

Influence of Brewing Method and Acidity Regulators on the Antioxidant Capacity of Coffee Brews

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The antioxidant capacity of coffee brews prepared with different coffeemakers (filter, plunger, mocha, and espresso) was measured by colorimetric (total phenolic compounds and ABTS) and electron spin resonance (ESR) spectroscopy techniques (Fremy's salt and TEMPO). The mocha coffeemaker had the highest yield in coffee antioxidant extraction per gram of ground roasted coffee, but espresso coffee was richest in terms of antioxidant intake (per milliliter of coffee brew) followed by mocha, plunger, and filter. Both Folin–Ciocalteu (total phenolic compounds) and ABTS assays reacted with standard solutions of chlorogenic acids (CGA) and melanoidins (MO-Ala and MO-Gly). However, Fremy's salt was mainly scavenged by chlorogenic acids, whereas the stabilized radical TEMPO was effectively scavenged by melanoidins, but not by chlorogenic acids. Thus, ESR spectroscopy allows distinguishing between phenolic and nonphenolic antioxidants. Moreover, the addition of pH-regulator agents to coffee, such as sodium carbonate (75 ppm) and bicarbonate (75 ppm), to extend its shelf life, slightly increases the pH, modifying the antioxidant capacity in those coffee brews with the highest capacity (mocha and espresso).

KEYWORDS: Coffee; antioxidants; phenolics; chlorogenic acids; melanoidins; additives; coffee brews

INTRODUCTION

Coffee is the second most consumed brew in the world, not only due to its pleasant taste and aroma but also because of its stimulating qualities. During the past few years, evidence of the healthy benefits (1) and the important contribution of coffee brew to the intake of antioxidants in the diet (2–4) has helped to increase coffee consumption. Several coffee compounds have been proposed as antioxidants. Some of them are phenolic compounds, such as chlorogenic and hydroxycinnamic acids, originally present in green coffee, which remain in smaller amounts in roasted coffee (5). Others, such as melanoidins and other Maillard reaction products (MRP), are developed during roasting (6–9). Other coffee components, such as caffeine (10) and volatile compounds (11–13), are also proposed as antioxidants in model systems, but their antioxidant activity is unclear in the coffee matrix (14). Moreover, during the past few years, some works have proposed that coffee melanoidins incorporate chlorogenic and hydroxycinnamic acids, contributing to the antioxidant capacity of coffee brews (15). At the same time, although the antioxidant capacity usually prevails in foods, it should be taken into account that some antioxidants and their decomposition products might also act as pro-oxidants depending upon the conditions (16).

The antioxidant activity of coffee has been studied using different methods. Traditionally, the methodologies were searching for the

evaluation of the overall antioxidant capacity, such as the Folin–Ciocalteu colorimetric method to routinely measure the total phenolic compounds. Other colorimetric reactions were developed to evaluate the ability of a food compound or product to quench and/or reduce radicals or metals using DPPH or ABTS radicals or the FRAP assay, respectively. The antioxidant capacity is normally compared to that of a very well-known antioxidant, such as vitamin E, using Trolox (a water-soluble vitamin E analogue). For this reason, these methodologies are usually called Trolox equivalent antioxidant capacity (TEAC) assays. These colorimetric methodologies are currently the most widespread, but all of them are based on similar electron-transfer redox reactions and evaluate more than phenolic compounds (17). During the past few years, a new methodology, electron spin resonance (ESR) spectroscopy, has been proposed as a much more specific technique, capable of distinguishing the contribution of phenolic and nonphenolic antioxidants in coffee by means of different stabilized radicals (18). The basis of ESR spectroscopy is the absorption of microwave energy by unpaired electrons, such as those of free radicals, when they are in a magnetic field. This technique has been frequently applied to measure the antioxidant capacity of a substance to scavenge a stabilized radical, such as Fremy's salt and TEMPO (19).

The antioxidant capacity of coffee can be influenced by several factors, such as the variety and origin of coffee (20), the roasting degree (6), the type of roast (natural or torrefacto) and their blends (14, 21), and the technological parameters applied for coffee brew extraction. The preparation of a cup of coffee can be

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made by several extraction methods that extract the potential antioxidants of coffee in different amounts (22). The most popular coffee brew preparation is the filter one, but during the past few decades the consumption of espresso coffee has increased. Moreover, in southern European countries such as Italy and Spain, the use of the mocha coffeemaker is much extended at the domestic level, and the plunger coffeemaker is being used more often for coffee aroma lovers (23). In each case, the technical conditions applied, such as the coffee/water ratio, water temperature, and water pressure, also contribute to the different chemical compositions of coffee brews (24–27).

In ready-to-drink coffee beverages, the preservation conditions (temperature, oxygen, etc.) could influence the degradation of phenolics and other antioxidants (28) and the antioxidant capacity (29). However, because of the sensory quality decrease and the limited shelf life of the coffee brew, other ingredients, such as milk derivatives or additives, are usually added. In a previous work carried out by our research group, the addition of certain pH-regulator agents to a black coffee has been proposed to extend shelf life (30), but the influence of these additives on the antioxidant capacity of ready-to-drink coffee brews has not yet been evaluated.

For all of these reasons, the aims of this work were (1) to compare the antioxidant capacity, measured by colorimetric and ESR techniques, of the coffee brews prepared with different coffeemakers (filter, plunger, mocha, and espresso) and to investigate the main responsible groups of antioxidants and (2) to study the effect of the addition of some acidity correctors on the antioxidant capacity of each coffee brew.

MATERIALS AND METHODS

Coffee. Vacuum-packed Colombian Arabica ground roasted coffee (2.25% water content, $L^* = 19.57 \pm 0.09$) was provided by a local factory and stored in darkness and at room temperature until the coffee brew preparation, < 1 month after roasting. The L^* value was analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in an 1 cm Petri plate, and the L^* value was measured in triplicate and on the CIELab scale.

Chemicals and Reagents. The methanol used was of spectrophotometric grade from Panreac (Barcelona, Spain). Pure reference standards of chlorogenic acids (CGAs) were obtained from Roth (Karlsruhe, Germany), gallic acid was obtained from Serva (Heidelberg, Germany), and TEMPO (2,2,6,6-tetramethyl-1-piperidin-1-oxyl), Fremy's salt (potassium nitrosodisulfonate), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (Steinheim, Germany). 2,2'-Azinobis(3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS) was also obtained from Sigma-Aldrich. Sodium carbonate and sodium bicarbonate were purchased from Panreac.

Preparation of Melanoidins. Melanoidins were prepared according to the method of Caemmerer and Kroh (31). As a carbonyl compound a maltooligosaccharide mixture DP 3–10 (MO) (Merck) was used, along with the amino acids D,L-alanine (Ala) (Fluka) or glycine (Gly) (Serva). For preparation of the melanoidins, 50 g of carbohydrate and 5 g of the amino acids were mixed thoroughly in solid state, and aliquots of 5 g were heated at 170 °C for 6 h (MO-Ala) and 10 h (MO-Gly), respectively. MO-Ala was dialyzed and freeze-dried according to the method of Caemmerer et al. (32). Finally, melanoidins were dissolved at 3.6 g/L with distilled water.

Coffee Brew Preparation. Four coffee-brewing procedures were selected: filter coffee machine, plunger coffeemaker, mocha coffeemaker, and espresso machine. The coffee brews were prepared as described by Lopez-Galilea et al. (22). The ground coffee packages were opened immediately before the preparation of the coffee brews to avoid oxidative damage.

The filter coffee brew was prepared from 24 g of ground roasted coffee for a volume of 400 mL, using a filter coffee machine (model K108, AKA). Extraction took 7 min at 90 °C.

The plunger coffee brew was prepared from 40 g of ground roasted coffee, which was extracted with 500 mL of water at 98 °C, using 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.

The mocha coffee brew was prepared from 40 g of ground roasted coffee for a volume of 500 mL, using a mocha coffeemaker (model Vitro-Fulgor, Varila, Spain). The heating temperature and extraction time were approximately 10 min at 93 °C.

The espresso coffee brew was prepared from 7 g of ground roasted coffee, for a volume of 40 mL, using an espresso machine (model Saeco Aroma, Italy). The water pump pressure was 15 bar, and the extraction time and temperature were approximately 30 s at 90 °C.

For the study of the influence of additives on the antioxidant capacity of coffee, three aliquots of each coffee brew were separated: one as reference, the second with the addition of 75 ppm of sodium carbonate, and the third with the addition of 75 ppm of sodium bicarbonate. The selection of the additives and their concentrations were reported in a previous paper (33).

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

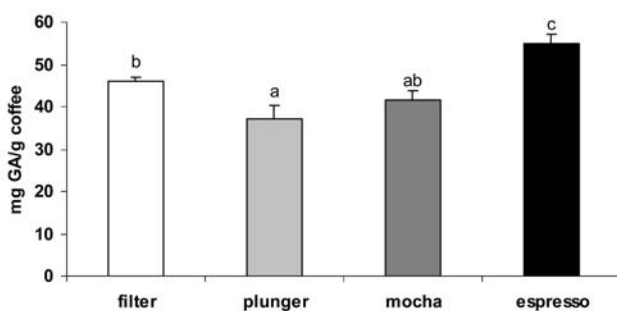
Total Phenolic Compounds. Total phenolic compounds were measured using the Folin–Ciocalteu reagent according to Singleton's method (34) and then calculated using gallic acid as standard. For every coffee sample, 1:10 dilutions with demineralized water were prepared, and 2.5 mL of Folin–Ciocalteu reagent (Merck) was added to 500 μ L of the coffee sample solution. After a 2 min delay, 2 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated at 50 °C for 15 min, cooled for 4 min in ice, and tempered at 25 °C during 5 min. The absorbance of the sample was measured at 760 nm in a spectrophotometer (Pharmacia LKB Biochrom, Germany). Gallic acid (GA) was used as reference, and the results were expressed as milligrams of GA per milliliter of coffee brew or per gram of ground coffee.

Determination of Antioxidant Capacity by ABTS Assay. The ABTS method for coffee brews was performed according to the method of Rohn et al. (35). The radicals $ABTS^{\bullet+}$ were generated by the addition of potassium persulfate to an ABTS solution prepared in phosphate-buffered saline (pH 7.4). An aliquot of 0.1 mL of each coffee brew sample diluted with demineralized water (1:200) was added to 0.5 mL of 0.5 mM ABTS solution. After the addition of 0.2 mL of potassium persulfate, the absorbance was then measured spectrophotometrically at 734 nm in a spectrophotometer (Analytic Jena, Jena, Germany), after exactly 6 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue), and total antioxidant capacity was expressed as micromoles of Trolox per milliliter of coffee brew or per gram of ground coffee.

Determination of 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. (36) and modified by Caemmerer and Kroh (18). For the investigation with Fremy's salt, 100 μ L of every coffee brew sample 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 μ L of coffee brew sample were allowed to react with 100 μ L 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 milliliter of coffee brew or per gram of ground coffee.

Statistical Analysis. Each parameter was analyzed in triplicate. Results are shown as means \pm standard deviations. A two-way analysis of variance (ANOVA) was performed to establish the impact of both the extraction method and the additive (sodium carbonate and bicarbonate) on antioxidant capacity parameters of coffee brew samples (Table 1). When interactions were significant, a one-way ANOVA was applied. A T-Tukey 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

a) Total Phenolic compounds



b) ABTS antioxidant capacity

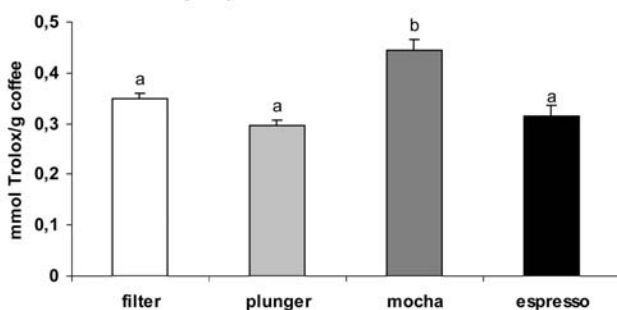


Figure 1. Total phenolic compounds (a) and ABTS antioxidant capacity (b) extracted from ground coffee by filter, plunger, mocha, and espresso methods. Bars with different letters are significantly different ($p < 0.05$).

correlation test. All statistical analyses were performed using the SPSS v.15.0 software package.

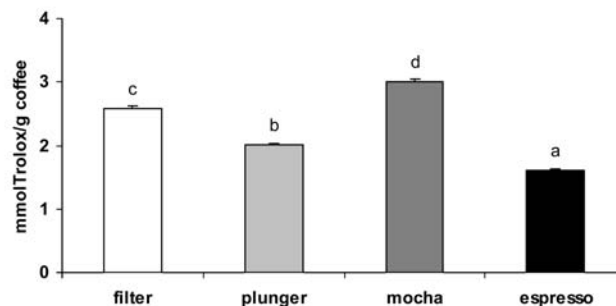
RESULTS AND DISCUSSION

Influence of Coffee Extraction Method. The effects of the application of different extraction methods for preparing a coffee brew on the antioxidant capacity measured by colorimetric assays (Folin–Ciocalteu and ABTS) and ESR (Fremy's salt and TEMPO) are shown in **Figures 1** and **2**, respectively. To determine the efficiency of each brewing method for extracting the potential antioxidants of coffee, the results were expressed per gram of ground roasted coffee.

The Folin–Ciocalteu assay is traditionally used for quantifying the total phenolic compounds in foods. Although the exact chemical nature of the Folin–Ciocalteu reagent is not known, it is believed that the molybdenum in the complex can be easily reduced by an electron-transfer reaction. Thus, the Folin–Ciocalteu reagent can be reduced by many electron-donor compounds (17). Total phenolic compounds of the coffee brews were in the range of 37–55 mg of GA/g of coffee (**Figure 1a**), similar to those results reported by other authors (37, 38).

The radical monocation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) generated by oxidation of ABTS with potassium persulfate is a stable free radical with strong absorption in the range of 600–750 nm. The radical cation reacts energetically with a hydrogen donor, such as phenolic compounds, being converted into a noncolored form of ABTS. The decay of the radical cation caused by the presence of antioxidants in a sample is monitored by the decolorization at 734 nm in a spectrophotometer and compared to those of a Trolox solution. **Figure 1b** shows the results of the ABTS assay of the coffee brews. The results were in the range of 0.296–0.445 mmol of Trolox/g of coffee, slightly higher than those reported by Brezova et al. (37), but 3–4-fold lower than those observed by Sanchez-Gonzalez et al. (38). This discrepancy could probably be due to the different coffee/water ratios and other technological parameters, but also

a) Fremy's Salt



b) TEMPO

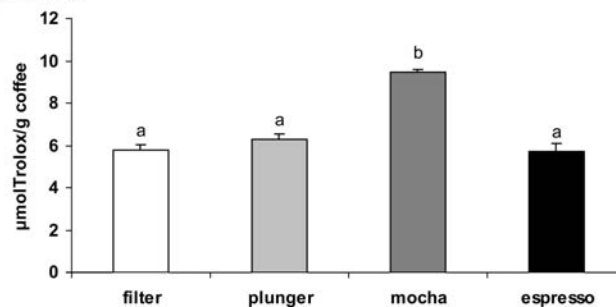


Figure 2. Fremy's salt (a) and TEMPO (b) antioxidant capacity extracted from ground coffee by filter, plunger, mocha, and espresso methods. Bars with different letters are significantly different ($p < 0.05$).

possibly the influence of the coffee type or blend. Thus, as reported by Lopez-Galilea et al. (21), blends with Torrefacto roasted coffee (roasted with sugar) showed higher antioxidant capacity than conventional roasted coffee. In this work, the coffee used was Colombian Arabica conventionally roasted coffee, whereas the coffee selected by Sanchez-Gonzalez et al. (38) was a blend with 30% of Torrefacto coffee.

As already stated, the basis of ESR spectroscopy is the absorption of microwave energy by unpaired electrons when they are in a magnetic field. This technique has been used for detecting and identifying free radicals generated by chemical reactions, including *in vivo*, but it is a rapid technique that is highly suitable for measuring the antioxidant capacity of a substance or product to scavenge a stabilized radical. The use of different radicals permits us to distinguish between antioxidants with different structural properties (19). A wide range of antioxidants, mainly phenolic compounds, can be detected when Fremy's salt is used as a stabilized radical, whereas TEMPO could be mainly scavenged by Maillard reaction products, such as melanoidins (18, 39). The scavenging of two different synthetic stabilized radicals, Fremy's salt (potassium nitrosodisulfonate) and TEMPO (2,2,6,6-tetramethyl-1-piperidin-1-oxyl), in coffee brews prepared with different extraction methods and measured by ESR spectroscopy is shown in **Figure 2**. The results were in the range of 1.6–2.6 mmol of Trolox/g of roasted coffee and 5.7–9.5 μmol of Trolox/g of ground roasted coffee in Fremy's salt and TEMPO antioxidant capacity. The TEMPO antioxidant capacity level was much lower than the antioxidant capacity level of Fremy's salt. This coincides with Bekedam et al. (39), who reported that the contribution of the roasting-induced antioxidants to the overall antioxidant capacity is rather limited and that phenolic antioxidants evaluated by Fremy's salt dominate the overall antioxidant capacity of coffee brews.

Several technological factors play an important role in the extraction of the antioxidant compounds. In previous works, Lopez-Galilea et al. (22) and Andueza et al. (24, 27) observed that the concentration of 5-caffeoylquinic acid, the most abundant

phenolic compound in coffee, and melanoidins, measured by the absorbance at 420 nm, increased with pressure and coffee/water ratio. The espresso coffeemaker had the highest water pressure of the coffeemakers (15 bar maximum pump power) and the highest coffee/water ratio (7 g/40 mL). Plunger and mocha are also pressure coffee extraction methods that work at 0.01–0.5 and 0.5 relative atmospheres, respectively (23), with the same coffee/water ratio (40 g/500 mL). Filter coffee is an infusion method obtained by gravity at atmospheric pressure where hot water flows through medium-ground roasted coffee (24 g/400 mL), allowing a longer contact between water and coffee particles. The extraction time, ranking from the fastest one, which is espresso (30 s), to the slowest one, which is mocha (10 min), through plunger (5 min) and filter (7 min), could also influence the extraction of soluble potential antioxidants, together with other technological factors (water temperature, etc.).

It was observed that the mocha coffeemaker extracted the highest amount of antioxidants per gram of ground roasted coffee, followed by the filter, espresso, and then plunger coffeemakers, in all antioxidant capacity parameters, except total phenolic compounds. Other authors also reported higher ABTS antioxidant capacity in filter and mocha coffees than in espresso (20, 38). However, there was apparently a discrepancy with the results of DPPH antioxidant capacity obtained by Lopez-Galilea et al. (22) that proposed the following order: filter < plunger ≤ mocha ≤ espresso. Despite the fact that DPPH and ABTS assays might show different results due to the use of different radicals, when results of the previous work (22) were expressed per gram of ground roasted coffee instead of per milliliter of coffee brew, DPPH antioxidant capacity maintained the same order and proportion as ABTS in this present work, showing that the mocha coffeemaker extracted the highest DPPH reducing antioxidants. Consequently, although the highest water pressure in espresso coffeemaker favors antioxidant extraction, the shortest water–coffee contact time and the highest coffee/water ratio decrease the extraction efficiency (per gram of ground roasted coffee) versus the other coffee brew techniques. In addition to the technological aspects of the coffee brew preparation, some considerations regarding techniques for the measurement of antioxidant capacity should be made to find possible explanations for the different order of coffee extraction methods obtained with the total phenolic compound results measured by the Folin–Ciocalteu technique (Figure 1a) versus the other antioxidant capacity parameters.

Elucidating the Antioxidants Extracted in Coffee Brews. To elucidate the main responsible coffee antioxidants evaluated by the colorimetric assays (total phenolic compounds and ABTS) and the ESR (Fremy's salt and TEMPO) and to attempt to clarify the discrepant results previously observed, three standard solutions (3.6 g/L) of chlorogenic acids (CGA), one dialyzed and one nondialyzed melanoidins (MO-Ala and MO-Gly, respectively) were analyzed. Both melanoidins were selected to compare the antioxidant capacity of the high molecular weight melanoidins (dialyzed MO-Ala) with the antioxidant capacity of a mixture of low, medium, and high molecular weight Maillard reaction products (nondialyzed MO-Gly). Figures 3 and 4 show the results obtained.

As shown in Figure 3a, although the colorimetric reaction was much higher in the CGA solution (0.42 mg of GA/mL), the melanoidin solutions also produced colorimetric reaction, acting as electron donors. Caemmerer and Kroh (18) and Bekedam et al. (39) proposed that the antioxidant capacity of coffee melanoidins measured by the Folin–Ciocalteu method could be due to both melanoidins and the phenolic compounds incorporated into the melanoidin skeleton. However, in the present experiment, the melanoidins were synthesized using only Maillard

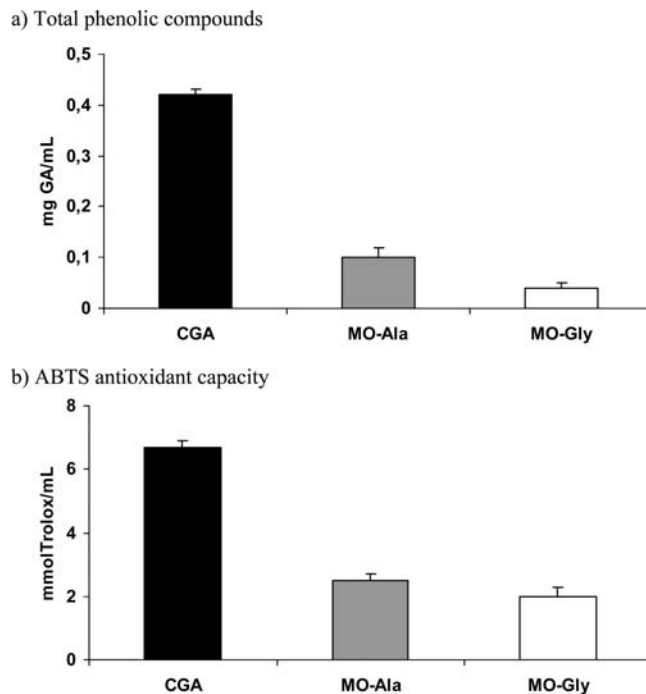


Figure 3. Total phenolic compounds (a) and ABTS antioxidant capacity (b) of the chlorogenic acid (CGA), the maltooligosaccharide–alanine (MO-Ala), and the maltooligosaccharide–glycine (MO-Gly) solutions (3.6 g/L).

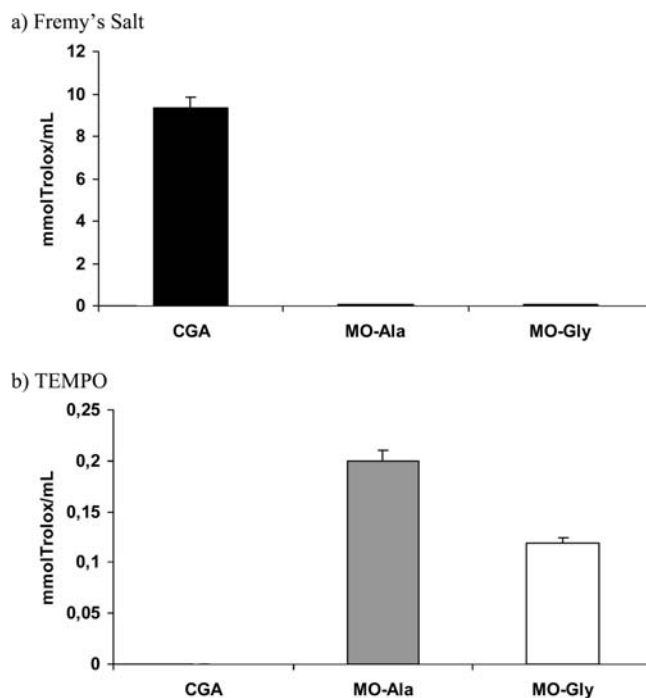


Figure 4. Fremy's salt (a) and TEMPO (b) antioxidant capacity of the chlorogenic acid (CGA), the maltooligosaccharide–alanine (MO-Ala), and the maltooligosaccharide–glycine (MO-Gly) solutions (3.6 g/L).

reaction precursors (maltooligosaccharides and alanine or glycine amino acids). Thus, the colorimetric reaction in the melanoidin model systems could only be due to the Maillard reaction products, not to the presence of phenolic compounds. Consequently, it is clear that the Folin–Ciocalteu assay evaluates not only the phenolic compounds but also the reducing or antioxidant capacity of other nonphenolic chemical compounds, such as Maillard reaction products including melanoidins.

Figure 3b shows the antioxidant capacity measured by the ABTS assay of the chlorogenic acids and melanoidin solutions. All of the solutions reacted with the ABTS^{•+}, but chlorogenic acids exhibited 3 times more antioxidant capacity than melanoidins. This coincides with the contribution of the melanoidins to the total antioxidant capacity in coffee measured by ABTS reported by other authors (9), showing their lower effectiveness as hydrogen donors versus phenolic compounds. It is well-known that the radical ABTS^{•+} reacts with any hydroxylated aromatic compound regardless of its antioxidant potential in vitro or in vivo. Even though at least 45 chlorogenic acids have been identified in coffee brews, caffeoylquinic acids (CQA), and mainly 5-CQA, are the most abundant phenolic compounds (around 86%) (5, 40). The antioxidant activity of these chlorogenic acids is due to the electron-donating effects on the ring of both the COOH—CH=CH— and the dihydroxylation in the 3,4-position in caffeic acid (3,4-dihydroxycinnamic acid) that enhance the availability as hydrogen donors (41).

Consequently, both Folin—Ciocalteu and ABTS assays, which are based on similar electron-transfer redox reactions, can be used to evaluate the overall antioxidant capacity in a simple way but they cannot distinguish antioxidant capacity due to phenolic and nonphenolic chemical compounds, such as Maillard reaction products. Moreover, the different Folin—Ciocalteu results obtained for both dialyzed and nondialyzed melanoidin model systems might partially explain the different order of coffee extraction methods in total phenolic compounds results because each method could favor the extraction of low, medium, or high molecular weight Maillard reaction products with different antioxidant activity (39).

ESR spectroscopy may be used to measure the degree of antioxidant effectiveness of a substance by the ability to scavenge a synthetic stabilized free radical, such as Fremy's salt, TEMPO, or others. In comparison to the more often used colorimetric assays, ESR is much more specific due to the formation of the characteristic signals. Moreover, ESR is a direct measurement of the unpaired electrons, avoiding the use of the indirect measurement of the discoloration of radicals. Although standardization is needed, it is less subjected to the influence of the medium conditions (pH, solvents, etc.). **Figure 4a** shows that Fremy's salt was mainly scavenged by chlorogenic acids, whereas only small traces were observed with melanoidins. Bekedam et al. (39) also observed that other antioxidants, such as Trolox and ascorbic acids, can scavenge this stabilized radical with reaction velocities similar to those of chlorogenic acid, in a nonspecific manner. However, **Figure 4b** shows that the stabilized radical TEMPO was effectively scavenged by both melanoidins, but not by chlorogenic acid. Therefore, by using both Fremy's salt and TEMPO as a stabilized radical, a distinction could be made between phenolic and nonphenolic antioxidants (18, 39), resulting in a more precise assessment of the antioxidant activity of the sample. Moreover, TEMPO results also show that melanoidins have different antioxidant capacities, depending on the structure and composition. MO-Gly was a nondialyzed melanoidin and, consequently, it had several low molecular weight Maillard reaction products that could interfere with the antioxidant capacity of the pure melanoidin, but this model system was more similar to the coffee matrix than the MO-Ala, which was a dialyzed melanoidin and contains mainly high molecular weight structures.

Taking into account the results from the solutions, and in comparison with the other coffeemakers, it could be said that it appears to be quite clear that the mocha coffeemaker extracted more antioxidant capacity per gram of ground roasted coffee due to both the chlorogenic acids (Fremy's salt) (**Figure 2a**) and the melanoidins (TEMPO) (**Figure 2b**). In fact, if results of the

Table 1. Two-Way ANOVA Results of Coffee Antioxidant Capacity Parameters

	additive effect		coffee extraction method effect		interaction effect	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
total phenolic compounds	19.097	<0.001	2910.130	<0.001	19.848	<0.001
ABTS	13.867	<0.001	248.237	<0.001	10.596	<0.001
Fremy's salt	34.127	<0.001	322.160	<0.001	17.519	<0.001
TEMPO	24.777	<0.001	184.074	<0.001	1.175	ns

previous work of Lopez-Galilea et al. (22) are expressed in terms of the extraction of 5-CQA, that is, milligrams per gram of coffee, it could be observed that filter and mocha coffeemakers extracted the highest amount of this chlorogenic acid (7.0 and 6.8 mg/g in conventional coffee, respectively) followed by plunger (5.5 mg/g) and espresso (3.9 mg/g), showing a similar behavior of the Fremy's salt antioxidant capacity (**Figure 2a**) that was mainly scavenged by chlorogenic acids. Moreover, in the same previous work of Lopez-Galilea et al. (22), when browned compound concentration was corrected in terms of the extraction of browned compounds, that is, per gram of ground roasted coffee, it could also be observed that mocha extracted the highest amount of browned compounds followed by the other coffeemakers (filter, plunger, and espresso), as in the TEMPO antioxidant capacity (**Figure 2b**). Thus, it seems clear that TEMPO is positively related to the extraction of browned compounds in coffee, mainly melanoidins.

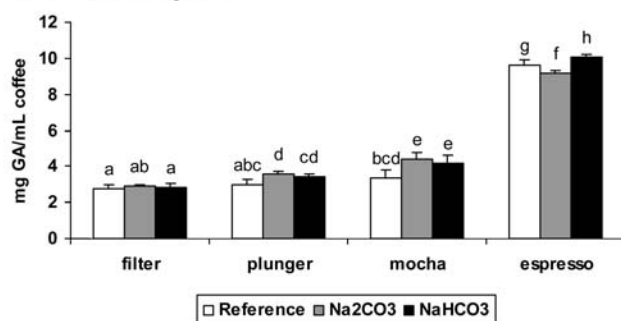
Influence of Addition of Acidity Correctors to Coffee Brew. Ready-to-drink coffee beverage consumption has been increased in past few decades (23). In this type of product, other ingredients or additives, mainly milk derivatives, are usually added to preserve the coffee brew quality, but no studies regarding their influence on the antioxidant capacity have been found. In a previous work carried out by our research group, the addition of sodium carbonate or sodium bicarbonate, as pH-regulator agents, to black coffee brews has been proposed to extend the shelf life (33).

For the study of the influence of the additives on the antioxidant capacity of coffee, three aliquots of each coffee brew were separated: one as reference, the second with the addition of 75 ppm of sodium carbonate, and the third with the addition of 75 ppm of sodium bicarbonate. The selection of the additives and their concentrations were mainly based on sensorial parameters and reported in a previous paper (33). Sodium carbonate (75 ppm) and sodium bicarbonate (75 ppm) water solutions were used as blanks, and no reactions in any of the antioxidant capacity parameters were observed. Thus, the differences in the antioxidant capacity among coffee brews prepared with the same coffeemaker, but with different additives, were due only to the influence of the additives on the antioxidant compounds involved in each technique.

A two-way ANOVA was performed to establish the impact of the additive addition and the brewing extraction method on the antioxidant parameters (**Table 1**). In most cases, significant interaction between the additive and the extraction method has been observed. TEMPO antioxidant capacity was significantly affected by both factors without interaction between them. Moreover, *F* values corresponding to extraction method were higher than the *F* values of the additives for all of the antioxidant capacity parameters, showing greater importance of the extraction method effect that has been previously discussed.

The effects of the acidity regulator addition on the antioxidant capacity measured by colorimetric assays (Folin—Ciocalteu and ABTS) and ESR (Fremy's salt and TEMPO) are shown in **Figures 5** and **6**, respectively. Results are expressed per milliliter

a) Total Phenolic compounds



b) ABTS antioxidant capacity

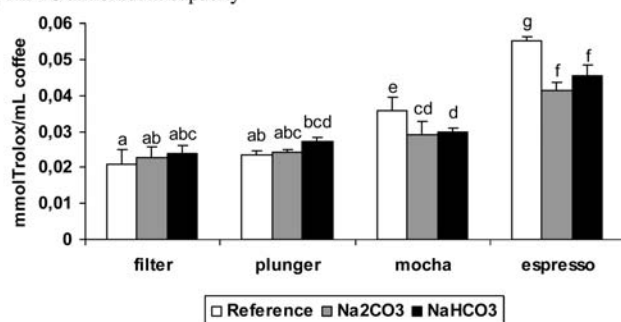
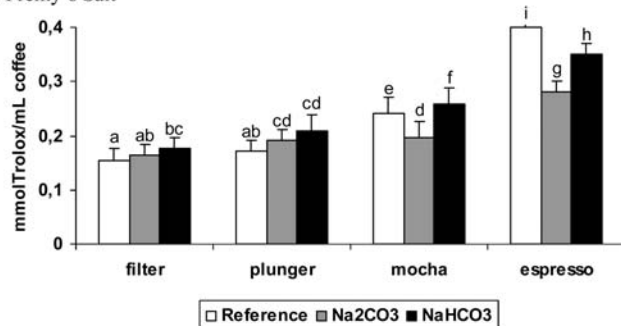


Figure 5. Effect of extraction method and sodium carbonate and bicarbonate addition on the total phenolic compounds (a) and ABTS antioxidant capacity (b) of coffee brews. Bars with different letters are significantly different ($p < 0.05$).

a) Fremy's Salt



b) TEMPO

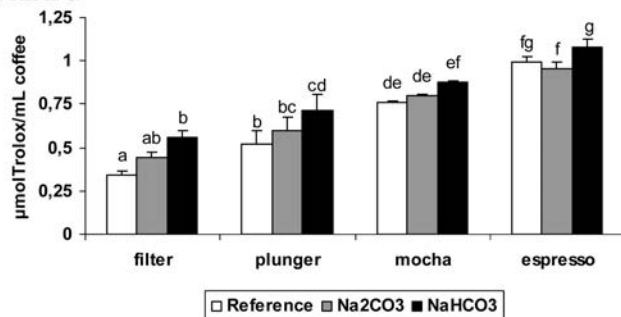


Figure 6. Effect of extraction method and sodium carbonate and bicarbonate addition on the (a) Fremy's salt and (b) TEMPO antioxidant capacity of coffee brews. Bars with different letters are significantly different ($p < 0.05$).

of coffee to facilitate the knowledge of the antioxidant intake. In all parameters, coffee extraction methods were ranked in the same order, showing the highest antioxidant capacity per milliliter in espresso coffee, followed by mocha, plunger, and filter.

Table 2. pH and Total Solids Results of the Coffee Brews with Additives

		pH	total solids (mg/mL)
filter	reference	5.2 ± 0.0	13.23 ± 0.13
	Na ₂ CO ₃	5.4 ± 0.0	
	NaHCO ₃	5.3 ± 0.0	
plunger	reference	5.2 ± 0.0	15.77 ± 0.07
	Na ₂ CO ₃	5.4 ± 0.0	
	NaHCO ₃	5.3 ± 0.0	
mocha	reference	5.1 ± 0.0	21.25 ± 0.12
	Na ₂ CO ₃	5.3 ± 0.0	
	NaHCO ₃	5.2 ± 0.0	
espresso	reference	5.1 ± 0.0	28.21 ± 0.15
	Na ₂ CO ₃	5.3 ± 0.0	
	NaHCO ₃	5.2 ± 0.0	

As discussed above, these results coincide with those reported by Lopez-Galilea et al. (22), using DPPH and redox potential antioxidant parameters, and by Sanchez-Gonzalez et al. (38), using the Folin–Ciocalteu technique.

Total phenolic compounds, with the exception of the filter and plunger coffee brews, were significantly different in coffee brews, with or without additives (Figure 5a). Taking into account the fact that the antioxidant quantities had to be the same in each extraction method because of the experimental design, it could be said that the slight increase of the pH in coffee brews with both pH-regulator agents (Table 2) could change the H-donating ability of the antioxidant compounds or induce some other phenolic or nonphenolic compounds into becoming potential reducers or oxidants.

When antioxidant capacity was measured according to the ABTS method, no significant differences were found between reference coffee brew and the coffee brews with additives (sodium carbonate and bicarbonate) in filter and plunger coffee brews. However, the addition of the pH-regulator agents significantly decreased the ABTS scavenging activity of coffee brews extracted with mocha and espresso coffeemakers, possibly because the addition of these additives might influence the hydrogen-donor capacity of the antioxidants when ABTS antioxidant capacity is higher and acidity is also slightly higher. The application of a Pearson correlation statistical test showed a highly significant excellent correlation between the total phenolic compounds and the radical scavenging ABTS activity (0.914, $p < 0.001$). This might be because both techniques are based on similar electron-transfer redox reactions, but also because both assays evaluate the antioxidant capacity of both phenolic and nonphenolic compounds as shown in Figure 3.

Figure 6a shows a similar behavior of the Fremy's salt antioxidant capacity of coffee brews in comparison to ABTS. Mocha and espresso coffee brews, with the highest antioxidant capacity, were also more affected by the addition of sodium carbonate and bicarbonate than the others. As Fremy's salt mainly evaluates the antioxidant capacity due to chlorogenic acids, this similar behavior between Fremy's salt and ABTS appears to point out that the acidity regulators influence the antioxidants electron distribution and, consequently, their hydrogen-donor capacity. Excellent correlations between Fremy's salt antioxidant capacity and total phenolic compounds (0.900, $p < 0.001$) and ABTS (0.819, $p < 0.01$) parameters in coffee brews were found. This could be because chlorogenic acids showed the highest antioxidant activity in these three assays.

The antioxidant capacity measured by the radical scavenging of TEMPO (Figure 6b) evaluates the antioxidant capacity due to

the Maillard reaction products (**Figure 4b**). As no statistical interaction between both factors, the extraction method and the additive, was found in two-way ANOVA, it is possible to study both variables separately. A gradual, but significant, increase in TEMPO antioxidant capacity was observed at the same time the browned compounds increased (22). When the effect of the additive was studied, it could be said that sodium bicarbonate increased the TEMPO antioxidant capacity, but no significant differences ($p < 0.05$) in sodium carbonate coffee brews were found. Although the change of pH might be a factor that influences the antioxidant activity of melanoidins or other Maillard reaction products, it could be said that it is not the most relevant. For this reason, further investigations of the effects of pH-regulator agents on the different coffee melanoidins or other Maillard reaction products should be carried out.

In conclusion, whereas the mocha coffeemaker seems to have the highest yield per gram of ground roasted coffee in coffee antioxidant extraction, espresso coffee is richest in terms of antioxidant intake (per milliliter of coffee brew). Moreover, the measurement of the coffee antioxidant ability to scavenge synthetic stabilized free radicals, such as Fremy's salt and TEMPO, by ESR spectroscopy allows us to distinguish between phenolic and nonphenolic antioxidants, such as Maillard reaction products and melanoidins. The addition of pH-regulator agents to coffee, such as sodium carbonate and bicarbonate, to extend its shelf life slightly changes the pH, modifying the antioxidant capacity in those coffee brews with the highest capacity (mocha and espresso). In addition to the importance of clarifying which antioxidant compounds are evaluated by each colorimetric or spectroscopic assays, the application of ESR may contribute to the knowledge of whether the antioxidant capacity is due to the presence of phenolic compounds that can be bioavailable (42) or to the melanoidins or other Maillard reaction products, the bioavailability of which is still unclear but which may act as prebiotic compounds (43).

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