast degree in the antiradical activity and TP contents of EC, several samples of arabica and robusta coffees were individually roasted at three different degrees. Results are reported in **Table 2**.

Beans' organic roast losses (ORL) increased during the roast (mean values of 7, 9, and 12%, for light, medium, and dark degrees, respectively), whereas a decrease in the color values of coffee powder (obtained from photometric analysis with infrared radiation) was observed: 171 for light roast, 127 for medium roast, and 95 for dark roast. When both coffee species were compared, statistical differences were not found ($p > 0.05$), except for the fact that light-roasted robustas showed a significantly darker color ($p < 0.05$) than light-roasted arabicas.

Robusta coffees showed higher ($p < 0.05$) DPPH• scavenging activities than arabicas, for all roast degrees (**Table 2**). Indeed, for light, medium, and dark roasts of robusta coffees mean values were 40, 42, and 43%, respectively, whereas for arabicas, correspondingly average activities of 32, 35, and 36% were found. Although a slight increase of scavenging activity with roast was observed for robusta samples, Dunnett's test showed that it was not significant ($p > 0.05$). For arabica samples, the scavenging activity of light-roasted brews was significantly lower ($p < 0.05$) than those of medium and dark roasts, whereas no differences ($p > 0.05$) were observed among these two last groups.

In relation to TP contents (**Table 2**), ECs prepared from robusta coffees contained higher levels: means of 482, 437, and 402 mg CGAE/cup, for light, medium, and dark roasts, in contrast with 361, 363, and 334 mg CGAE/cup for arabica ECs. These results were expected because robusta beans might contain almost double the amount of phenolic compounds compared with arabica beans (*5*). According to Dunnett's test, the TP content of robusta ECs significantly decreased ($p < 0.05$) along the three roast degrees, with dark-roasted brews containing approximately 16% lower levels than light ones. For arabica samples, lower average values were found for dark ECs, when compared with other roast degrees (~7% less). However, this decrease was not statistically significant ($p > 0.05$). This might be due to a higher resistance of arabica phenolics to roast, compared with robusta samples, probably related to intrinsic characteristics of the beans, namely, size (higher in arabicas).

Results in the literature concerning the antioxidant activity of coffee species are variable according to the methodology used, but higher activities for robusta coffees were also reported by other authors. Moreira et al. (*23*) found a higher iron-reducing activity (FRAP assay) in robusta coffee beverages (~50%). Daglia et al. (*24*) reported higher ex vivo antihydroxyl radical activity (assessment of cell viability by MTT colorimetric assay) for roasted robusta coffees, whereas no significant differences between in vitro antihydroxyl radical activities (by deoxyribose assay) of both species were detected. The same group also found more reducing substances in robusta coffees than in arabicas (*25*). Richelle et al. (*12*), using an in vitro low-density lipoprotein (LDL) oxidation assay, reported 2-fold higher antioxidant activity for green robusta beans compared to arabica ones, but after roasting that difference was no longer significant. A high variability in the evolution of antioxidant capacity during roasting was also observed according to the methodologies used. According to Cämmerer et al. (*10*) a significant loss of antioxidant activity (~40%) with roast was observed using the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, although the activity for roasted coffee was higher than that of green coffee, probably due to a release of highly active low molecular weight phenols from the green coffee constituents with moderate heating or melanoidins formation. These authors also tested solid phase electron paramagnetic ressonance (EPR) spectroscopy with different stabilized radicals, namely, Fremy's salt (potassium nitrosodisulfonate) and 2,2,6,6-tetramethyl-1-piperidin-1-oxyl (TEMPO), and results differed depending on which radical was used: in contrast to the results obtained with the stabilized radical Fremy's salt, the antioxidant activity using TEMPO increased with higher roasting degree. Summa et al. (*11*), also by means of EPR spectroscopy with Fremy's salt, reported a decrease in antioxidant activity with roast as well. According to Richelle et al. (*12*) the increase of roast degree slightly reduced the antioxidant activity of coffee using the in vitro LDL oxidation assay. Del Castillo et al. (*15*) observed a higher activity in ABTS assay for medium-roasted coffees. The same results were obtained

by Nicoli et al. (*14*) by testing oxygen-scavenging and chain-breaking capacities of coffee beverages.

Anese et al. (*13*) analyzed DPPH• scavenging activities of brews prepared from the same coffee blend at different roast degrees. No statistical differences were observed between green coffee and light/medium roasts, but an increase was detected for dark-roasted brews. In a general way, these observations are in accordance with ours (**Table 2**), which show that dark-roasted beans, although having a lower TP content, might originate brews with a higher antiradical potential than light-roasted ones. Nevertheless, ECs prepared from dark-roasted robusta beans still have higher TP levels than a light-roasted arabica brew, probably due to the higher content of phenolics in the former. Therefore, it can be assumed that Maillard products (generated during the roast) also contribute, to a certain extent, to the values measured in DPPH• assay (because the decrease in phenolic contents of the brews was not accompanied by a decrease in the DPPH• assay values), compensating and even increasing the antiradical activity. Indeed, the antioxidant properties of melanoidins were already reported by other authors (*6*, *8*).

The differences observed between results from several authors are obviously due to the methodologies assayed, the distinct phenolics content of samples, and the different rates at which these are lost and other antioxidants are formed (these last two, according to the roasting conditions). This shows the difficulty in evaluating the antioxidant activity of a sample, especially when a mixture of different antioxidant compounds is present, as in the case of coffee.

LC-DAD-ESI-MS Analysis of Espresso Coffee. To ascertain the influence of both coffee species and roasting in the phenolic profile of the brew, 30 mL ECs from two arabica (Honduras A and Honduras B) and two robusta samples (Ivory Coast A and Vietnam A), each one light and dark roasted, were chosen to be analyzed by LC-DAD-ESI-MS.

Peak assignments were performed by comparison of UV absorption spectra, MS data, and retention times with standards or bibliographic sources (*7*, *26*−*28*).

Figure 2 shows the chromatograms, recorded at 320 and 280 nm, of EC extracts prepared with light-roasted (**A**) and dark-roasted (**B**) robusta beans (compound identification is detailed in **Table 3**). It was possible to identify not only hydroxycinnamic derivatives (peaks 3−8 and 10−26) but also nonphenolic compounds such as caffeine (CAF; peak 9), trigonelline (TRG; peak 1), and hydroxymethylfurfural (HMF; peak 2). CAF and TRG (both present in green beans) are of interest due to their antioxidant properties (*29*). HMF is formed during roasting, being known as a potentially harmful compound (*30*). Therefore, the evolution with roast of these three additional compounds was also followed.

Within the 23 hydroxycinnamic derivatives identified in ECs (**Figure 2**; **Table 3**), three main groups were observed, as expected: CGA (peaks 3−8, 10, 12−14, 18, 19, 21−23, and 25), chlorogenic acid lactones, also known as 1,5-y-quinolactones, (CGL; peaks 11, 15−17, and 20), and cinnamoyl−amino acid conjugates (peaks 24 and 26).

With regard to CGA, the following subgroups were detected in brews prepared from either arabica or robusta species, both light and dark roasted: caffeoylquinic acids (CQA; peaks 3, 6, and 8), feruloylquinic acids (FQA; peaks 4, 7, 12, and 13), *p*-coumaroylquinic acids (CoQA, peaks 5, 10, and 14), dicaffeoylquinic acids (diCQA, peaks 18, 19, and 21), diferuloylquinic acids (diFQA, peak 25), and caffeoylferuloylquinic acids (CFQA, peaks 22 and 23). Two subgroups of CGL were also identified, namely, caffeoylquinides (CQL; peaks 11 and 15) and feruloylquinides (FQL; 16, 17, and 20). Perrone et al. (*27*) reported 3,4-di-*p*-coumaroylquinic acid, *p*-coumaroylquinides, and 3,4-dicaffeoylquinide in roasted samples of *C. arabica* and *C. canephora*, but those compounds were not detected in our espressos. Clifford et al. (*26*) found tryptophan, tyrosine, and phenylalanine conjugates with hydroxycinnamic acids in green robusta beans. In our brews, we found only caffeoyltryptophan (CTrp) and *p*-coumaroyltryptophan (CoTrp).

In the MS spectra of phenolic compounds (**Table 3**), protonated molecular ions $[M + H]^+$ were observed, as well as adducts with sodium $[M + Na]^+$ and ion fragments such as $[M - \text{quinic acid}]^+$, $[M - H_2O]^+$, or $[M - \text{amino acid}]^+$. Ion fragments at m/z 163, 177, and 147 were representative of caffeoyl, feruloyl, and *p*-coumaroyl moieties, respectively. In the MS spectra of nonphenolic compounds (TRG, CAF, and HMF), dominant protonated molecular ions $[M + H]^+$ were observed. In **Figure 3**, typical UV absorption spectra of the analyzed compounds can be observed.

Caffeic, ferulic, and *p*-coumaric acids can be esterified by their carboxyl group to one or more hydroxyls (at positions 1, 3, 4, and 5) of quinic acid, originating a series of positional isomers (*5*). As described in **Table 3**, mass spectra obtained in positive mode were identical for positional isomers (e.g., peaks 3, 6, and 8), hindering their identification. For that reason, isomers in **Table 3** were classified on the basis of their elution order in similar reversed phase HPLC systems described in the Literature Cited (*7*, *27*). Moreover, CGA and CGL nomenclature was the same as used by Farah et al. (*31*), in which the IUPAC numbering system was adopted for CGA and the same numbering of the carbon atoms as for the acid precursors was used for lactones.

Although six CFQA isomers have been found in green coffee (*32*), only two peaks corresponding to CFQA were present in our extracts (peaks 22 and 23, **Figure 2**). In this case, we were not able to identify to which isomer each peak corresponds.

The aim of this study was not to quantify the different phenolics individually but to analyze the EC phenolics profile, as influenced by roast and species. Therefore, we used chromatographic peak areas (measured at 320 nm) to evaluate those parameters. As mentioned, also nonphenolic compounds (TRG, CAF, and HMF) were additionally considered in this study. In this case, peak area measurements were performed at 280 nm. Results are presented in **Table 4**.

In a general way, the phenolic profiles obtained for EC are in agreement with those reported in the literature for coffee beans (*27*, *31*, *32*).

Robusta ECs contained mean higher levels of total CGA, especially when light roast was applied to the beans. For both species, a decrease of ∼60% was observed from light- to dark-roasted beverages.

CQA represented the most abundant group of CGA, 5-CQA being the major compound of all ECs. Although higher levels of CQA have been reported for roasted robusta beans (*31*), in this work, no significant differences ($p > 0.05$) were found between ECs prepared from different species roasted to the same degree. Moreover, a similar degradation profile with roast was observed for both species, with dark brews containing <65% of the CQA as light ones. Indeed, the differences found between total CGA of arabica and robusta ECs seem to be essentially due to minor CGA, namely, FQA, diCQA, CFQA, and diFQA.

FQA were the second most abundant group within CGA, 5-FQA being the major isomer (∼49%), whereas 1-FQA was present in lower levels (∼3%). Robusta ECs contained double amounts of total FQA compared with arabicas, for both roast degrees, and their levels decreased about 50% with roast, for both species. However, in the specific case of 1-FQA, no significant differences ($p > 0.05$) were observed between distinct species or roasts. Unlike the remaining FQA, this compound is present in only roasted coffee, not in green coffee beans (*27*), probably the result of some isomerization of other FQA. Therefore, their different behavior from other FQA is not surprising.

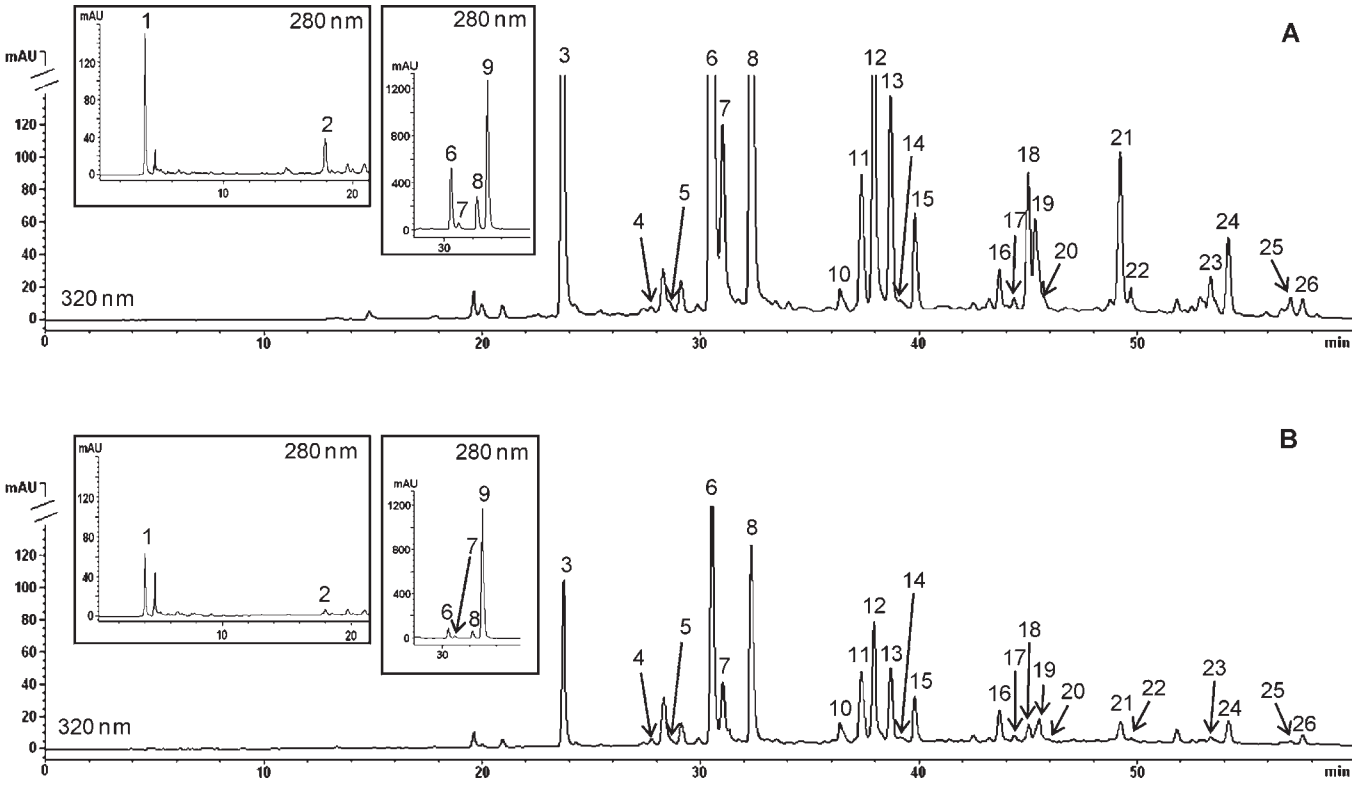

Figure 2. Chromatograms, recorded at 320 and 280 nm, of an EC extract prepared with light-roasted (**A**) and dark-roasted (**B**) beans of *Coffea canephora* var. *robusta* from Ivory Coast (compound identification is detailed in **Table 3**). The main peaks are shown off-scale to evidence the minor ones.

Table 3. LC-DAD-MS Characteristics of Compounds Identified in EC Extracts

peak	t_R[a]	compound	abbreviation	$[M + H]^+$	$[M + Na]^+$	fragment ions	UV spectrum[b]
1	3.907	trigonelline	TRG	138			E
2	17.834	5-hydroxymethylfurfural	HMF	127			G
3	23.643	3-caffeoylquinic acid	3-CQA	355	377	163	A
4	27.698	1-feruloylquinic acid	1-FQA	369	391	177	A
5	28.550	3-*p*-coumaroylquinic acid	3-CoQA	339	361	147	B
6	30.451	5-caffeoylquinic acid	5-CQA	355	377	163	A
7	30.968	3-feruloylquinic acid	3-FQA	369	391	177	A
8	32.275	4-caffeoylquinic acid	4-CQA	355	377	163	A
9	32.975	caffeine	CAF	195			F
10	36.383	5-*p*-coumaroylquinic acid	5-CoQA	339	361	147	B
11	37.352	3-caffeoyl-1,5-quinide	3-CQL	337	359	163	A
12	37.902	5-feruloylquinic acid	5-FQA	369	391	177	A
13	38.658	4-feruloylquinic acid	4-FQA	369	391	177	A
14	39.117	4-*p*-coumaroylquinic acid	4-CoQA	339	361	147	B
15	39.808	4-caffeoyl-1,5-quinide	4-CQL	337	359	163	A
16	43.647	3-feruloyl-1,5-quinide	3-FQL	351	373	177	A
17	44.315	4-feruloyl-1,5-quinide	4-FQL	351	373	177	A
18	44.972	3,4-dicaffeoylquinic acid	3,4-diCQA	517	539	499, 163	A
19	45.285	3,5-dicaffeoylquinic acid	3,5-diCQA	517	539	499, 163	A
20	45.457	1-feruloyl-1,5-quinide	1-FQL	351	373	177	A
21	49.186	4,5-dicaffeoylquinic acid	4,5-diCQA	517	539	499, 163	A
22	49.670	caffeoylferuloylquinic acid	CFQA (1)	531	553	513, 177, 163	A
23	53.326	caffeoylferuloylquinic acid	CFQA (2)	531	553	513, 177, 163	A
24	54.159	caffeoyltryptophan	CTrp	367	389	163	C
25	56.990	3,4-diferuloylquinic acid	3,4-diFQA	545	567	527, 177	A
26	57.548	*p*-coumaroyltryptophan	CoTrp	351	373	147	D

[a] t_R = retention time (in minutes). [b] Typical UV spectra of the analyzed compounds are depicted in **Figure 3**.

The third most abundant CGA group was constituted by diCQA, which provided 5−10% of total CGA. Robusta ECs contained approximately double the amounts of diCQA as arabica ones, and a general decrease of ∼70% was observed with roast. CoQA contributed 2−4% of total CGA, 5-CoQA being the major isomer. CFQA contents were significantly higher ($p < 0.05$) in robusta ECs, for both types of roast, as well as those of 3,4-diFQA.

With regard to CGL, total levels were higher in light robusta ECs. CQL represents the most abundant group of CGL

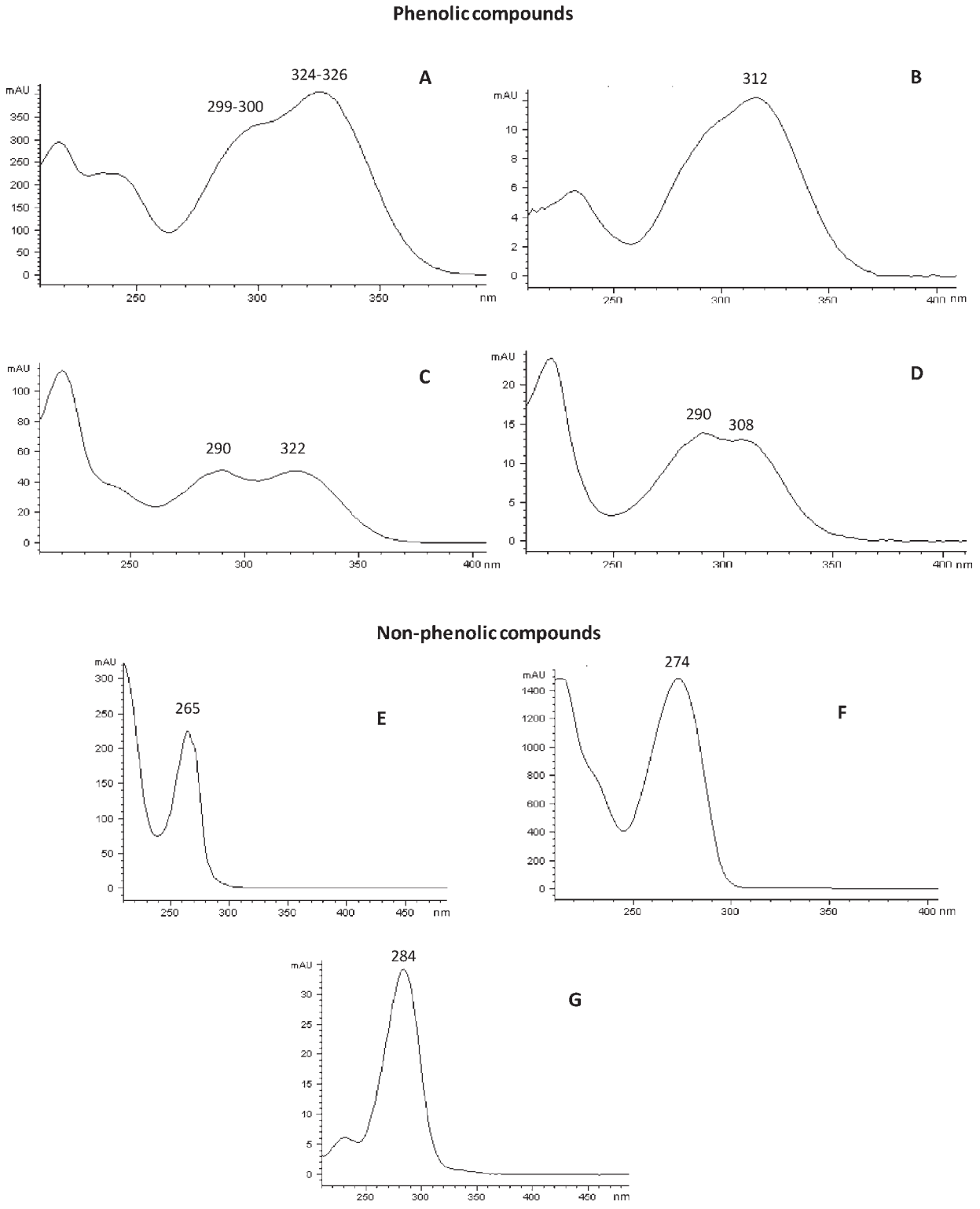

Figure 3. UV spectra of the compounds identified in EC extracts (described in **Table 3**).

(70−80%), 3-CQL being the main CQL isomer present in ECs (~60% of CQL).

FQL represented 20−30% of total CGL. Their amounts increased with roast in arabica brews, whereas a decrease was observed for robustas. Nevertheless, robusta ECs contained ~2-fold higher FQL amounts than arabicas.

With regard to the cinnamoyl−tryptophan conjugates, significantly higher ($p < 0.05$) contents were found in robusta brews. With respect to CoTrp, robustas contained ~30- and ~2-fold higher amounts than arabicas for the same roast degrees. Unlike Okada et al. (*33*), who found only CoTrp in robusta coffees, concluding that it was an indicator of robusta beans, we identified a small chromatographic peak in arabica samples corresponding to this compound, showing that although present in lower amounts, it also exists in arabica coffees.

Arabica light brews contained significantly higher ($p < 0.05$) TRG contents, and a decrease of 40% with roast occurred for both species. Robusta samples contained 1.5-fold the CAF contents of arabica ECs and, for this compound, no influence of roast degree was noted, as expected (*34*).

Table 4. Peak Areas ($\times 10^3$) of the Compounds Identified in EC Extracts and HMF Contents in ECs[a]

	arabica ECs		robusta ECs	
compound	light roasted	dark roasted	light roasted	dark roasted
3-CQA	5.73 ± 0.88	2.13 ± 0.34	6.06 ± 0.44	2.23 ± 0.38
4-CQA	7.44 ± 0.92	2.82 ± 0.50	7.88 ± 0.36	2.88 ± 0.83
5-CQA	13.52 ± 1.97	4.59 ± 0.86	13.53 ± 0.07	4.33 ± 1.32
CQA	26.69 ± 3.77 a	9.54 ± 1.70 b	27.48 ± 0.87 a	9.44 ± 1.99 b
1-FQA	0.12 ± 0.07	0.09 ± 0.03	0.12 ± 0.05	0.11 ± 0.08
3-FQA	1.01 ± 0.04	0.40 ± 0.03	2.04 ± 0.18	1.03 ± 0.34
4-FQA	0.83 ± 0.08	0.55 ± 0.07	2.00 ± 0.25	1.07 ± 0.30
5-FQA	1.72 ± 0.13	1.02 ± 0.09	3.87 ± 0.07	1.80 ± 0.25
FQA	3.68 ± 0.33 b	2.06 ± 0.22 c	8.02 ± 0.41 a	4.01 ± 1.07 b
3-CoQA	0.16 ± 0.01	0.11 ± 0.02	0.15 ± 0.01	0.08 ± 0.04
4-CoQA	0.11 ± 0.02	0.09 ± 0.03	0.18 ± 0.05	0.02 ± 0.01
5-CoQA	0.37 ± 0.01	0.35 ± 0.03	0.40 ± 0.09	0.38 ± 0.10
CoQA	0.63 ± 0.02 b	0.55 ± 0.02 c	0.73 ± 0.05 a	0.48 ± 0.13 c
3,4-diCQA	0.61 ± 0.01	0.22 ± 0.03	1.31 ± 0.32	0.34 ± 0.18
3,5-diCQA	0.57 ± 0.15	0.14 ± 0.03	0.91 ± 0.32	0.21 ± 0.09
4,5-diCQA	0.74 ± 0.02	0.29 ± 0.02	1.77 ± 0.46	0.46 ± 0.10
diCQA	1.92 ± 0.12 b	0.66 ± 0.05 d	4.00 ± 1.10 a	1.01 ± 0.34 c
3,4-diFQA	0.05 ± 0.02 b	0.02 ± 0.00 c	0.27 ± 0.07 a	0.05 ± 0.00 b
CFQA (1)	0.11 ± 0.04	0.01 ± 0.00	0.50 ± 0.19	0.15 ± 0.06
CFQA (2)	0.08 ± 0.01	0.03 ± 0.01	0.53 ± 0.14	0.12 ± 0.07
CFQA	0.19 ± 0.05 b	0.04 ± 0.02 c	1.02 ± 0.33 a	0.27 ± 0.12 b
total CGA	33.17 ± 3.93 b	12.88 ± 1.96 c	41.52 ± 2.83 a	15.26 ± 2.06 c
3-CQL	1.25 ± 0.18	1.29 ± 0.15	1.31 ± 0.06	1.06 ± 0.12
4-CQL	0.80 ± 0.09	0.80 ± 0.11	1.01 ± 0.09	0.66 ± 0.10
CQL	2.05 ± 0.27 ab	2.09 ± 0.25 ab	2.33 ± 0.15 a	1.72 ± 0.21 b
1-FQL	0.06 ± 0.04	0.08 ± 0.01	0.17 ± 0.02	0.10 ± 0.05
3-FQL	0.18 ± 0.00	0.29 ± 0.01	0.45 ± 0.07	0.43 ± 0.04
4-FQL	0.16 ± 0.01	0.17 ± 0.04	0.56 ± 0.03	0.29 ± 0.09
FQL	0.40 ± 0.03 d	0.55 ± 0.05 c	1.18 ± 0.08 a	0.82 ± 0.17 b
total CGL	2.45 ± 0.25 b	2.64 ± 0.30 b	3.50 ± 0.23 a	2.54 ± 0.37 b
CTrp	0.10 ± 0.00 c	0.07 ± 0.00 d	1.13 ± 0.36 a	0.49 ± 0.24 b
CoTrp	0.01 ± 0.00 c	0.01 ± 0.00 c	0.32 ± 0.12 a	0.18 ± 0.07 b
TRG	1.45 ± 0.20 a	0.87 ± 0.10 c	1.12 ± 0.05 b	0.67 ± 0.12 c
CAF	10.59 ± 1.61 b	11.09 ± 1.27 b	16.49 ± 0.15 a	16.46 ± 0.59 a
HMF[b]	2.60 ± 0.71 a	0.84 ± 0.41 c	1.29 ± 0.38 b	0.68 ± 0.32 c

[a] Data are expressed as mean ± standard deviation of chromatographic peak areas ($\times 10^3$) of EC extracts prepared from two arabica (Honduras A and B) and two robusta (Ivory Coast A and Vietnam A) samples, light and dark roasted. Data followed by different letters within each row are significantly different at $p < 0.05$. [b] HMF data are expressed in mg/EC (30 mL).

To ascertain the HMF intake through EC, the quantification of this compound was performed (**Table 4**). Its level was significantly higher ($p < 0.05$) in arabica light ECs, ranging from 2.60 to 0.84 mg, for light and dark roasts, respectively, and from 1.29 to 0.68 mg, for light- and dark-roasted robustas, correspondingly.

In conclusion, the different technological factors analyzed influenced, to greater or lesser extents, the antiradical activity, phenolics levels, and antioxidants profile of EC. Lower $DPPH^{\bullet}$ scavenging activities and phenolics amounts were found for decaffeinated ECs. A long espresso (70 mL) more than doubles the phenolics amount of a short one (20 mL). Robusta brews showed higher antiradical activities and phenolics contents than arabica ones, for all roast degrees. No significant differences ($p > 0.05$) were observed for scavenging activity of robusta brews from different roast degrees, but a decrease in total phenolics with roast was noted. For arabica ECs, an increase of scavenging activity in medium-dark brews was observed, whereas no significant differences ($p > 0.05$) in total phenolics were found between brews from different roasts. Robusta ECs contained upper levels of total CGA, and a decrease of ~60% from light to dark roasts occurred for both arabica and robusta brews. CQA were the most abundant group, but ECs contents were not influenced by species, showing that the differences found between total CGA were mainly due to FQA, diCQA, CFQA, and diFQA. Total CGL were significantly higher ($p < 0.05$) in light-roasted robusta ECs. Robusta brews contained higher levels of CTrp and CoTrp than arabica ones, independent of roast degree. Besides, superior levels of TRG and HMF were found in arabica ECs, whereas robusta brews contained more CAF. Unlike the latter, levels of TRG and HMF decreased from light to dark roast (40 and 70%, respectively).

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