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Evaluation of Spent Coffee Obtained from the Most Common Coffeemakers as a Source of Hydrophilic Bioactive Compounds

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ABSTRACT: The main hydrophilic antioxidant compounds (3-, 4-, and 5-monocaffeoylquinic and 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids, caffeine, and browned compounds, including melanoidins) and the antioxidant capacity (Folin−Ciocalteu, ABTS, DPPH, Fremy's salt, and TEMPO) were evaluated in Arabica and Robusta spent coffee obtained from the preparation of coffee brews with the most common coffeemakers (filter, espresso, plunger, and mocha). All spent coffee grounds, with the exception of those from the mocha coffeemaker, had relevant amounts of total caffeoylquinic acids (6.22−13.24 mg/g of spent coffee), mainly dicaffeoylquinic acids (3.31−5.79 mg/g of spent coffee), which were 4−7-fold higher than in their respective coffee brews. Caffeine ranged from 3.59 to 8.09 mg/g of spent coffee. The antioxidant capacities of the aqueous spent coffee extracts were 46.0−102.3% (filter), 59.2−85.6% (espresso), and <42% (plunger) in comparison to their respective coffee brews. This study obtained spent coffee extracts with antioxidant properties that can be used as a good source of hydrophilic bioactive compounds.

KEYWORDS: *coffee, spent coffee, byproducts, bioactive compounds, chlorogenic acids, antioxidant*

INTRODUCTION

Millions of coffee cups are consumed every day around the world, producing spent coffee grounds in tons in restaurants and cafeterias and also at domestic levels. Traditionally these residues have been considered as wastes, or occasionally used as fertilizer. During the past decades, at the industrial level several applications have been proposed to add value to coffee byproducts originated from the fruit to roasted coffee (coffee pulp, husk, and silverskin) and even soluble coffee (spent coffee).[1,2] For example, spent coffee from soluble coffee industry might be used for animal feed;[3] for biofuel, biodiesel, or bioethanol production;[4,5] as adsorbent and activated carbon;[6,7] and also to extract antioxidants or other bioactive compounds.[8,9]

Although the production of soluble (or instant) coffee originates a highly effective extraction of coffee bioactive compounds, such as caffeine and chlorogenic acids, limited amounts of these compounds remain in spent coffee grounds and might be extracted with solvents (ethanol, methanol, etc.).[8,10] Similarly, the spent coffee grounds originated by the preparation of a cup of coffee might also contain relevant amounts of bioactive compounds. However, a direct transfer of the results of industry soluble coffee extraction to coffee brew preparation is not possible because, despite the type of coffee, there is a clear influence of (i) different coffee brewing procedures, including different coffeemakers,[11,12] and also (ii) technological factors, such as water temperature and pressure, coffee/water ratio, and grinding, on the extraction of coffee compounds.[13−16] Currently, to our best knowledge, only a few studies about the presence of phenolics in spent coffee grounds from espresso coffee[17,18] and the preparation of extracts with antioxidant capacity from spent coffee grounds obtained by filter coffee brewing have been found.[19]

On the other hand, the increasing demand for foodstuffs free of artificial additives or with nutritional or healthy added values induces the food industry (and also the pharmaceutical industry) to find new sources of antioxidants and bioactive compounds. For that reason, knowledge of the main bioactive compounds extracted from spent coffee grounds would give them a higher value. Therefore, the aim of this work was to quantitate the main bioactive compounds (caffeoylquinic acids, caffeine, and browned compounds, including melanoidins) in spent coffee obtained from the preparation of coffee brews with the most common coffeemakers (filter, espresso, plunger, and mocha) and to evaluate their antioxidant capacity. This study allows us to know whether the residues generated during the coffee brewing procedure, produced in large amounts in cafeterias and restaurants or at domestic levels, can be considered as a source of natural antioxidants.

MATERIALS AND METHODS

Chemicals and Reagents. The methanol used (spectrophotometric and HPLC grade) and Folin−Ciocalteu reagent were from Panreac (Barcelona, Spain). 2,2′-Azinobis(3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, Fremy's salt

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(potassium nitrosodisulfonate), and 2,2,6,6-tetramethyl-1-piperidin-1-oxyl (TEMPO) were purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid was from Fluka (Madrid, Spain). Pure reference standards of 5-caffeoylquinic acid (5-CQA) and caffeine were obtained from Sigma-Aldrich, and pure reference standards of 3,4-, 3,5-, and 4,5-dicaffeoylquinic acids were purchased from Phytolab (Vestenbergsgreuth, Germany). A mixture of 3-CQA, 4-CQA, and 5-CQA was prepared from 5-CQA using the isomerization method of Trugo and Macrae,[20] also described in Farah et al.[21]

Spent Coffee Preparation. Roasted coffee (without defective beans) from Guatemala (*Coffea arabica*, named Arabica, 3.03% water content, $L^* = 25.40 \pm 0.69$, roasted at 219 °C for 905 s) and Vietnam (*Coffea canephora* var. robusta, named Robusta, 1.59% water content, $L^* = 24.92 \pm 0.01$, roasted at 228 °C for 859 s) was provided by a local factory. Coffee beans were ground for 20 s using a grinder (model Moulinex super junior "s", París, France). The L^* value was analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant and CIE 1931 standard observer. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in a 1 cm Petri plate, and the L^* value was measured in triplicate on the CIELab scale. Water content was measured by weight loss after drying for 2 h at 102 ± 3 °C in a JP Selecta oven (Barcelona, Spain).

Four coffee brewing procedures were selected to obtain the spent coffee: filter coffeemaker, espresso coffeemaker, plunger coffeemaker, and mocha coffeemaker. The coffee brews were prepared as described by López-Galilea et al.[11] with some modifications. Filter coffee brew was prepared from 24 g of ground roasted coffee for a volume of 400 mL of water, using a filter coffeemaker (model Avantis 70 Inox, Ufesa, Spain). Extraction took approximately 6 min at 90 °C. Espresso coffee brew was prepared from 7 g of ground roasted coffee for a volume of 40 mL using an espresso coffeemaker (model Saeco Aroma, Italy). The water pump pressure was 15 bar. Extraction took approximately 24 s at 90 °C. Plunger coffee brew was prepared from 40 g of ground roasted coffee, which was extracted by adding 500 mL of water at 98 °C in a plunger coffeemaker (model Bistro Nouveau Coffee Maker, Bodum, 1 L capacity). The water and the coffee powder were kept in contact for 5 min before the plunger was slowly pushed down. Mocha coffee brew was prepared from 36 g of ground roasted coffee for a volume of 450 mL, using a mocha coffeemaker (model Bra, Spain). The heating temperature and extraction time were approximately 10 min at 93 °C.

Ground roasted coffee after brewing procedures, called spent coffee, was dried to a constant weight for 2 h at 102 ± 3 °C in a JP Selecta oven.

Spent Coffee Extract Preparation. Spent coffee extracts were prepared according to the method described by Bravo et al.[19] Briefly, first, dried spent coffee was defatted with petroleum ether (1:11, w/v) for 3 h at 60 °C in a Soxhlet extraction system (Extraction Unit B-811 Standard Büchi, Flawil, Switzerland). Then, 24 g of spent coffee was extracted with a volume of 400 mL of water using a filter coffeemaker (model Avantis 70 Inox). Extraction took approximately 6 min at 90 °C. Aqueous spent coffee extracts were lyophilized using a Cryodos Telstar (Terrassa, Spain).

Caffeoylquinic Acids and Caffeine. Extract preparation and cleanup were carried out according to the method of Bicchi et al.[22] The compounds were analyzed by HPLC following the method described by Farah et al.,[21] with some modifications. HPLC analysis was achieved with an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump and an automated sample injector. A reversed-phase Hypersil-ODS (5 μm particle size, 250 × 4.6 mm) column was used at 25 °C. The sample injection volume was 100 μL. The chromatographic separation was performed using a gradient of methanol (solvent A) and Milli-Q water acidulated with phosphoric acid (pH 3.0, solvent B) at a constant flow of 0.8 mL/min starting with 20% solvent A. Then solvent A was increased to 50% within 15 min, maintained at 50% for 9 min, and, finally, returned to initial conditions (20% solvent A) in 3 min. Detection was accomplished with a diode array detector, and chromatograms were recorded at 325 nm for caffeoylquinic acids and at 276 nm for caffeine. Identification of caffeoylquinic acids and caffeine was performed by comparing the retention time and the UV−vis spectra with those of their reference compounds. Quantitation of 5-caffeoylquinic (5-CQA) and caffeine was made by comparing the peak areas with those of the standards.

The method was validated by obtaining a linear relationship between the concentrations of each compound and the respective UV absorbance ($r = 0.999$). The recovery values were 101.3 ± 1.3% for 5-CQA and 106.3 ± 2.4% for caffeine. Results for repeatability showed good precision of the method with coefficient of variation values below 5%. A narrow dispersion of values was also observed for intermediate precision, with coefficients of variation between 0.11 and 4.96%. The detection limits were 1.18 and 0.83 μg/mL for 5-CQA and caffeine, respectively. The quantitation limits were 3.95 μg/mL for 5-CQA and 2.76 μg/mL for caffeine.

Quantitation of the other caffeoylquinic acids was performed using the area of 5-CQA standard combined with molar extinction coefficients of the respective caffeoylquinic acid as reported by Trugo et al.[20] and Farah et al.,[21] using the equation

$$C = \frac{\mathrm{RF} \times \varepsilon_1 \times M_{r2} \times A}{\varepsilon_2 \times M_{r1}}$$

where C is the concentration of the isomer in milligrams per milliliter, RF is the response factor of the 5-CQA standard (concentration in milligrams per milliliter per unit area), ε_1 is the molar extinction coefficient of 5-CQA, ε_2 is the molar extinction coefficient of the isomer in question, M_{r1} is the relative molecular mass of 5-CQA, M_{r2} is the relative molecular mass of the isomer in question, and A is the area of the peak corresponding to the isomer in question. Molar extinction coefficients ($\times 10^4$) were as follows (at λ_{max} 330 nm): 5-CQA = 1.95, 4-CQA = 1.80, 3-CQA = 1.84, 3,4-diCQA = 3.18, 3,5-diCQA = 3.16, and 4,5-diCQA = 3.32.

Results were expressed as milligrams of each compound per gram of spent coffee dry matter (spent coffee extracts) or per gram of coffee (coffee brews).

Browning Index (Abs 420 nm). Fifty microliters of the sample (spent coffee extract or coffee brew) was diluted to 2 mL with demineralized water. Browning index was measured by reading the absorbance of samples at 420 nm, after exactly 2 min in a 3 mL capacity cuvette (1 cm length) with a Lambda 25 UV−vis spectrophotometer (Perkin-Elmer Instruments, Madrid, Spain) connected to a thermostatically controlled chamber (25 °C) and equipped with UV WinLab software (Perkin-Elmer). This index measures the browned compounds developed during caramelization and Maillard reactions, including melanoidins.[23]

Antioxidant Capacity by Folin−Ciocalteu (FC) Assay. The Folin−Ciocalteu reducing capacity of coffee was performed according to Singleton's method.[24] Spent coffee extracts and coffee brews were diluted 3:10 and 1:10, respectively, in demineralized water prior to analysis. A volume of 500 μL of Folin−Ciocalteu reagent (Panreac) was added to a mixture of 100 μL of the extract sample and 7.9 mL of demineralized water. After a 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a Lambda 25 UV−vis spectrophotometer (Perkin-Elmer Instruments). Gallic acid (GA) was used as reference, and the results were expressed as milligrams of GA per gram of spent coffee dry matter (mg GA/g spent coffee dm) or per gram of coffee (mg GA/g coffee).

Antioxidant Capacity by ABTS Assay. The ABTS antioxidant capacity was performed according to the method of Re et al.[25] with some modifications. The radicals $ABTS^{\bullet+}$ were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS solution prepared in phosphate-buffered saline (PBS) (pH 7.4), and the $ABTS^{\bullet+}$ solution was stored in darkness for 12 h. The $ABTS^{\bullet+}$ solution was adjusted with PBS to an absorbance of 0.700 (±0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV−vis spectrophotometer, Perkin-Elmer Instruments). Spent coffee extracts and coffee brews were diluted 3:100 and 15:1000, respectively, in demineralized water prior to analysis. Samples (50 μL) were added

Table 1. Caffeoylquinic Acids (Milligrams per Gram) in Spent Coffee and Coffee Brews[a]

	spent coffee				coffee brews			
	filter	espresso	plunger	mocha	filter	espresso	plunger	mocha
3-CQA								
Arabica	1.64 ± 0.00 d	1.29 ± 0.03 c	1.10 ± 0.03 b	nd a	3.54 ± 0.00 ab	3.08 ± 0.07 a	3.91 ± 0.22 b	5.29 ± 0.29 c
Robusta	0.68 ± 0.03 b	0.83 ± 0.03 c	0.63 ± 0.02 b	nd a	2.26 ± 0.13 b	1.81 ± 0.09 a	2.03 ± 0.11 ab	3.43 ± 0.05 c
4-CQA								
Arabica	2.51 ± 0.02 d	2.03 ± 0.02 c	1.75 ± 0.10 b	nd a	5.50 ± 0.00 b	3.97 ± 0.05 a	6.01 ± 0.32 b	7.67 ± 0.41 c
Robusta	0.97 ± 0.04 b	1.16 ± 0.01 c	0.99 ± 0.05 b	nd a	2.93 ± 0.17 b	2.45 ± 0.02 a	3.01 ± 0.18 b	4.03 ± 0.02 c
5-CQA								
Arabica	3.59 ± 0.02 d	2.84 ± 0.04 c	2.48 ± 0.07 b	nd a	7.42 ± 0.01 a	6.89 ± 0.13 a	8.57 ± 0.43 b	10.72 ± 0.61 c
Robusta	1.26 ± 0.06 b	1.56 ± 0.05 c	1.18 ± 0.05 b	nd a	4.38 ± 0.21 b	3.70 ± 0.14 a	3.63 ± 0.20 a	6.25 ± 0.07 c
total CQAs								
Arabica	7.74	6.16	5.33	nd	16.46	13.94	18.49	23.68
Robusta	2.91	3.55	2.80	nd	9.57	7.96	8.67	13.71
3,4-diCQA								
Arabica	2.53 ± 0.03 c	2.34 ± 0.02 b	2.46 ± 0.06 c	nd a	0.22 ± 0.00 a	0.47 ± 0.02 b	0.57 ± 0.03 c	0.89 ± 0.05 d
Robusta	1.49 ± 0.07 b	1.80 ± 0.01 c	1.99 ± 0.10 d	nd a	0.29 ± 0.01 a	0.29 ± 0.00 a	0.30 ± 0.02 a	0.52 ± 0.00 b
3,5-diCQA								
Arabica	1.17 ± 0.01 c	1.09 ± 0.02 b	1.26 ± 0.05 d	nd a	nd a	0.25 ± 0.01 c	0.22 ± 0.01 b	0.42 ± 0.02 d
Robusta	0.62 ± 0.03 b	0.74 ± 0.01 c	0.70 ± 0.04 c	nd a	0.18 ± 0.01 c	0.14 ± 0.00 a	0.16 ± 0.01 b	0.32 ± 0.00 d
4,5-diCQA								
Arabica	1.80 ± 0.03 c	1.46 ± 0.02 b	2.07 ± 0.10 d	nd a	nd a	0.35 ± 0.01 b	0.37 ± 0.02 b	0.63 ± 0.03 c
Robusta	1.20 ± 0.05 b	1.40 ± 0.03 c	1.41 ± 0.08 c	nd a	nd a	0.26 ± 0.00 b	0.26 ± 0.01 b	0.74 ± 0.00 c
total diCQAs								
Arabica	5.50	4.89	5.79	nd	0.22	1.07	1.16	1.94
Robusta	3.31	3.94	4.10	nd	0.47	0.69	0.72	1.58
total CQAs + diCQAs								
Arabica	13.24	11.05	11.12	nd	16.68	15.01	19.65	25.62
Robusta	6.22	7.49	6.90	nd	10.04	8.65	9.39	15.29

[a]All values are shown as the mean ± SD ($n = 3$). In each row, different letters indicate significant differences ($p < 0.05$) among different spent coffee or coffee brews. CQA, monocaffeoylquinic acids; diCQA, dicaffeoylquinic acids; nd, not detected.

to 2 mL of $ABTS^{\bullet+}$ solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue), and the antioxidant capacity was expressed as micromoles of Trolox per gram of spent coffee dry matter (μmol Trolox/g spent coffee dm) or per gram of coffee (μmol Trolox/g coffee).

Antioxidant Capacity by DPPH Assay. The antioxidant capacity was also measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) decolorization assay (Brand-Williams et al.[26] with modifications). A 6.1×10^{-5} mol/L $DPPH^{\bullet}$ methanolic solution was prepared immediately before use. The $DPPH^{\bullet}$ solution was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV−vis spectrophotometer, Perkin-Elmer Instruments). Spent coffee extracts and coffee brews were diluted 3:100 and 15:1000, respectively, in demineralized water prior to analysis. Samples (50 μL) were added to 1.95 mL of the $DPPH^{\bullet}$ solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue). The antioxidant capacity was expressed as micromoles of Trolox per gram of spent coffee dry matter (μmol Trolox/g spent coffee dm) or per gram of coffee (μmol Trolox/g coffee).

Antioxidant Capacity by Electron Spin Resonance (ESR) Spectroscopy. The ESR spectroscopy measurements were performed with Fremy's salt and TEMPO as stabilized radicals with the same procedure as described by Roesch et al.[27] and modified by Cämmerer and Kroh.[28] For the investigation with Fremy's salt, 100 μL of every spent coffee extract diluted 100-fold with demineralized water was allowed to react with an equal volume of an aqueous 1 mM Fremy's salt solution prepared in 50 mM phosphate buffer (pH 7.4). ESR spectra were recorded every 35 s for 30 min. For the investigation with TEMPO, aliquots of 200 μL of spent coffee extract were allowed to react with 100 μL of 1 mM TEMPO solution. ESR spectra were obtained after 30 min, by which time the reaction was complete. Microwave power was set at 10 dB. Modulation amplitude, center field, and sweep width were set at 1.5, 3397, and 71 G, respectively. Both Fremy's salt and TEMPO antioxidant activity were calculated as Trolox equivalents and expressed as micromoles of Trolox per gram of spent coffee dry matter (μmol Trolox/g spent coffee dm).

Statistical Analysis. Each parameter was analyzed in triplicate. Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter; the source of variation was the different brewing procedure for obtaining spent coffee. A Tukey test was applied as a test a posteriori with a level of significance of 95%. All statistical analyses were performed using the SPSS v.15.0 software package.

RESULTS AND DISCUSSION

Caffeoylquinic Acids. Caffeoylquinic acids are the most abundant phenolic compounds in coffee. These chlorogenic acids are water-soluble esters formed between quinic acid and one or two moieties of caffeic acid, a *trans*-cinnamic acid. Monocaffeoylquinic acids (3-CQA, 4-CQA, 5-CQA) and dicaffeoylquinic acids (3,4-diCQA, 3,5-diCQA, 4,5-diCQA) were identified and quantified in spent coffee obtained from different coffeemakers (filter, espresso, plunger, and mocha) and in their respective coffee brews. The results are shown in Table 1.

All spent coffee grounds, with the exception of those from the mocha coffeemaker, had relevant amounts of total

Table 2. Caffeine (Milligrams per Gram) and Melanoidins (Absorbance 420 nm) in Spent Coffee and Coffee Brews[a]

	spent coffee				coffee brews			
	filter	espresso	plunger	mocha	filter	espresso	plunger	mocha
caffeine								
Arabica	5.20 ± 0.04 d	3.59 ± 0.00 b	3.76 ± 0.12 c	nd a	10.08 ± 0.04 a	10.07 ± 0.17 a	11.83 ± 0.65 b	15.64 ± 0.86 c
Robusta	7.53 ± 0.35 c	8.09 ± 0.05 c	5.73 ± 0.31 b	nd a	22.08 ± 0.06 c	17.73 ± 0.14 b	15.64 ± 0.84 a	22.68 ± 0.02 c
melanoidins								
Arabica	0.165 ± 0.006 d	0.102 ± 0.001 b	0.114 ± 0.001 c	tr a	0.155 ± 0.003 a	0.315 ± 0.008 b	0.401 ± 0.009 c	0.803 ± 0.011 d
Robusta	0.145 ± 0.005 d	0.133 ± 0.001 c	0.118 ± 0.000 b	tr a	0.197 ± 0.001 a	0.418 ± 0.008 b	0.647 ± 0.006 c	0.847 ± 0.009 d

[a]All values are shown as the mean ± SD (n = 3). In each row, different letters indicate significant differences ($p < 0.05$) among different spent coffee or coffee brews. nd, not detected; tr, traces.

Table 3. Antioxidant Capacity of Spent Coffee and Coffee Brews[a]

	spent coffee				coffee brews			
	filter	espresso	plunger	mocha	filter	espresso	plunger	mocha
Folin−Ciocalteu (mg GA/g)								
Arabica	24.60 ± 0.18 d	15.79 ± 0.08 c	13.87 ± 0.16 b	1.36 ± 0.03 a	26.70 ± 0.07 b	23.13 ± 0.23 a	33.29 ± 0.13 c	50.16 ± 0.07 d
Robusta	17.54 ± 0.26 c	19.12 ± 0.14 d	13.22 ± 0.20 b	1.88 ± 0.05 a	38.16 ± 1.31 b	30.55 ± 0.06 a	51.41 ± 1.02 c	60.92 ± 0.46 d
ABTS (μmol Trolox/g)								
Arabica	215.12 ± 2.18 d	131.83 ± 1.49 c	120.98 ± 1.81 b	8.09 ± 0.33 a	242.08 ± 3.54 b	206.12 ± 1.68 a	280.11 ± 7.01 c	456.33 ± 5.79 d
Robusta	167.24 ± 5.78 c	177.63 ± 1.23 d	118.23 ± 3.05 b	22.17 ± 0.30 a	310.93 ± 2.27 b	299.86 ± 2.69 a	493.97 ± 1.27 c	622.45 ± 6.16 d
DPPH (μmol Trolox/g)								
Arabica	112.06 ± 2.21 d	74.57 ± 2.99 c	60.96 ± 1.75 b	2.56 ± 0.18 a	109.49 ± 2.80 b	87.13 ± 0.40 a	145.94 ± 3.66 c	208.18 ± 9.39 d
Robusta	74.09 ± 1.00 c	83.59 ± 2.39 d	53.99 ± 0.25 b	5.18 ± 0.06 a	128.18 ± 5.20 a	121.72 ± 4.69 a	231.06 ± 8.64 b	243.46 ± 0.75 b

[a]All values are shown as the mean ± SD (n = 3). In each row, different letters indicate significant differences ($p < 0.05$) among different spent coffee or coffee brews.

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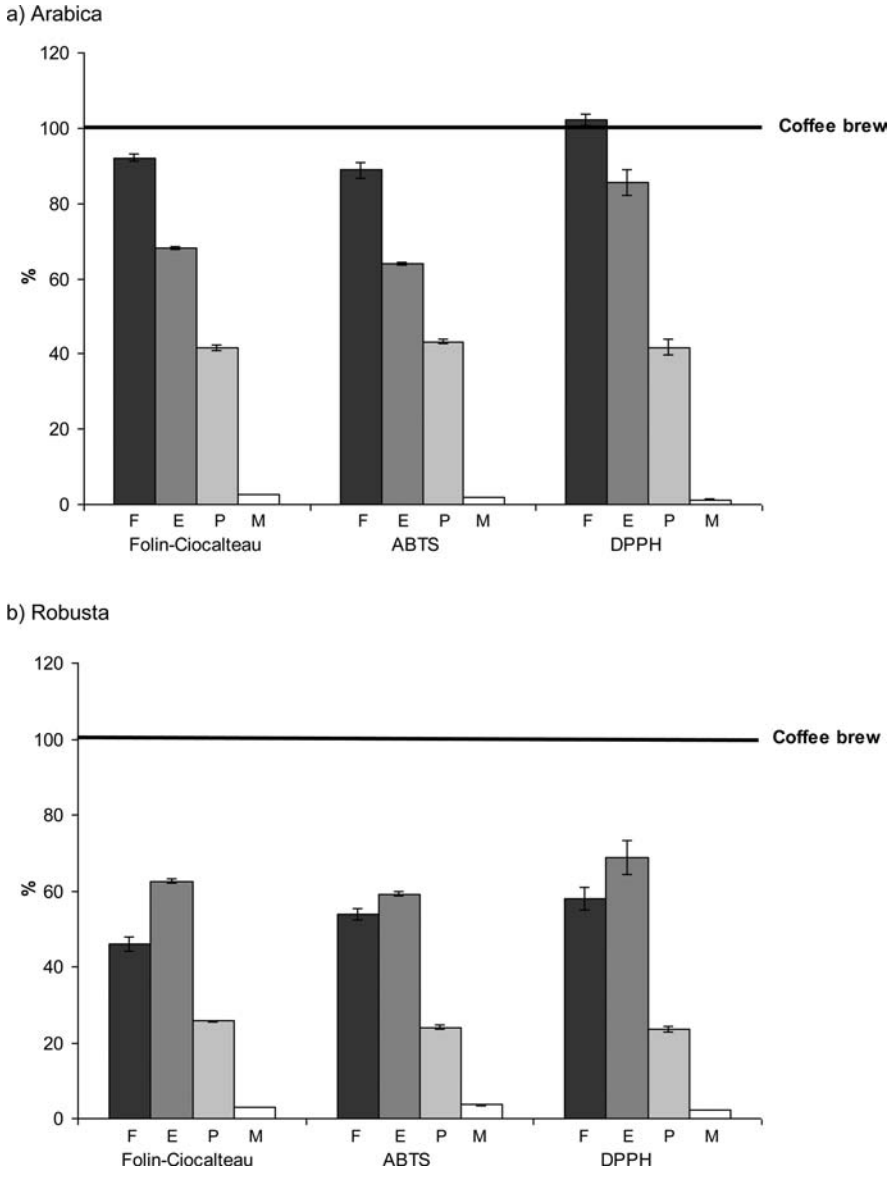

Figure 1. Antioxidant capacity percentage of Arabica (a) and Robusta (b) spent coffee obtained from different coffeemakers in comparison to their respective coffee brew (expressed as 100%). F, filter; E, espresso; P, plunger; M, Mocha.

caffeoylquinic acids ranging from 11.05 mg (espresso) to 13.24 mg (filter) per gram of Arabica spent coffee and from 6.22 mg (filter) to 7.49 mg (espresso) per gram of Robusta spent coffee. These amounts correspond to 56.6−86.6% of those caffeoylquinic acids obtained for the coffee brews. In espresso coffee residues, Cruz et al.[17] reported similar amounts of 5-CQA. However, in the present study the total CQA and diCQA values reach higher results than total CGA in the Cruz et al. study.[17] This discrepancy could be probably due to the different type of coffee, Arabica or Robusta, in the present study or Arabica/Robusta blends in the Cruz et al. work[17] and also to other technological factors, such as roasting degree and extraction methodology. Despite these results, to our knowledge, the present study is the first to report the quantity of individual mono- and dicaffeoylquinic acids present in spent coffee obtained from coffeemakers different from espresso ones.

Coffee brews prepared with the mocha coffeemaker showed the highest content in both monocaffeoylquinic (23.68 and 13.71 mg/gg for Arabica and Robusta, respectively) and dicaffeoylquinic (1.94 and 1.58 mg/g for Arabica and Robusta, respectively) acids, suggesting a practically complete extraction of caffeoylquinic acids, the amount present in the spent coffee grounds being negligible. In a previous work, we also observed that a second successive aqueous extraction from the spent coffee grounds contributed to only a small amount of phenolic compounds (<15% of those obtained in the first extraction).[19] Therefore, it could be considered that the total content of caffeoylquinic acids present in coffee has been practically extracted with the preparation of the coffee brew and the first aqueous extraction of spent coffee. This was also corroborated by the fact that the sum of the total caffeoylquinic acids of each coffee brew and its respective spent coffee was quite similar among different coffeemakers, ranging from 15.29 mg (mocha) to 16.29 mg (plunger) per gram for Arabica and from 25.62 mg (mocha) to 30.77 mg (plunger) per gram for Robusta coffee. These results are also in agreement with the total chlorogenic acid amounts reported for roasted coffees from different origins and varieties.[29,30] Although higher amounts of caffeoylquinic acids in Robusta coffees than in Arabica ones have been extensively reported,[21] other authors have found lesser amounts

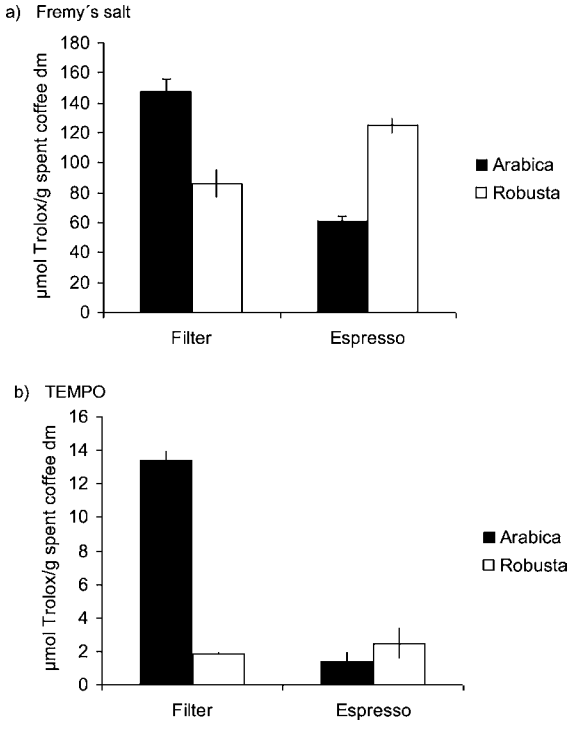

Figure 2. Fremy's salt (a) and TEMPO (b) antioxidant capacity of spent coffee obtained from filter and espresso coffeemakers.

of CQAs in Robusta coffees.[31,32] This could be due to both the origin of coffee and the higher loss of chlorogenic acids in Robusta coffee during the roasting process.[33,34]

In spent coffee, 5-CQA and 3,4-diCQA were the most abundant caffeoylquinic acids, whereas 3,5-diCQA and 3-CQA were the least ones. Moreover, the content of total diCQAs was quite similar and even higher than total monoCQAs. This is due to 3−6-fold, and even higher for the filter coffeemaker, amounts of total diCQAs in spent coffee in comparison to their respective coffee brews. In contrast, in coffee brews the monoCQA content was 12-fold or even higher than diCQAs, 5-CQA being the most and 3,4-diCQA the least abundant. Therefore, the monoCQAs are mainly extracted during coffee brewing, whereas the diCQAs need further conditions to be extracted. Other authors also observed that diCQAs or dicaffeoylquinides were extracted rather slowly from coffee in comparison to monoCQAs.[32,35] The esterification of an additional caffeic acid moiety in diCQAs increases the number of hydroxyl groups that might be bound to the amide carbonyls of the peptide bond in melanoidins.[36,37] Also, other authors[38] corroborated this hypothesis that the chlorogenic acids are incorporated into melanoidins via hydroxycinnamic acid (such as caffeic acid) moieties, mainly through nonester linkages upon coffee roasting. Therefore, the release of diCQAs bound to melanoidins is rather more difficult than that of monoCQAs. However, as proposed by Ludwig et al.,[32] the use of an extraction system with turbulences during a few minutes facilitates the contact of grounds and water, a polar solvent, and favors the caffeoylquinic acid extraction, also diCQAs. Taking into account all of these considerations, the application of the appropriate conditions allowed us to obtain spent coffee extracts that can be considered as a good source of caffeoylquinic acids, mainly diCQAs.

Caffeine. The presence of caffeine in spent coffee grounds has been proposed as one of the main problems for use in agriculture because of its toxicity.[39] Although some studies have reported the content of caffeine in spent coffee,[8,10,17] to our knowledge, the present study is the first to report the amount of caffeine in spent coffee grounds from coffeemakers different from espresso ones. The results of the amount of caffeine in spent coffee and in their respective coffee brews are shown in Table 2.

All spent coffee grounds, with the exception of those from the mocha coffeemaker, had concentrations of caffeine ranging from 3.59 mg (espresso) to 5.20 mg (filter) per gram of Arabica spent coffee and from 5.73 mg (plunger) to 8.09 mg (espresso) per gram of Robusta spent coffee. Quite similar amounts of caffeine were reported by Cruz et al.[17] for espresso coffee residues obtained from Arabica/Robusta blends. The caffeine amounts in spent coffee were 2−3-fold lower than those obtained for their respective coffee brews. Taking into account that a second successive aqueous extraction of spent coffee grounds extracted <3% of the caffeine concentration found in the first extraction, the total content of caffeine present in roasted coffee has been practically extracted with the preparation of the coffee brew and the first aqueous extraction of spent coffee. In fact, the sum of caffeine in coffee brews and spent coffee ranged from 13.66 to 15.64 mg/g for Arabica coffee and from 21.37 to 29.61 mg/g for Robusta coffee. These results are in agreement with those of the literature, which extensively reports that Robusta coffees are richer in caffeine than Arabica ones.[40]

Similar to caffeoylquinic acids, the mocha coffeemaker extracted the majority of the caffeine present in coffee, the mocha coffee brews being the richest in caffeine and leaving no detectable caffeine amounts in the spent coffee grounds. Consequently, spent coffee grounds from the mocha coffeemaker and spent coffee grounds from the other coffeemakers after one extraction with water can be considered as caffeine-free coffee byproducts.

Browned Compounds. Browned compounds, mainly melanoidins, are originated by Maillard reactions during the coffee roasting process. They contribute to the antioxidant and other functional properties of coffee by themselves and because of the presence of phenolics in their skeleton.[41,42] Spent coffee extracts obtained from the filter coffeemaker showed the highest browning index (Table 2; 0.165 and 0.145 for Arabica and Robusta coffees, respectively). These results were similar to those of the filter coffee brews. In contrast, browned compounds in spent coffee extracts from espresso and plunger coffeemakers were 3−5-fold lower than in their respective coffee brews. Similar to the other bioactive compounds (caffeoylquinic acids and caffeine), aqueous soluble browned compounds were mainly extracted in coffee brews when the mocha coffeemaker was used, releasing only traces in spent coffee. Yen et al.[10] also reported a lower browning index in aqueous extracts from soluble spent coffee grounds in comparison to that from roasted coffee. Therefore, although melanoidins and other browned compounds may act as antioxidants, because of their lower amounts in spent coffee, their contribution to the antioxidant capacity could be rather limited in comparison to that in coffee brews, where they reach up to 80%.[43]

Antioxidant Capacity of Spent Coffee. The presence of antioxidant compounds, such as chlorogenic acids, caffeine, and Maillard reaction products, has been extensively associated with the health benefits of coffee, such as cardiovascular and neurodegenerative disease prevention and anticarcinogenic and

antimutagenic activities.[44,45] Besides these healthy properties, antioxidant capacity may contribute to the preservation of coffee against oxidative damage throughout the storage time. Antioxidant activity has been also reported by other authors in extracts of spent coffee grounds from the soluble coffee production,[2,8,46] from espresso coffee,[17,18] and from filter coffee in a previous work by our group.[19] However, to our knowledge, the antioxidant capacity of spent coffee grounds obtained from other different coffeemakers (plunger and mocha) using an Arabica and a Robusta coffee has not been reported previously. For those reasons, the antioxidant capacity was evaluated using three different colorimetric assays (Folin−Ciocalteu, ABTS, and DPPH) in spent coffee obtained from different coffeemakers (filter, espresso, plunger, and mocha) and in their respective coffee brews. The results of the antioxidant capacity are shown in Table 3.

As was expected because of the presence of phenolic and nonphenolic antioxidants, all spent coffee, except that from the mocha coffeemaker, showed relevant antioxidant capacity. Arabica spent coffee obtained from the filter coffeemaker showed the highest values in all assays, followed by Robusta spent coffees from espresso and filter coffeemakers and Arabica spent coffee from the espresso coffeemaker, in that order. The results of the Folin−Ciocalteu assay of spent coffee from the espresso coffeemaker (15.79 and 19.12 mg GA/g of spent coffee for Arabica and Robusta coffees, respectively) were similar to those found by other authors.[18] On the other hand, spent coffee obtained from the plunger coffeemaker showed less antioxidant capacity, and no significant differences between Arabica and Robusta coffees were found.

These antioxidant capacity results seem to be due to a balance between caffeoylquinic acids, more abundant in Arabica spent coffee, and caffeine and browned compounds, mainly present in the Robusta coffee. In fact, caffeine was in higher amounts in Robusta spent coffee than in Arabica, Robusta spent coffee from the espresso coffeemaker being that with the highest content (8.09 mg/g of spent coffee). Other authors also found high correlations between antioxidant capacity of coffee brews and caffeine, suggesting that caffeine might be a good contributor to the antioxidant capacity or reducing power of coffee brews,[11,31,32] whereas other authors[43] find it for the melanoidin content.

The comparison between the antioxidant capacity of the spent coffee with their respective coffee brews (expressed as 100%) is shown in Figure 1. The antioxidant capacity of spent coffee obtained from the filter coffeemaker was quite similar (88.9−102.3%) to that of the coffee brew for Arabica coffee, whereas for Robusta coffee it was only 46.0−57.8%. However, for spent coffee grounds obtained with the espresso coffeemaker, the antioxidant capacity was 59.2−68.7 and 64.0−85.6% for the Robusta and Arabica coffee brews, respectively. These results might be partially explained by the higher browning index in both espresso coffee brews (0.315 and 0.418) and, in less extent, in Robusta filter coffee brew (0.197) that reduce the amount of melanoidins and other browned compounds to be extracted from spent coffee. Moreover, it should be also taken into account that other minor phenolic (hydroxycinnamic acids, feruloylquinic acids, and other chlorogenic acids) and nonphenolic (volatiles, etc.) compounds might contribute to the antioxidant capacity. Thus, to go deeper into the knowledge of the contribution of phenolic and nonphenolic compounds to the antioxidant capacity of spent coffee, ESR spectroscopy was used to measure the antioxidant capacity of spent coffee obtained from filter and espresso coffeemakers.

Mainly phenolic compounds can be detected when Fremy's salt is used as the stabilized radical, whereas the TEMPO radical is mainly scavenged by Maillard reaction products (MRP), such as melanoidins.[12,28] As shown in Figure 2, the ESR antioxidant capacity of Arabica spent coffee from the espresso coffeemaker was lower than that from the filter one, whereas for Robusta spent coffee these results were reversed. The behavior of the results of antioxidant capacity measured by Fremy's salt technique (Figure 2a) was similar to those obtained by the colorimetric assays, but rather different from that of the amounts of caffeoylquinic acids in spent coffee. This may be explained because, although caffeoylquinic acids are the most abundant phenolics in coffee, other minor hydroxycinnamic acids, such as caffeic acid, ferulic acid, and its derivatives by esterification with quinic acid, might also contribute to the Fremy's salt antioxidant capacity of spent coffee. Moreover, although Fremy's salt is mainly scavenged by chlorogenic acids, other antioxidants, including melanoidins, can scavenge this stabilized radical in a nonspecific manner and in a very low proportion.[12,47] On the other hand, the TEMPO scavenging capacity (Figure 2b) was at least 10-fold lower than Fremy's salt, showing that the contribution of roasting-induced antioxidants is rather limited in spent coffee. Similar behavior was also observed in coffee brews by other authors.[12,32,47] For that reason, all TEMPO results should be considered as very low, including the significantly higher TEMPO antioxidant capacity of the aqueous extracts obtained from Arabica spent coffee grounds from the filter coffeemaker. This higher result might be explained by the slightly higher browning index (Abs 420 nm = 0.165) but also because melanoidins have different antioxidant capacities, depending on the structure, their composition, and the phenolics bound in their skeleton.[12,47−49]

In conclusion, spent coffee obtained from the most common coffeemakers used at domestic and cafeteria levels (filter and espresso), and in less proportion from plunger ones, could be considered as a good potential source of hydrophilic bioactive compounds. These compounds are easily extracted with water to obtain extracts with antioxidant properties that might be used in food and pharmaceutical industries to increase food nutritional values or product stability or to develop new healthy products. In contrast, spent coffee grounds from the mocha coffeemaker have only traces of hydrophilic bioactive compounds because this coffeemaker is very effective in extracting them in coffee brews. Moreover, these spent coffee grounds and the spent coffee grounds from the other coffeemakers after one extraction with water might be subsequently used for agriculture because they are practically caffeine-free coffee byproducts. Therefore, this study allows us to know that it is possible to consider spent coffee as an added-value byproduct because it can be used as a good source of bioactive compounds and after that in agriculture. However, further research is needed to identify and quantitate the minor antioxidant compounds present in spent coffee and to demonstrate their functional or healthy properties.

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Notes

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