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Correlation of Selected Constituents with the Total Antioxidant Capacity of Coffee Beverages: Influence of the Brewing Procedure

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Relationships between volatile and nonvolatile compounds and the antioxidant capacity of coffee brews prepared from commercial conventional and torrefacto roasted coffees, employing commonly used doses and prepared by four brewing procedures (filter, plunger, mocha, and espresso machine) were assessed. Significant correlations between volatile Maillard reaction products and antioxidant capacity (measured by both 2,2-diphenyl-1-picrylhydrazyl radical and redox potential methods) were not observed. Highly positive correlations between browned compounds and caffeine with both antioxidant capacity parameters were reported. Principal component analysis allowed coffee brews separation according to coffee roasting processes (PC1) and brewing procedures (PC2), showing that in all cases coffee brews from torrefacto roasted coffee were more antioxidant that those extracted from conventional ones; also, coffee brews extracted by an espresso machine were more antioxidant than those extracted by mocha, plunger, and filter machines.

KEYWORDS: Coffee; antioxidants; torrefacto; brewing procedure

INTRODUCTION

Brewed coffee is one of the most consumed beverages in the world due to its pleasant taste and aroma, as well as its stimulating qualities. However, the types of coffee beverages and the modality of consumption are tightly associated with social habits and cultures of the different countries. Recently, several studies indicated the important contribution of coffee brews to the intake of the antioxidants in the diet, especially in countries such as Spain, Norway, or Italy (*1*−*3*). Furthermore, some coffee compounds such as caffeine (*4*), phenolic compounds such as chlorogenic acids (*5*), hydroxycinnamic acids (*6*), or compounds developed along Maillard reactions (MRs) such as melanoidins (*7*, *8*) have been identified as antioxidants.

In the last few years, volatile compounds derived from MRs during the roasting process have been studied as a new source of natural antioxidants. They are a reliable alternative to the synthetic ones, since the latter present some disadvantages (*9*). Severini et al. (*10*) observed that certain volatile compounds produced in the roasting process of almonds displayed an antioxidant effect. Volatile heterocyclic compounds, in particular heterocyclic flavor chemicals, obtained from a sugar/amino acid model system have been reported to inhibit the oxidation of both lipids (*11*) and hexanal (*12*). Moreover, some typical volatile heterocyclic compounds found in coffee brew have been examined for antioxidant activity. Fuster et al. (*13*) and Yanagimoto et al. (*14*) assayed, one by one, the antioxidant activity of some isolated volatile compounds in a dichloromethane solution of hexanal. In these experiments, pyrroles, furans, and thiophenes, assayed at concentrations not comparable with those present in coffee brews, exhibited antioxidant activities. Later, Yanagimoto et al. (*15*) studied the antioxidant activities of chromatographic fractions obtained from a dichloromethane extract of coffee brews. This work suggested that some heterocyclic volatile compounds present in these fractions were potentially responsible for the antioxidant activities. Thus, even though the potential antioxidant activity of each isolated volatile compound has been demonstrated, the lack of consideration of both the coffee matrix effect and the antagonistic/synergistic effects of the overall volatile compounds does not allow us to consider volatile Maillard reaction products (MRPs) as antioxidants in "veritable" coffee brews.

Coffee compounds can be affected differently by the roasting process. Phenolic compounds are partially degraded and/or bound to polymer structures depending on the roasting conditions (*16*). Whereas some compounds are degraded, many others are developed or enhanced deriving from MRs, carbohydrate caramelization, and pyrolysis of organic compounds (*17*).

Torrefacto is a roasting process in which sugar is added to Robusta coffee. The influence of the torrefacto roast in the chemical composition of coffee brews (*18*), in the coffee brew aromatic profile (*19*), and in the antioxidant capacity of ground roasted coffee (*20*) has been previously reported. The addition of sugar at the end of the torrefacto roasting process might intensify the development of MRs and, consequently, MRPs development.

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As it has been recently reported (*21*, *22*), the brewing procedure plays a non-negligible role in the antioxidant capacity of coffee brews. Coffee compounds, such as caffeine (*23*) or polyphenols (*21*), are extracted differently depending on the brewing procedure.

For the exposed above, this work is aimed at studying the relationships between volatile and nonvolatile compounds and the antioxidant capacity of coffee brews prepared from commercial conventional and torrefacto-roasted coffees extracted by four brewing procedures (filter, plunger, mocha, and espresso machines). Multivariate statistical techniques were applied to the obtained results.

MATERIALS AND METHODS

Chemicals and Reagents. The methanol used was of spectrophotometric grade from Panreac (Barcelona, Spain). Pure reference standards of pentoxifylline, caffeine, trigonelline, 5-caffeoylquinic acid (5-CQA), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), furan, 2-methylfuran, thiophene, 2-furfurylthiol, furfural, and 5-methylfurfural were purchased from Aldrich (St. Quentin Fallavier, France).

Coffee Samples. Three commercially roasted coffee samples from the same brand were purchased in a local market: a conventionally roasted coffee blend of Arabica/Robusta named as 0, a blend of Arabica/Robusta with 30% torrefacto-roasted coffee (*30*), and a 100% torrefacto-roasted coffee of the Robusta variety (*100*) as whole beans. All were ground, for 40 s, using a home grinder (model Moulinex 980 26-F).

Coffee Brew Preparation. Several coffee-brewing procedures were selected as follows: filter coffee machine, plunger coffeemaker, mocha coffeemaker, and espresso machine.

Filter Coffee Brew. The filter coffee brews (F) were prepared from 24 g of ground-roasted coffee for a volume of 400 mL using a filter coffee machine (model KF 147 aroma Select, Braun). Extraction took 7 min at 90 °C.

Plunger Coffee Brew. The plunger coffee brews (P) were prepared from 40 g of ground-roasted coffee, which was extracted with 500 mL of water at 98 °C. The hot water was added to the coffee powder in the plunger coffeemaker (model Eileen French Press coffee maker, 1 L capacity). The water and the coffee powder were kept in contact for 5 min before the plunger was pushed down slowly.

Mocha Coffee Brew. The mocha coffee brews (M) were prepared from 40 g of ground-roasted coffee for a volume of 500 mL, with the use of a mocha coffeemaker (model Vitro-Fulgor, Valira, Spain). The heating temperature and extraction time were approximately 10 min at 93 °C.

Espresso Coffee Brew. The espresso coffee brews (E) were prepared from 7 g of ground-roasted coffee; the dosage was selected according to Petracco (*24*), for a volume of 40 mL using an espresso coffee machine (model Saeco Aroma, Italy). Fixed espresso coffee-brewing conditions were followed using a pressure in the espresso machine pump equal to 15 bar and extraction times around 30 s at 90 °C.

pH, Total Solids, Extraction, and Concentration. Coffee brew samples were rapidly cooled at 20 °C, and the pH (Orion 420 A Benchtop pH meter) was measured. The total solids were determined by oven drying 40 mL of coffee brew to a constant weight (14 h, 102 ± 3 °C). The extraction was defined as the percentage of total solids with respect to ground-roasted coffee dose. The concentration was defined as the percentage of total solids with respect to the coffee brew volume.

Caffeine and Trigonelline. Extract preparation, cleanup, and high-performance liquid chromatography (HPLC) analysis were already described by Maeztu et al. (*18*). HPLC analysis was achieved with an analytical HPLC unit equipped with a binary pump, an UV-diode array detector (DAD), and an automated sample injector (Hewlett-Packard 1100). A reversed-phase Hypersil-ODS (5 μm particle size, 250 mm × 4.6 mm) column was used. The mobile phase was acetonitrile/water (15:85) in isocratic conditions at a constant flow rate of 2.0 mL min^{-1} at 25 °C. Detection was accomplished with a DAD, and chromatograms were recorded at 280 nm.

5-CQA. Extraction of 5-CQA, cleanup, and HPLC analysis were carried out according to the method of Bichi et al. (*25*). The HPLC equipment was as described above. The conditions of the gradient solvent system used were 100% citrate−acetic acid buffer solution (pH 3.0) for 2 min and 85:15 buffer/methanol for 8 min, both at a flow rate of 0.8 mL min^{-1}, and 85:15 buffer/methanol for 5 min at a flow rate of 1.2 mL min^{-1}. The wavelength of detection was set at 325 nm.

Browned Compounds (Abs 420 nm). Fifty microliters of coffee brews was diluted up to 2 mL with deionized water. Browned compounds were measured by measuring the absorbance of sample at 420 nm after exactly 1 min, in a 3 mL capacity glass 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 measurement was employed as a convenient index of the development of caramelization and MRs.

Color Analysis. Color analysis was carried out on coffee brews using a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan). The instrument was standardized against a white tile before sample measurements. Color was expressed in L^*, a^*, and b^* Cielab scale parameters.

Volatile Compounds. The profiles of volatile compounds were obtained with the method described by Sanz et al. (*26*) adapted to coffee brews. HS-GC analysis was achieved with a fused silica capillary column DB-Wax (J&W Scientific, i. d. 0.25 mm, 60 m, film thickness = 0.5 μm) in a HP 6890 gas chromatograph equipped with a static headspace sampler (Hewlett-Packard model 7694). Mass spectrometry was performed on a mass selective detector model 5973 (Hewlett-Packard) operated in the electron impact mode (70 eV). The mass spectrometer scanned mass from m/z 33 to 300. The ion source temperature was set at 230 °C.

Identification of the Volatile Compounds. The identification of the volatile compounds was carried out by comparison of their mass spectra with those of the pure reference compounds and Wiley library and, in addition, by comparing their retention indices (RIs) with those of standard compounds and data from the literature. Linear RIs of the compounds were calculated using a series of *n*-alkanes injected in the same chromatographic conditions and compared with available literature data.

Quantitative Measurements. The identified coffee aroma compounds were quantified by gas chromatography−mass spectrometry. The areas of the peaks were measured by calculating of the total ionic current. Results from volatile analysis are provided in total area counts × 10^{-6} (**Table 3**).

Antioxidant Capacity. The antioxidant capacity was measured by using the DPPH• decolorization assay (*27*). A 6.1 × 10^{-5} M DPPH• methanol solution was prepared immediately before use. The DPPH• solution was adjusted with methanol to an absorbance of 0.7 (±0.02) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV−vis spectrophotometer, Perkin-Elmer Instruments). Coffee brews were diluted 1:50 in water prior to analysis. Samples (20 μL) were added to the DPPH• solution (1.98 mL). After mixing, the absorbance was measured at 515 nm after exactly 1 min and then every minute for 18 min. Reaction rates were calculated using the equation proposed by Manzocco et al. (*28*)

$$1/\mathrm{Abs}^3 - 1/\mathrm{Abs}_o^3 = -3kt$$

where k is the DPPH• bleaching rate, Abs^3 is the initial absorbance value, and Abs^3 is the absorbance at increasing time, t. The antioxidant capacity was expressed as the slope obtained from the equation (−Abs^{-3} min^{-1}) per mL of sample.

Pro-oxidant Capacity. The pro-oxidant capacity was determined using crocin as a radical quencher, according to the method described by Manzocco et al. (*29*). Crocin was isolated from commercial saffron. Saffron (0.6 g), previously washed with ethyl ether, was extracted with methanol. The crocin solution was adjusted with 0.1 M phosphate buffer (pH 7.0) to an absorbance of 1.8 (±0.02) at 443 nm in a 3 mL capacity cuvette (1 cm length) at 40 °C (Lambda 25 UV−vis spectrophotometer Perkin-Elmer Instruments). Coffee brews were diluted 1:50 in water

Table 1. Effects of Coffee Roasting Process and Coffee Extraction Method on Physicochemical Parameters, on Coffee Volatile Compounds, and on Antioxidant, Pro-oxidant, and Redox Potential Values of Coffee Brews (*F* and *p* Values)

	coffee roasting process effect		coffee brewing procedure effect		interaction effect	
	F	*p*	*F*	*P*	*F*	*p*
pH	147.355	<0.001	581.995	<0.001	80.177	<0.001
total solids	162.621	<0.001	2331.021	<0.001	164.350	<0.001
extraction	176.374	<0.001	1130.557	<0.001	116.163	<0.001
concentration	162.629	<0.001	2331.434	<0.001	164.387	<0.001
caffeine	9426.382	<0.001	4192.187	<0.001	783.780	<0.001
trigonelline	19193.181	<0.001	5891.218	<0.001	4077.738	<0.001
5-CQA	8351.230	<0.001	1203.268	<0.001	187.858	<0.001
browned compounds	1698.596	<0.001	2871.497	<0.001	299.939	<0.001
L	69.941	<0.001	206.977	<0.001	50.277	<0.001
*a**	787.383	<0.001	1668.921	<0.001	519.570	<0.001
*b**	745.887	<0.001	1907.097	<0.001	525.625	<0.001
furan	483.191	<0.001	786.947	<0.001	463.947	<0.001
2-methylfuran	1892.167	<0.001	970.049	<0.001	593.504	<0.001
thiophene	404.055	<0.001	506.394	<0.001	332.904	<0.001
2-methylthiophene	573.515	<0.001	393.782	<0.001	288.683	<0.001
1-mehtyl-1H-pyrrole	1892.863	<0.001	339.832	<0.001	287.328	<0.001
2-furfurylthiol	4893.211	<0.001	678.987	<0.001	658.340	<0.001
furfural	2617.437	<0.001	345.866	<0.001	183.003	<0.001
2-acetylfuran	1761.973	<0.001	629.762	<0.001	812.333	<0.001
pyrrole	204.928	<0.001	226.603	<0.001	341.012	<0.001
5-methylfurfural	1751.560	<0.001	379.224	<0.001	437.292	<0.001
furfuryl alcohol	384.295	<0.001	110.124	<0.001	107.630	<0.001
antioxidant capacity (DPPH)	244.036	<0.001	324.689	<0.001	12.867	<0.001
pro-oxidant activity	745.477	<0.001	306.589	<0.001	144.506	<0.001
redox potential	1419.839	<0.001	1357.750	<0.001	43.556	<0.001

prior to analysis. Samples (20 μL) were added to the crocin solution (1.9 mL) together with phosphate buffer (80 μL). The absorbance was monitored at 443 nm for 10 min. The decrease in absorption at 443 nm, 5 min after the addition of sample, was used for the calculation of the pro-oxidant capacity. The pro-oxidant capacity was expressed as the decrease in crocin absorbance at 443 nm after 5 min of reaction per mL of sample (ΔOD 5 min mL^{-1}).

Redox Potential. The redox potential measurements of the coffee brew were made with a platinum-indicating electrode connected to a voltmeter (model 5261, Crison, Spain). Calibration was performed against 220 and 468 mV redox standard solutions at room temperature (Crison). Electrodes were placed in a 50 mL three-neck flask containing a volume of 16 mL of coffee brew together with 20 mL of deionized water. Prior to analysis, oxygen was removed from the system by continuous nitrogen flushing for a period of 10 min. Millivolt values were recorded for at least 15 min at room temperature, until a stable potential was reached. A stable redox potential was arbitrarily defined as a change of less than 1 mV in a 3 min period.

Statistical Analysis. All of the analyses were performed at least in triplicate. Results are shown as means ± standard deviations. The global effect of the coffee-roasting process and brewing procedure on physicochemical and antioxidant parameters of coffee brew samples was analyzed by a two-way analysis of variance (ANOVA). As interactions occurred between the two variables (**Table 1**), a one-way ANOVA was applied for each brewing procedure. The source of variation was the different type of roasted coffee. Tukey's test was applied as a test a posteriori with a level of significance of 95%. Correlations among variables were assessed by means of the Pearson's correlation test (**Table 4**). Principal component analysis (PCA) was applied to the analytical data (based on the Pearson correlation matrix) to observe differences among coffee brew samples. Statistical analyses were performed using the SPSS version 14.0 software package for Windows.

RESULTS AND DISCUSSION

Physicochemical Parameters in Coffee Brews. A two-way ANOVA was performed to establish the impact of the coffee-roasting process and the brewing procedure on the physicochemical parameters, on the coffee volatile compounds, and on the antioxidant, pro-oxidant, and redox potential values of coffee brew samples (**Table 1**). In all cases, significant interactions between the coffee-roasting process and the coffee-brewing procedure were observed. Thus, the effect of the roasting process was studied deeper in each coffee-brewing procedure. **Table 2** shows the physicochemical parameter results. pH values were in the range of acceptance proposed by other authors (*30*). Total solids and concentration yields depend on both the coffee/water ratio and the brewing procedure used. The higher the coffee dose is, the greater the total solids content is (*31*). As regards the brewing procedure, filter coffee is an infusion method and plunger and mocha coffeemakers work at 0.5−1 bar, respectively; an espresso machine works at a pressure of 15 bar that allows it to extract more total solids.

The percentages of extraction ranging from 18 to 22% have been proposed as the most acceptable; the coffee brews below 16% are considered to be underdeveloped, and those above 24% are considered to be overextracted (*32*). In the present work, the percentages of extraction for coffee brews prepared from the mocha coffeemaker were outside this range. Contrarily to total solids and concentration, extraction was not a dose-dependent parameter (*31*).

Nitrogenous compounds, caffeine and trigonelline, presented higher values in 100 torrefacto coffee brew samples followed by 30 torrefacto coffee brew samples and by conventional ones (0). The increase in both compounds with the percentage of torrefacto-roasted coffee could be explained because torrefacto is a Robusta coffee variety, which presents higher amounts of caffeine and trigonelline than an Arabica variety (*33*), whereas conventionally roasted coffee samples are blends of Arabica/Robusta varieties. 5-CQA values in torrefacto coffee brews were approximately half those of conventional ones. Although Robusta coffee usually has higher amounts of chlorogenic acids (*34*), two factors could contribute to the lower values in the 100 torrefacto coffee brews. On the one hand, the roasting

Table 2. Physicochemical Parameters of Coffee Brews[a]

	filter			plunger			mocha			espresso		
	0	30	100	0	30	100	0	30	100	0	30	100
pH	5.47 ± 0.05 b	5.40 ± 0.00 a	5.40 ± 0.00 a	5.14 ± 0.01 a	5.34 ± 0.00 c	5.27 ± 0.00 b	5.18 ± 0.05 b	5.21 ± 0.00 b	5.01 ± 0.00 a	5.23 ± 0.01 b	5.27 ± 0.00 c	5.11 ± 0.00 a
total solids (mg/mL)	17.24 ± 0.36 b	17.69 ± 0.07 c	16.24 ± 0.30 a	20.00 ± 0.20 c	16.87 ± 0.07 a	19.11 ± 0.16 b	22.99 ± 0.23 a	27.33 ± 0.36 c	25.25 ± 0.55 b	36.48 ± 0.31 b	40.24 ± 1.16 c	27.11 ± 1.92 a
extraction (%)	22.98 ± 0.48 b	23.59 ± 0.09 c	21.65 ± 0.40 a	24.33 ± 0.25 c	21.69 ± 0.08 a	23.24 ± 0.19 b	27.59 ± 0.28 a	30.74 ± 0.40 c	28.40 ± 0.62 b	20.84 ± 0.18 b	22.99 ± 0.66 c	15.48 ± 1.09 a
concentration (%)	1.72 ± 0.04 b	1.77 ± 0.01 c	1.62 ± 0.03 a	2.00 ± 0.02 c	1.69 ± 0.01 a	1.91 ± 0.02 b	2.30 ± 0.02 a	2.73 ± 0.04 c	2.52 ± 0.05 b	3.65 ± 0.03 b	4.02 ± 0.12 c	2.71 ± 0.19 a
caffeine (mg/mL)	0.22 ± 0.00 a	1.05 ± 0.03 b	1.10 ± 0.00 c	0.20 ± 0.00 a	1.12 ± 0.06 b	1.36 ± 0.03 c	0.28 ± 0.00 a	1.59 ± 0.13 b	1.92 ± 0.01 c	0.63 ± 0.01 a	2.41 ± 0.03 b	3.75 ± 0.03 c
trigonelline (mg/mL)	0.00 ± 0.00 a	0.36 ± 0.02 b	1.06 ± 0.00 c	0.00 ± 0.00 a	0.40 ± 0.02 b	1.33 ± 0.02 c	0.00 ± 0.00 a	0.57 ± 0.04 b	1.67 ± 0.06 c	0.00 ± 0.00 a	0.99 ± 0.02 b	5.60 ± 0.13 c
5-CQA (mg/mL)	0.42 ± 0.00 c	0.23 ± 0.00 b	0.22 ± 0.00 a	0.44 ± 0.01 b	0.23 ± 0.00 a	0.24 ± 0.00 a	0.54 ± 0.01 c	0.25 ± 0.01 a	0.27 ± 0.00 b	0.67 ± 0.01 b	0.32 ± 0.01 a	0.31 ± 0.01 a
browned compounds (Abs 420 nm)	0.29 ± 0.01 a	0.34 ± 0.01 b	0.47 ± 0.01 c	0.30 ± 0.00 a	0.38 ± 0.01 b	0.60 ± 0.01 c	0.65 ± 0.02 a	0.68 ± 0.00 b	0.92 ± 0.02 c	0.78 ± 0.02 a	0.84 ± 0.04 a	1.67 ± 0.07 b
L	20.92 ± 0.02b	20.75 ± 0.08 a	21.22 ± 0.05 c	23.16 ± 0.08 b	21.68 ± 0.19 a	21.50 ± 0.25 a	23.34 ± 0.03 c	22.91 ± 0.43 b	22.05 ± 0.18 a	21.20 ± 0.55 a	22.34 ± 0.06 b	20.76 ± 0.12 a
*a**	1.06 ± 0.06 b	0.29 ± 0.02 a	0.30 ± 0.02 a	0.88 ± 0.01 a	0.86 ± 0.05 a	1.07 ± 0.05 b	2.54 ± 0.10 c	2.08 ± 0.06 b	1.02 ± 0.10 a	1.06 ± 0.07 b	2.13 ± 0.04 c	0.74 ± 0.04 a
*b**	0.49 ± 0.01 b	0.82 ± 0.02 c	0.43 ± 0.03 a	0.80 ± 0.03 a	0.99 ± 0.02 b	1.47 ± 0.13 c	3.22 ± 0.05 c	2.90 ± 0.03 b	1.10 ± 0.17 a	1.03 ± 0.11 b	2.24 ± 0.04 c	0.63 ± 0.05 a

[a] All values are shown as means ± standard deviations ($n = 6$). In each row, different superscripts indicate significant differences ($p < 0.05$) among different roasted coffees in each brewing procedure.

process could produce a greater decrease in these compounds in Robusta coffee than in Arabica coffee (*35*). On the other hand, chlorogenic acids could react in the early stage of the MR by donation of a carbonyl residue (*36*).

With respect to the coffee-brewing procedure, and as was observed by Andueza et al. (*31*), caffeine, trigonelline, and 5-CQA contents increased when the coffee/water ratio was higher. As was previously discussed, the different pressure conditions of the brewing procedure may also play an important role.

Browned compounds are measured as a convenient index of the development of caramelization and MRs. As can be observed, the greatest production of browned compounds took place in the 100 torrefacto coffee brew samples; intermediate values were reported in the 30 torrefacto coffee brew samples, and the lowest values were in the conventional ones (0). Beyond the effect that the addition of sugar might have on the rate of MRs and, consequently, in the development of MRPs such as melanoidins, the caramelization of sugar contributes to the brownish color of the torrefacto coffee brews. With respect to the brewing procedure, the increases of the coffee/water ratio and the pressure employed by each coffeemaker are positively associated with the browned compounds measured.

Volatiles in Coffee Brews. Different authors (*13*−*15*) examined the antioxidant activities of pure volatile compounds that can be developed in MRs and presented a series of potential antioxidant volatile compounds. In the present study, the HS-GC/MS analysis of volatile compounds present in coffee brews showed 11 of these volatile compounds previously reported as antioxidants by the cited authors (**Table 3**). In general, the volatile compounds of coffee brews from conventionally roasted coffee were higher than in the 100 torrefacto coffee brews. Moreover, pressure-brewing procedures, such as espresso and mocha, extracted more volatiles than plunger and filter procedures. Similar results were observed by López-Galilea et al. (*19*). Among the identified furans, 2-methylfuran has been observed as a powerful antioxidant at high concentrations, exhibiting a dose-dependent activity (*14*). Nevertheless, in our study carried out in coffee brews, the presence of 2-methylfuran was not significantly correlated with antioxidant capacity parameters measured by DPPH$^{\bullet}$ and redox potential methods ($r = 0.179$ and -0.091, respectively) (**Table 4**). In the same way, furan, 2-acetylfuran, and furfuryl alcohol, which have been previously observed as antioxidants (*13*, *14*, *37*), did not present significant correlations with antioxidant capacity parameters with *r* values ranging from 0.476 to −0.345 in all cases. Following the literature data, pyrroles and thiophenes related with antioxidant capacity could be expected to be observed (*13*, *14*). However, results from the present study showed that 1-methyl-1H-pyrrole and pyrrole, as well as thiophene and 2-methylthiophene, were not related with antioxidant parameters (*r* values ranging from 0.050 to 0.395 for DPPH$^{\bullet}$ results and from −0.279 to 0.062 for redox potential results). Consequently, the antioxidant capacity of the volatile compounds reported by other authors in model systems may be attributed to the high levels of volatile compounds assayed in those experiments (concentration ranging from 5 to 500 μg/mL), considerably higher than levels present in coffee brews (ranging from micrograms to milligrams/kilogram). Furthermore, in a recent study conducted with ground-roasted coffee, a significant correlation between the antioxidant capacity of ground-roasted coffee and the volatile compounds was not shown (*38*).

Table 3. Chromatographic Areas ($\times 10^{-6}$) of Volatile Compound Identified in Coffee Brews[a]

			filter			plunger			mocha			espresso		
RI[b]	ID[c]		0	30	100	0	30	100	0	30	100	0	30	100
716	A	furan	1.91 ± 0.07 b	1.92 ± 0.07 b	1.29 ± 0.04 a	3.55 ± 0.05 c	1.30 ± 0.02 a	2.72 ± 0.14 b	4.32 ± 0.13 b	1.08 ± 0.04 a	5.39 ± 0.01 c	5.22 ± 0.29 c	4.76 ± 0.16 b	2.50 ± 0.06 a
832	A	2-methylfuran	5.97 ± 0.39 b	6.26 ± 0.23 b	3.41 ± 0.11 a	11.35 ± 0.42 c	3.86 ± 0.04 a	6.91 ± 0.03 b	18.02 ± 0.24 c	3.88 ± 0.07 a	12.48 ± 0.32 b	14.12 ± 0.15 c	10.70 ± 0.26 b	5.22 ± 0.28 a
1021	A	thiophene	0.23 ± 0.01 b	0.26 ± 0.01 c	0.16 ± 0.01 a	0.41 ± 0.01 c	0.16 ± 0.00 a	0.33 ± 0.03 b	0.62 ± 0.01 b	0.17 ± 0.00 a	0.73 ± 0.04 c	0.52 ± 0.02 c	0.43 ± 0.02 b	0.24 ± 0.01 a
1097	B	2-methylthiophene	0.06 ± 0.00 b	0.06 ± 0.00 b	0.04 ± 0.00 a	0.11 ± 0.01 c	0.04 ± 0.00 a	0.07 ± 0.00 b	0.18 ± 0.01 c	0.03 ± 0.00 a	0.16 ± 0.01 b	0.12 ± 0.00 c	0.10 ± 0.00 b	0.05 ± 0.00 a
1149	B	1-mehtyl-1H-pyrrole	0.26 ± 0.01 c	0.13 ± 0.00 b	0.08 ± 0.01 a	0.26 ± 0.01 c	0.08 ± 0.00 a	0.18 ± 0.00 b	0.42 ± 0.01 c	0.07 ± 0.00 a	0.29 ± 0.00 b	0.48 ± 0.02 c	0.23 ± 0.00 b	0.09 ± 0.00 a
1472	A	2-furfurylthiol	0.03 ± 0.00 a	ND	0.33 ± 0.01 b	ND	ND	0.31 ± 0.01 a	ND	ND	0.10 ± 0.00 a	0.02 ± 0.00 a	ND	0.05 ± 0.00 b
1490	A	furfural	0.62 ± 0.02 b	0.34 ± 0.01 a	0.64 ± 0.04 b	0.70 ± 0.01 b	0.13 ± 0.00 a	0.69 ± 0.01 b	0.89 ± 0.03 c	0.12 ± 0.00 a	0.54 ± 0.01 b	1.17 ± 0.01 c	0.48 ± 0.01 a	0.65 ± 0.01 b
1536	B	2-acetylfuran	0.08 ± 0.00 c	0.05 ± 0.00 a	0.06 ± 0.00 b	0.09 ± 0.00 c	0.01 ± 0.00 a	0.05 ± 0.00 b	0.12 ± 0.01 b	0.01 ± 0.00 a	0.21 ± 0.00 c	0.15 ± 0.01 c	0.06 ± 0.00 b	0.04 ± 0.00 a
1542	B	pyrrole	0.19 ± 0.01 b	0.21 ± 0.01 c	0.12 ± 0.01 a	0.20 ± 0.00 c	0.08 ± 0.00 a	0.15 ± 0.01 b	0.24 ± 0.01 b	0.07 ± 0.00 a	0.39 ± 0.02 c	0.37 ± 0.02 c	0.28 ± 0.01 b	0.12 ± 0.01 a
1605	A	5-methylfurfural	0.41 ± 0.01 c	0.21 ± 0.00 a	0.32 ± 0.01 b	0.46 ± 0.01 c	0.08 ± 0.00 a	0.29 ± 0.01 b	0.61 ± 0.02 b	0.08 ± 0.00 a	0.85 ± 0.04 c	0.76 ± 0.02 b	0.28 ± 0.01 a	0.24 ± 0.02 a
1686	B	furfuryl alcohol	0.52 ± 0.01 c	0.38 ± 0.00 b	0.36 ± 0.01 a	0.56 ± 0.03 c	0.13 ± 0.00 a	0.31 ± 0.03 b	0.72 ± 0.04 b	0.16 ± 0.01 a	0.85 ± 0.07 c	0.93 ± 0.07 c	0.48 ± 0.00 b	0.33 ± 0.00 a

[a] All values are shown as means ± standard deviations ($n = 3$). In each row, different superscripts indicate significant differences ($p < 0.05$) among different roasted coffees in each brewing procedure. [b] Retention index determined on HP-Wax capillary column. [c] Identification proposals are indicated by the following: A, mass spectrum agreed with standards injected in the same conditions; B, tentative identification by comparing mass spectrum with Wiley mass spectral database and retention indexes with literature data. ND, not detected.

Table 4. Pearson Correlation Coefficients between Volatile and Nonvolatile Compounds and Antioxidant Capacity (Measured by DPPH• and Redox Potential Methods) in Coffee Brews[a]

coffee brew compound	DPPH•	redox potential
caffeine	0.826*	−0.844*
trigonelline	0.744*	−0.767*
5-CQA	−0.057	0.140
browned compounds	0.916*	−0.908*
furan	0.476	−0.345
2-methylfuran	0.179	−0.091
thiophene	0.395	−0.279
2-methylthiophene	0.238	−0.136
1-methyl-1H-pyrrole	0.050	0.062
2-furfurylthiol	0.066	−0.145
furfural	0.130	−0.073
2-acetylfuran	0.312	−0.169
pyrrole	0.317	−0.168
5-methylfurfural	0.237	−0.105
furfuryl alcohol	0.195	−0.054

[a] The symbol * indicates significance at the 0.01 probability level.

Antioxidant and Pro-oxidant Parameters of Coffee Brews. The antioxidant capacity was measured by a chain-breaking activity that allows one to evaluate the quenching rate of coffee compounds toward a reference radical (DPPH•). The results are shown in **Figure 1**. The antioxidant capacity of coffee brews was increased with the percentage of torrefacto coffee. These results, in agreement with Sánchez-González et al. (*21*), could be explained by the enhancement of MRs with sugar additions in the torrefacto-roasting process. MRPs, like melanoidins, are the prevailing contributors to the maintenance of the antioxidant activity of coffee brews (*5*). Thus, highly significant ($p < 0.01$) and excellent ($r > 0.75$) correlations between both antioxidant capacity parameters (DPPH• and redox potential) and browned compound contents (0.916 and −0.908, respectively) were found in coffee brews (**Table 4**). As was observed by other authors (*39*, *40*), the development of browned compounds is associated with an increase in the antioxidant properties in systems where MRs are the prevalent reactions. There were significant correlations between the caffeine content and the antioxidant capacity measured by the DPPH• method (0.826) and the redox potential (−0.844), suggesting that caffeine was likely a significant contributor to the antioxidant capacity in the coffee brews. The role of caffeine as an antioxidant compound has been previously discussed. While Devasagayam et al. (*4*) observed caffeine as an antioxidant compound, a work carried out with ground-roasted coffee has not observed caffeine positively correlated with antioxidant capacity (*38*). Moreover, Parras et al. (*22*) showed different decaffeinated coffee brew behaviors depending on the free radical scavenging assay used. With regard to the coffee-brewing procedure, the order of antioxidant capacity established in all coffee brew samples tested was espresso > mocha > plunger > filter and pointed out the relevance of both the coffee/water ratio and the pressure of the coffeemaker.

The pro-oxidant capacity was evaluated by the crocin-bleaching test, and these results are shown in **Figure 2**. The assessment of the pro-oxidant activity by crocin bleaching allows one to evaluate the quenching rate of coffee compounds toward a reference radical quencher (crocin). As can be observed in **Figure 2**, the influence of the coffee-roasting process in pro-oxidant capacity is noticeably higher than the coffee-brewing procedure. In agreement with antioxidant capacity results, lower values of pro-oxidant capacity in torrefacto coffee brews were observed in all brewing procedures. According to Manzocco et

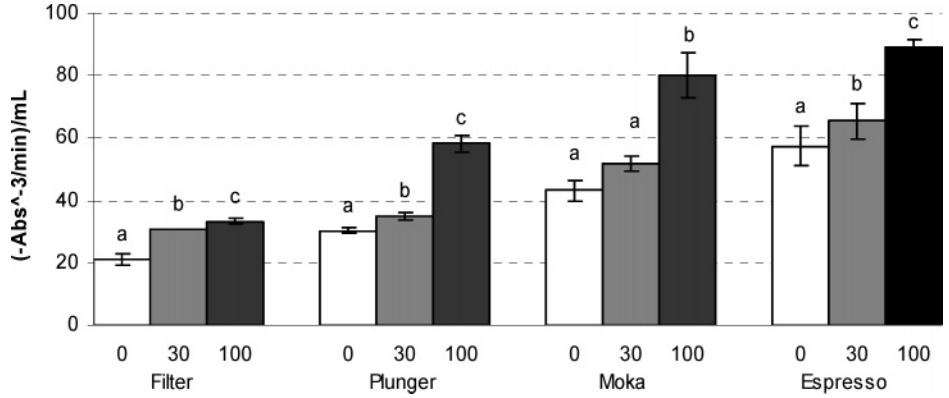

Figure 1. Antioxidant capacity of coffee brews (measured by DPPH• method). All values are shown as means ± standard deviations ($n = 6$). Different letters indicate significant differences ($p < 0.05$) among different roasted coffees in each brewing procedure.

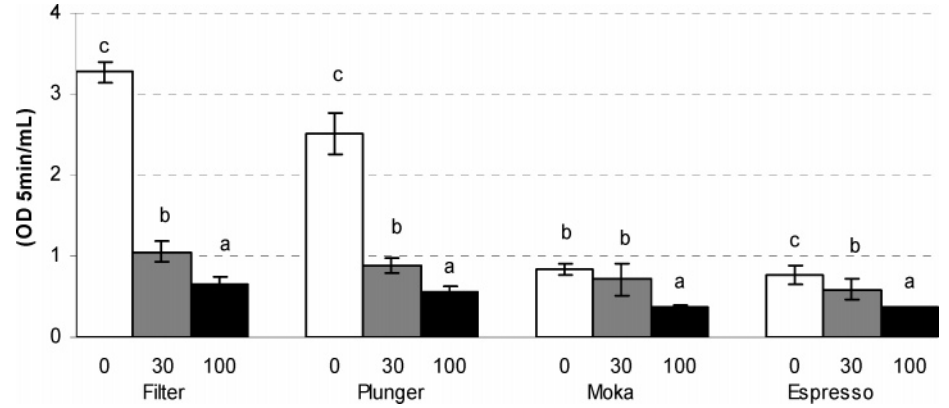

Figure 2. Pro-oxidant capacity of coffee brews. All values are shown as means ± standard deviations ($n = 6$). Different letters indicate significant differences ($p < 0.05$) among different roasted coffees in each brewing procedure.

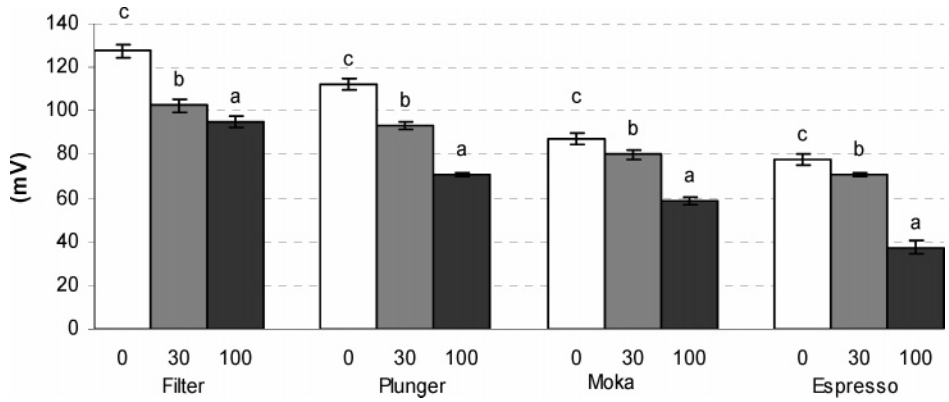

Figure 3. Redox potential values of coffee brews. All values are shown as means ± standard deviations ($n = 6$). Different letters indicate significant differences ($p < 0.05$) among different roasted coffees in each brewing procedure.

al. (*29*), highly reactive radicals are formed in the early phases of the MR whereas strong antiradical properties are attributable to the high molecular weight brown compounds formed in the advanced phases of the reaction. Therefore, when the roasting process has been completed, pro-oxidant radicals could be quenched by MRPs, decreasing the pro-oxidant activity with the torrefacto roast.

The balance between antioxidant and pro-oxidant activities is expressed by the redox potential. The results can be observed in **Figure 3**. With regard to the coffee-roasting process, significant differences among coffee brews were reported. According to the higher antioxidant capacity, a marked tendency to decrease the redox potential values with the increase of torrefacto coffee was observed. In terms of coffee-brewing procedure, the different samples were sorted according to their redox potential values as follows, in a decreasing order: filter > plunger > mocha > espresso, opposite to the brewing procedure order established for antioxidant capacity values.

PCA was applied to examine relationships between antioxidant capacity parameters and chemical compositions of coffee brew samples. Four principal components (PC) with eigenvalues greater than 1 and explaining 91% of the total variance of the data were obtained. **Figure 4** shows the bidimensional representation for all of the variables and coffee samples defined by the two first PCs. PC1, which explained 49.2% of the total variance, is mainly characterized by coffee aroma compounds.

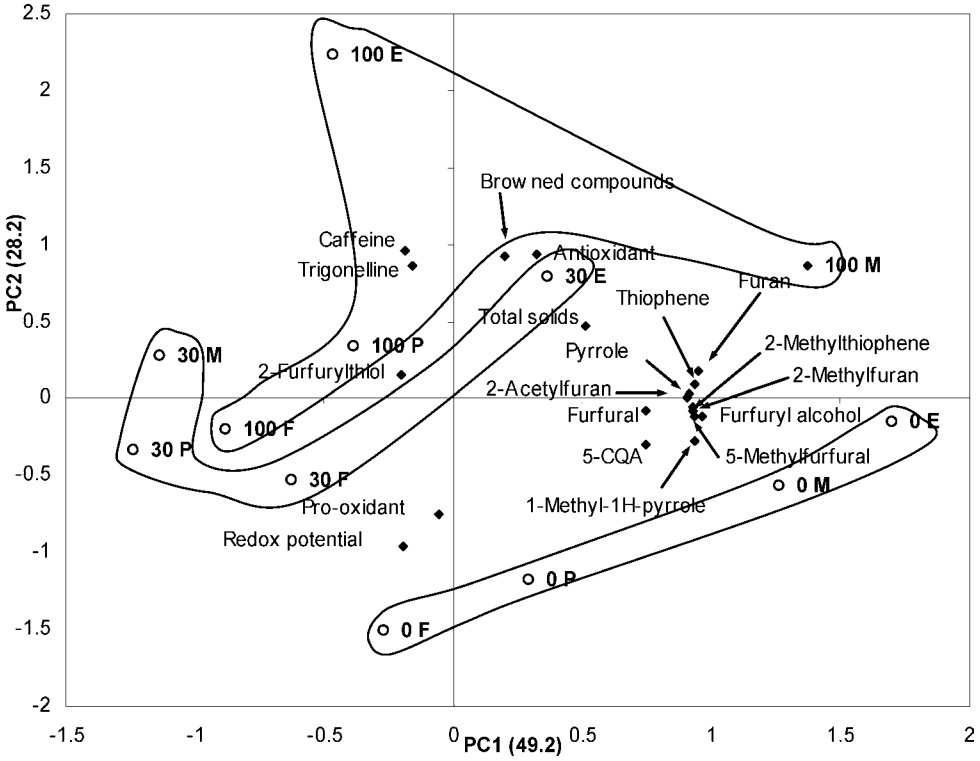

Figure 4. PCA of the coffee brews.

As can be seen, coffee brews from conventionally roasted coffees are, in almost all cases, found on the positive values of PC1 (right half of the graphic), whereas torrefacto coffee brews are mainly placed on the negative half of the graphic. In fact, as previously observed by López-Galilea et al. (*19*), coffee brews extracted from torrefacto ground-roasted coffee were less aromatic than conventional ones.

PC2, which explained 28.2% of the total variance, is mainly characterized by antioxidant capacity (DPPH$^{\bullet}$ and redox potential), pro-oxidant capacity, browned compounds, trigonelline, and caffeine. Coffee brews from torrefacto or blend torrefacto/conventional roasted coffee are generally localized on the top showing the highest antioxidant capacity. Coffee brews from conventionally roasted coffee are mainly placed on the bottom of the graphic because of their lowest antioxidant capacity.

In this paper, correlations between the volatile and nonvolatile compounds and the antioxidant capacity of coffee brews prepared from different commercially roasted coffees (conventional vs torrefacto) and prepared by four commonly used brewing procedures (filter, plunger, mocha, and espresso machines) were assessed. No significant correlations between coffee brew volatile compounds, previously reported as potential antioxidant compounds (*13*−*15*), and antioxidant capacities of coffee brews were observed. Highly significant correlations between browned compounds and caffeine with the antioxidant capacity parameters were reported. PCA allowed coffee brew separations according to coffee-roasting processes (PC1) and brewing procedures (PC2), showing that in all cases coffee brews from torrefacto coffees were more antioxidant that those extracted from conventional ones; also, coffee brews extracted by espresso machines were more antioxidant than those extracted by mocha, plunger, and filter ones. Thus, a combination of torrefacto-roasted coffee and an espresso machine results in a beverage possessing a great antioxidant capacity.

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