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Coffee capsules: implications in antioxidant activity, bioactive compounds, and aluminum content

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Abstract

The effect of new coffee brewing, coffee capsules, on the antioxidant activity (ORAC and ABTS), total phenolic content, chlorogenic acid, caffeine, and melanoidin concentration was assessed and compared with the conventional coffeemakers (filter, mocha, and expresso). In addition, the aluminum content was also analyzed to investigate the potential migration of this metal from the capsule to the beverages. The capsule method showed the lowest values of antioxidant capacity and total phenolic content. Capsule coffeemaker had the lowest extraction yield of the main coffee antioxidants (chlorogenic acid and melanoidins). On average, the highest amount of aluminum was obtained in decaffeinated coffees. Moreover, despite the fact that the coffee is considered to be a poor source of aluminum for humans, when the sample is decaffeinated, especially by Swiss Water® method and prepared by capsule machine, its aluminum content notably increases. The data collected provide useful insights for the selection of the type of coffee beverage with the major content of bioactive compounds and the minor content of harmful components for health.

Keywords Coffee capsule · Coffee antioxidants · Chlorogenic acid · Melanoidins · Aluminum content

Abbreviations

GAE Gallic acid equivalent
HMW High molecular weight
TE Trolox equivalent
TPC Total phenolic content

Introduction

Coffee is one of the highly consumed beverages in the world for its specific flavor and positive effects on human health, and the most important non-alcoholic beverage on a commercial perspective [1]. The consumption of coffee worldwide has risen rapidly in the last 5 years from 151 to 164 million bags (60 kg) [2]. This increase is to a great extend due to the new coffee consumption formats, such as single-dose capsules, which offer a fast and clean way to consume this beverage. In fact, seven out of ten homes in Spain have a capsule machine nowadays [3]. Furthermore, in 2016, the total revenue in coffee capsules in Spain was of 552 million euros, compared to 661 million euros spent in coffee traditional formats [4]. Currently, coffee capsules are produced in two types of materials, the polybutylene terephthalate (PBT), capsules that use a polyester plastic material which is resistant to solvents and high temperatures, and the "In Mold Labeling" (IML), capsules that use aluminum foil in their composition [5]. However, both types of single-dose capsule have an aluminum foil as a cover.

Aluminum is a nonessential metal with toxic properties that have been related to its prooxidant, mutagenic, or cytotoxic effects in humans [6]. The body presence of aluminum seems to trigger the development of neurological disorders, reproductive dysfunction, autoimmune/inflammatory syndrome induced by adjuvants, microcytic anemia, atherosclerosis plaques formation, osteopenia, and breast cancer [7]. The major route of exposure to aluminum for the general population is through diet and the origin of the aluminum in foods might be from the natural occurrence, the use of food additives containing aluminum and from the presence of aluminum in food contact materials such as pots, pans, and foil [8]. In this context, single-dose coffee capsules could be

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considered a new source of human exposure to aluminum because of their boosted consumption.

Some antioxidant components derived from foods have demonstrated a powerful antioxidant action that can protect against diseases caused by the onset of oxidative stress. One of the postulated mechanisms of action is through the reduction of oxidative stress [9, 10]. In this regard, coffee is one of the greatest sources of antioxidant compounds in the diet due to its high content of bioactive compounds such as chlorogenic acid, caffeine, and melanoidins among others [11, 12]. There is a wide spectrum of coffee-preparation methods such as boiled, drip filtered, instant or soluble, espresso, Turkish, and mocha, and several factors are involved in the extraction of these phytochemicals in the coffee brewing process, including the origin, species or variety of the coffee bean, the roasting, and grinding degree [13–15]. Many other works already compare coffee brews in terms of antioxidant capacity, caffeine, etc. using the conventional coffeemakers [16–22], but only a few employed espresso capsules [23–25]. However, none of these studies include the aluminum content in the coffee beverages and this result is of interest in order to know the consumption of this metal by consumers. Additionally, to the best of our knowledge, this is the first study which compares the antioxidant capacity and bioactive compounds present in a wide range of commercial coffee capsules in the Spanish market. Since coffee capsule consumption has highly increased in recent years, it is important to investigate and characterize the new coffee consumption method to know their impact on human health.

Therefore, the purpose of our study was: (1) to analyze the antioxidant capacity and composition in bioactive compounds of coffee obtained by capsule machine and compare it with the conventional methods, (2) to investigate the antioxidant capacity and composition in bioactive compounds of the most consumed coffee capsules in Spain, and (3) to evaluate the potential migration of aluminum to coffee beverage after using single-dose coffee capsules and compare it with conventional methods.

Materials and methods

Reagents

Folin–Ciocalteu reagent, gallic acid, dibasic potassium phosphate, dibasic sodium phosphate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH), formic acid, fluorescein, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), chlorogenic acid (5-O-caffeoilquinic acid), caffeine, and potassium persulfate were purchased from Sigma Aldrich (Madrid, Spain). Sodium carbonate was supplied by Panreac (Barcelona, Spain). Acetonitrile HPLC grade was purchased from VWR (Madrid, Spain).

Coffee samples

To determine the changes in antioxidant capacity and bioactive compounds concentration between conventional (mocha, filter and espresso) and new methods (single-dose capsule), a ground caffeinated coffee (CC) and decaffeinated coffee (DC) were used (objective 1). Aluminum content was also evaluated in both samples under all extraction methods.

First, CC was composed by a blend of *Coffea arabica* (arabica) and *Coffea canephora* (robusta) with a medium roasted and the followed grinding specifications: < 0.25 mm of particle $= 27.6\%$; $0.25–0.5$ mm of particle $= 65\%$; $0.5–0.8$ mm of particle: 7.3%; and > 0.8 mm of particle $= 0.01\%$ (information provided by the company). CC was also packed in IML and PBT capsules for use in capsule machine.

DC has the following characteristics: decaffeinated by the organic solvent (methylene chloride), composed by a mixture of arabica and robusta coffees, medium roasted, and the same grinding specifications than CC. DC was also packed in IML and PBT capsules for use in capsule machine.

Second, to study the antioxidant activity and the main bioactive compounds in usually consumed coffee capsules (objective 2), six packs of different brands of coffee capsules (caffeinated and ristretto type) were purchased from a local supermarket (Madrid, Spain). They were named as C1, C2, C3, C4, C5, and C6.

Third, to examine the effect of decaffeination method plus the extraction method employed in the aluminum concentration of beverages obtained (objective 3), two decaffeinated coffee were added to the experiment:

- Decaffeinated coffee by Swiss Water® (DCSW) ground and packed in IML capsules.
- Decaffeinated coffee by supercritical fluid (DCSF) ground.

Brewing methods

Several methods were used to prepare coffee samples which are explained as follows:

Mocha: coffee was made in a stainless-steel mocha pot (Vev Vigano, Casatenovo, Italy) and extraction took 5 min and 50 s at approximately 76 °C.

Filter: coffee was obtained in a filter machine (Melitta, Minden, Germany) using 1×4 Melitta® filter papers. Extraction took 3 min at 75 °C.

In both described methods, the ratio coffee/water was calculated following the Specialty Coffee Association of

Europe & Nordic Barista Cup indication, which is: 8.25 g of ground coffee per 150 mL of water. [26].

Espresso: coffee was made in a Ruby Pro machine (Quality Espresso, Barcelona, Spain) and extraction took 17 s at 65 °C. The concentration coffee/water was made according to the Specialty Coffee Association of America which was 7 g of coffee for 64 mL of water [27].

Single-dose capsule: ristretto coffee was performed in a capsule machine (Krups, Solingen, Germany) which pours 15 mL of water. Each capsule has approximately 5 g of ground coffee, so the ratio for this type of coffee was 5 g/15 mL. Extraction took 15 s at 62 °C.

Three coffee beverages were made for each sample in each coffee method. All beverages were collected in 50 mL falcon tubes and were aliquoted in Eppendorf tubes (2 mL), and subsequently frozen to -20 °C until their analysis.

To standardize brewing conditions, the coffees were made in a laminar flow hood (Telstar, Barcelona, Spain), and bottled water was used for preparing all the samples (Solán de Cabras, Cuenca, Spain). In addition, before and after each sample was brewed, the machines were cleaned with bottled water without coffee to get rid of any remains and impurities of previous samples.

ORAC antioxidant capacity

The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out following the protocol described by [28] and modified by [29]. Samples were defrosted and centrifuged at 11200g for 2 min at room temperature. Each sample was diluted in different concentrations in PBS buffer [1:1000, 1:2000, 1:3000, 1:4000, and 1:5000 (v/v) to create a concentration curve with the area under the curve of each measurement]. All samples and reagents were dissolved in phosphate buffer (75 mM; pH 7.4). The reaction was performed in a final volume of 200 µL: 20 µL test samples, or 20 µL Trolox solutions (0.2–2 nM) for the calibration curve, 120 µL fluorescein solution (1.17 mM), and 60 µL AAPH (1.3% solution) were added to the wells of a black 96-well plate (Corning, Kennebunk, United States). The fluorescence was recorded at 37 °C every 55 s for 95 min using a fluorimeter (SpectraMax M2; Molecular Devices), with excitation and emission wavelengths of 480 and 520 nm, respectively. Three independent trials were carried out and samples were tested in triplicate in each trial. ORAC values were expressed as µmol Trolox equivalents (TE)/g of coffee.

ABTS antioxidant capacity

The ABTS assay was performed according to [30] and modified by [31] for its use in microplate. Samples were defrosted and centrifuged at 11200 g for 2 min at room temperature. Coffee samples were diluted according to the coffee maker used and its concentration, to adapt absorbance of all samples to the spectrophotometer spectrum. Coffee capsules were diluted in PBS buffer 1:1000 (v/v), mocha, filter, and espresso 1:200 (v/v). An ABTS•+ stock solution was prepared by adding 44 µL of potassium persulfate (140 mmol/L) to a 2.5 mL ABTS•+ aqueous solution (7 mmol/L). The working solution of the radical ABTS + was prepared by diluting the stock solution 1:75 (v/v) in a sodium phosphate buffer (5 mmol/L, pH 7.4) to obtain an absorbance value of 0.7 ± 0.02 at 734 nm. Samples (30 µL) were added to 270 µL of the working solution of ABTS•+ in a microplate. Absorbance was measured at 734 nm and 30 °C for 20 min, every 5 min in a Synergy HT plate spectrophotometer (Biotek Instruments, Winoosky, USA). A calibration curve was made with Trolox (20–250 µM). All samples were analyzed in triplicate. Results were expressed in µmol equivalents of Trolox (TE)/g coffee.

Total phenolic content (TPC)

The TPC was determined according to the Folin–Ciocalteu method described by [32] and modified by [33]. Samples were defrosted and centrifuged at 11200g for 2 min at room temperature. Coffee samples were diluted according to the coffee maker used and its concentration, to adapt absorbance of all samples to the spectrophotometer spectrum. Coffee capsules were diluted in Milli-Q water 1:50 (v/v), mocha, filter, and espresso 1:20 (v/v). The assay was carried out in 96 polystyrene transparent polystyrene plates (Corning), 24 µL of the sample, standard or water (blank), and 47 µL of the Folin–Ciocalteu reagent were added to each well. Next, 189 µL of sodium carbonate (700 mM) was added to each well. After incubation at room temperature for 2 h, the absorbance at 765 nm was recorded in a microplate reader (Biotek Instruments). A calibration curve was constructed with gallic acid (20–1500 µM). Three independent trials were carried out and samples were tested in triplicate in each trial. Results were expressed as µmol equivalent of gallic acid (GAE)/g coffee.

Analysis by high-performance liquid chromatography (HPLC)

The chlorogenic acid and caffeine content in coffee beverages was analyzed by HPLC. Coffee beverages were centrifuged at 11200 g for 2 min at room temperature and supernatants were filtered using a 0.45 µm pore-size PVDF membrane syringe filter (Análisis Vínicos, Ciudad Real, Spain). Subsequently, filtered samples were diluted (1:10 for capsules and 1:2 for mocha, filter, and espresso) with Milli-Q water. An Agilent 1260 Infinity Liquid Chromatograph (Agilent, Santa Clara, CA, USA) equipped with a Diodo Array Detector and an autosampler were used.

Analytical conditions consisted of a Poroshell 120 EC-C18 (4.6 mm × 250 mm and 4 μm of particle diameter) column using 0.1% (v/v) formic acid in Milli-Q water and 0.1% (v/v) formic acid in acetonitrile (HPLC grade) as A and B mobile phases, respectively. Elution was carried out according to the following gradient: 0 min, 10% B; 40 min, 14% B; 50 min, 50% B; 60 min, 5% B until the end in minute 65. Optimum flow rate was 0.8 mL/min, whereas the injection volume was 50 μL. The UV/Vis spectrophotometer detector recorded the wavelengths at 270 nm for caffeine and 330 nm for chlorogenic acid. Column and autosampler compartments were kept at room temperature. Quantitation of standard solutions were used to create a calibration curve (10–360 μg/mL) of chlorogenic acid and caffeine, and samples were analyzed via area peak using the software Agilent OpenLAB CDS ChemStation (Agilent). The results were expressed as mg of chlorogenic acid/g coffee and mg of caffeine/g coffee, respectively.

Melanoidin determination

The presence of melanoidins in coffee beverages was analyzed as described [34]. First, after defrosting, coffee samples were subjected to ultrafiltration using an Amicon®Ultra-10 regenerated cellulose 10 kDa (Millipore, Bedford, MA) at 14,000g for 20 min. Retentates, containing the high molecular weight (HMW) fraction corresponding to coffee melanoidins, were washed three times with Milli-Q water (0.5 mL). After that, retentates were recovered by inverting the Amicon device and centrifuged at 1000g for 10 min. Finally, the HMW fraction was resuspended in 0.5 mL of distilled water and measured spectrophotometrically at 400 nm in a microplate reader (BioTEK Instruments,). Sample reporting absorbance values higher than 1 was additionally diluted (1:20 for capsule samples and 1:10 for the rest of the samples). Determinations were assayed in triplicate. The specific extinction coefficient (K_{mix}) was calculated using the law of Lambert–Beer as described in [28]. The melanoidin content was expressed as mg of melanoidin/g of coffee.

Aluminum determination

Total aluminum concentration in coffee samples was carried out in the Elemental Analysis Unit of the Autonomous University of Madrid (Madrid, Spain) using inductively coupled plasma mass spectrometry (ICP-MS). Samples were centrifuged at 11,200g and were prepared in 1% HNO3 using Milli-Q water. To verify the recovery of the aluminum in the coffee beverages, coffee solutions containing known concentrations of Al were used. The instrument employed was a NexION 300XX (Perkin-Elmer, MA, USA). The data

obtained correspond to aluminum in all its forms. The results were expressed as μg of aluminum/L of coffee beverages.

Statistical analysis

Results are presented as mean ($n = 3$) ± SD. Due to the number of samples to study if the distribution of the data was Gaussian, the Kolmogorov–Smirnov test with Dallal–Wilkinson–Lilliefor p value was carried out. As the data passed the normality test ($\alpha = 0.05$), results were analyzed by One-Way Analysis of Variance (ANOVA). Differences between the coffee samples were assessed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 5 software (San Diego, CA, USA).

Results

Antioxidant capacity

Figure 1 shows the values of antioxidant capacity measured by ORAC and ABTS for CC (Fig. 1a) and DC (Fig. 1b) using four types of brewing methods. CC exhibited the highest antioxidant capacity in mocha and filtered methods for both assays. Overall, antioxidant capacity in expresso and capsule samples showed values approximately 50% lower than filtered and mocha coffees. Regarding DC coffee beverages, filtered method presented the best antioxidant capacity in ORAC and ABTS tests. Results in DC capsule coffee were very different when comparing both assays, ORAC value was the lowest one, and ABTS value was near to filtered coffee.

Total phenolic content

Figure 2 shows the influence of each brewing method (filter, mocha, capsule, and expresso) on the TPC in CC (Fig. 2a) and DC (Fig. 2b) coffee samples. Filtered coffees of CC and DC presented the major content of phenolic compounds with values ranged between 215 and 300 μmol GAE/g coffee. In contrast, coffees obtained by capsule machine exhibited the lowest content of phenolic compounds (20–150 μmol GAE/g coffee). DC mocha and expresso beverages exhibited similar phenolic contents. However, CC sample showed significant differences between these extraction methods. Total phenolic content results are in line with antioxidant capacity measured by ORAC assay.

Chlorogenic acid and caffeine content

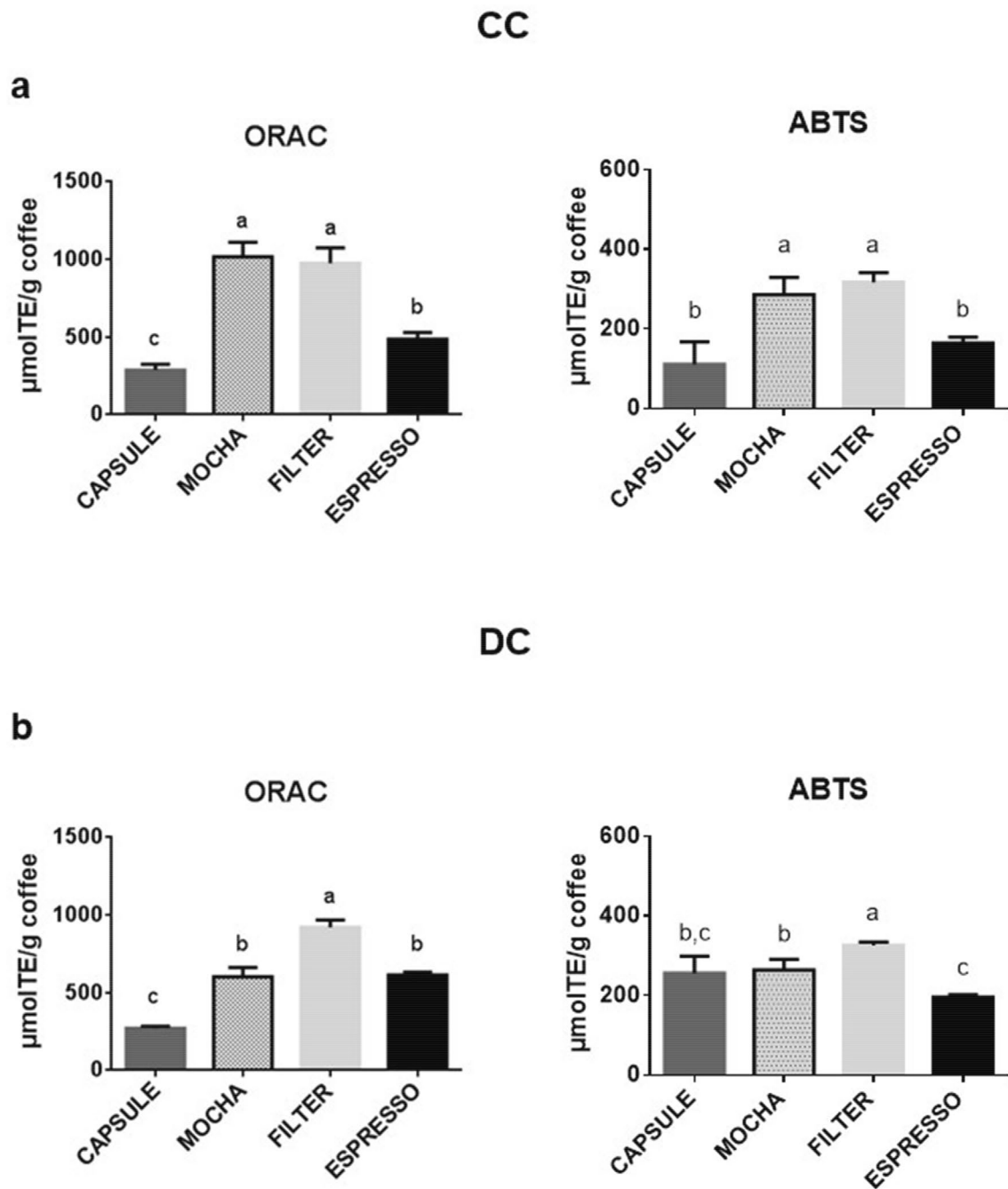The contents of chlorogenic acid and caffeine for CC (Fig. 3a) and DC (Fig. 3b) by diverse coffee makers are

Fig. 1 Overall antioxidant capacity measured by ORAC and ABTS of caffeinated coffee (CC) and decaffeinated coffee (DC) performed by capsule, mocha, filter, and espresso. Data are expressed as mean ± SD ($n=3$). Different letters mean that values are significantly different (Tukey test. $p < 0.05$). TE, Trolox equivalent

shown in Fig. 3. CC and DC filtered coffee contained the major amount of chlorogenic acid (7–8.5 mg/g) than the other extraction methods that ranged between 2 and 6 mg/g. In the case of CC beverage obtained with mocha pot presented similar amount of chlorogenic acid (6.8 mg/g) than filter machine (6.5 mg/g). For CC, single-dose capsules (2.5 mg/g) as well as espresso (2.0 mg/g) showed twofold

less chlorogenic acid extraction than mocha and filtered coffee. In DC beverage, chlorogenic acid highlights in filtered coffee (8 mg/g) and in single-dose capsules (6.2 mg/g), followed by mocha (3.9 mg/g) and espresso (1.8 mg/g).

CC mocha and filtered coffees had a notably higher amount of caffeine per g of coffee (20.7 mg/g) followed by capsule (9.2 mg/g) and expresso (5.25 mg/g) (Fig. 3a).
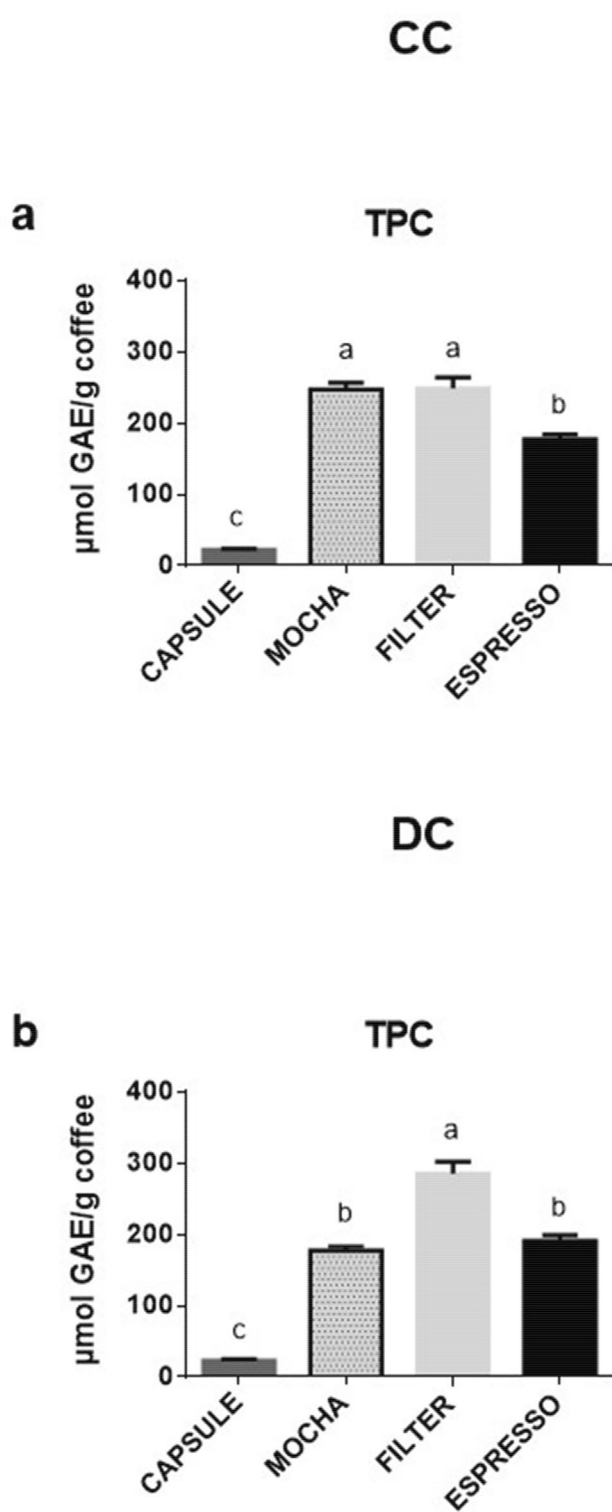
Fig. 2 Total polyphenol content of caffeinated coffee (CC) and decaffeinated coffee (DC) performed by capsule, mocha, filter, and espresso. Data are expressed as mean±SD (*n*=3). Different letters mean that values are significantly different (Tukey test. *p* < 0.05). *GAE* gallic acid equivalent

Determination of melanoidins

Figure 4 shows the values of melanoidins (MW > 30 KDa) in CC (Fig. 4a) and DC (Fig. 4b) coffees by diverse extraction methods. Espresso machine significantly produced the coffee beverages with the major content in melanoidins. The order of brown compounds extraction in both samples by coffee method was: expresso > filter > mocha > capsule.

Evaluation of antioxidant capacity, total phenolic content, and bioactive compounds of commercial brewed-coffee capsules

The study of health-promoting compounds in normally consumed caffeinated coffee capsules in Spain, designated here as C1–C6, is shown in Fig. 5. All coffee capsule samples showed a similar capacity of scavenging oxygen radicals (ORAC) (Fig. 5a). However, the capacity of scavenging the ABTS radical was considerably lower in C1 and C2 (Fig. 5b). The TPC of all coffee capsule samples ranged between 80 and 140 μmol GAE/g with the exception of C1 and C2 that showed 20 μmol GAE/g (Fig. 5c). In line with antioxidant capacity, the TPC values were notably higher for traditional extraction methods (200–300 μmol GAE/g) than for commercial coffee capsules (22–130 μmol GAE/g). As in ABTS assay, C3, C4, C5, and C6 exhibited a significant higher content of phenolic compounds than C1 and C2. The chlorogenic acid content appears to be significantly lower in sample C5 (1.4 mg/g) compared with C6 which had the highest content (3.6 mg/g); once again, capsules exhibited lower extraction yield than mocha and filtered coffee (7–8.5 mg/g). For caffeine content, C3 and C4 reached the highest values (16 mg/g), followed by C5 (11.8 mg/g) and C2 in last place (8 mg/g).

Interestingly, the highest melanoidin content was also in samples C3, C4, and C6. Appears to be a direct relation among ORAC, TPC, and melanoidin content values.

Total aluminum content

The total aluminum content of coffee beverages made by different extraction methods (mocha, filter, and capsule) is indicated in Table 1. As can be observed, aluminum values were undetected in both coffees prepared with mocha pot. It is important to note that DC capsule IML and PBT exhibited much higher content of aluminum than caffeinated ones. However, the content of aluminum in the rest of the samples was insignificant.

These results pushed us to add to our study new coffee samples decaffeinated by different process [Swiss Water® (DCSW) and supercritical fluid (DCSF)] to investigate the total aluminum content between the most used decaffeinated methods. These data are shown in Table 2. All coffee
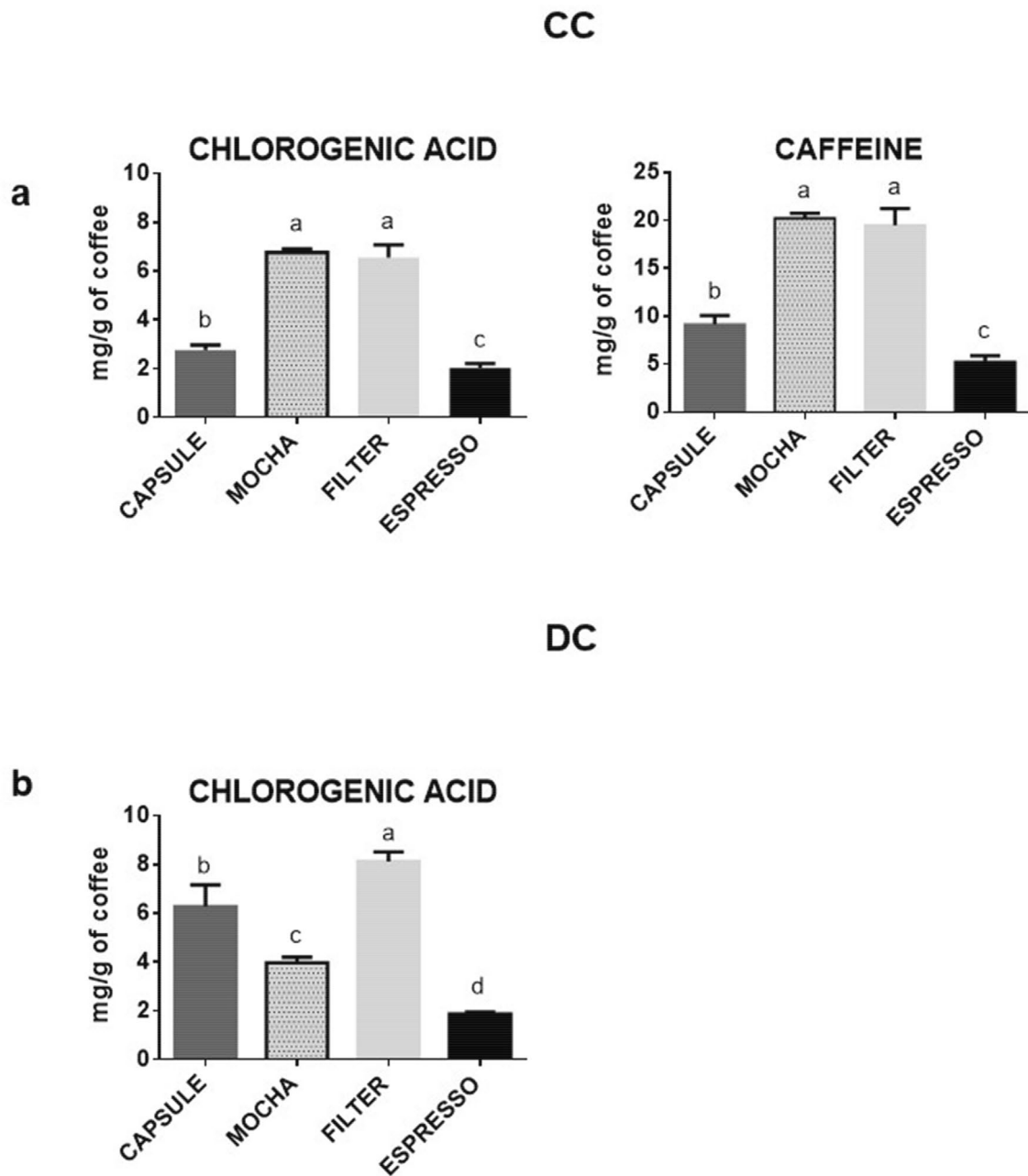
CC

Fig. 3 Chlorogenic acid and caffeine content of caffeinated coffee (CC) and decaffeinated coffee (DC) performed by capsule mocha, filter, and espresso. Data are expressed as mean ± SD ($n = 3$). Different letters mean that values are significantly different (Tukey test. $p < 0.05$)

beverages obtained from DCSW sample (mocha, filter, and capsule) showed notably higher values than coffee samples decaffeinated by DCSF extraction and organic solvents. Interestingly, capsule coffeemaker extracted the highest content of aluminum (1916.9 µg/mL) in the decaffeinated coffee sample by Swiss Water® process. Swiss Water® decaffeinated method added to capsule format produces coffees beverages with 80-fold higher concentration of total aluminum than supercritical fluid one. In addition, DCSW filter

and capsule samples had the lowest pH values, in line with aluminum content.

Discussion

It is important to pinpoint that single-dose capsules have shown exponential growth in recent years, both in the domestic and world market. The major factors that encourage
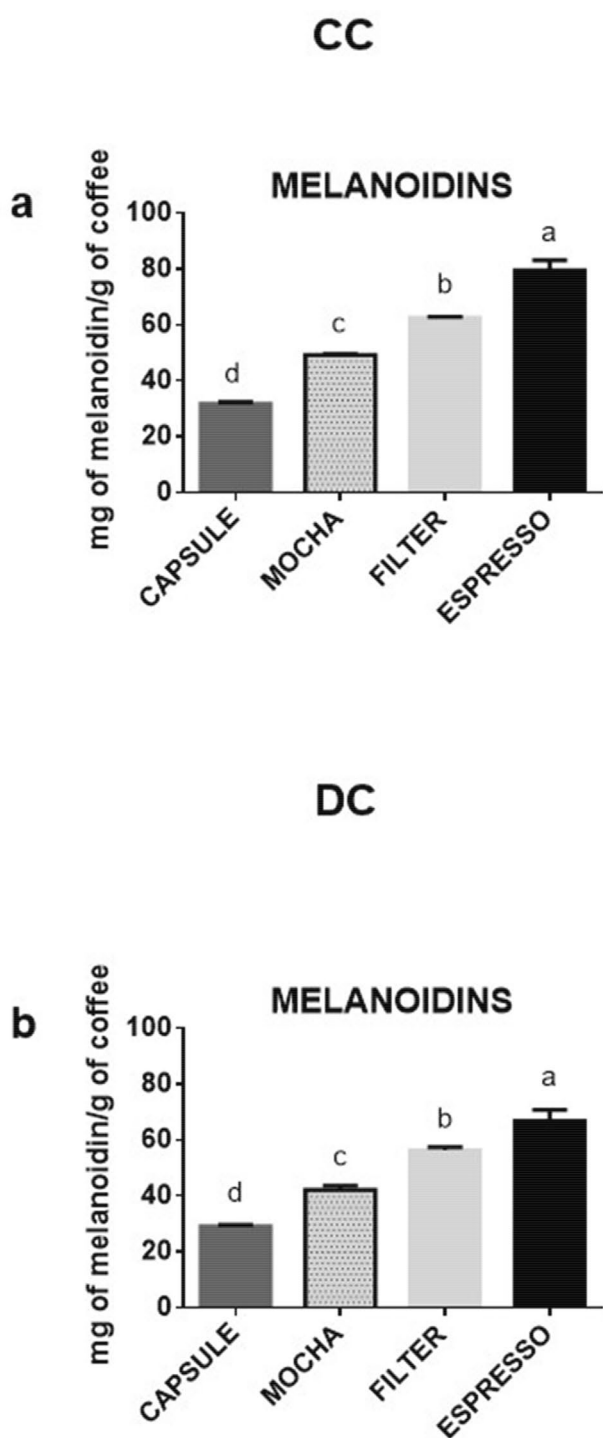
CC

DC

Fig. 4 Melanoidin content of caffeinated coffee (CC) and decaffeinated coffee (DC) performed by filter, mocha, capsule, and espresso. Data are expressed as mean±SD (*n*=3). Different letters mean that values are significantly different (Tukey test. *p*<0.05). *Abs* absorbance

coffee capsules consumption are quality, friendly-user, and, for instance, the attractive designs of coffeemakers [35]. Thus, the coffee consumption is changing and the intake

of bioactive compounds due to its antioxidant properties from this source could be different from the conventional beverages (filter, mocha, and espresso). Moreover, this new extraction coffee method uses aluminum foils and this could constitute a new source of exposure to this toxic metal to humans.

In a first step, the aim of this study was to evaluate the antioxidant activity of single-dose capsules compared with various coffee brews made by different brewing methods. For that, the overall in vitro antioxidant activity was evaluated by different assays (ORAC and ABTS) as recommended in the literature [36], since plant-based polyphenols are multifunctional components acting as reducing agents, hydrogen atom donators, and singlet oxygen scavengers. ORAC determines the ability of coffee antioxidants to inhibit peroxyl radical-induced oxidations by hydrogen atom transfer [11, 37]. The reactions of antioxidants with ABTS radicals involve hydrogen atom transfer and single electron transfer [37]. However, this test employs the radical ABTS which is not representative of biomolecules as peroxyl radicals generated in ORAC assay. Therefore, it is frequent to use at least two different methods to measure antioxidant activity. Regarding antioxidant activity tested by ORAC, the rank of coffee brews was: filter ≥ mocha ≥ espresso > capsule. The order was slightly different with ABTS assay being: filter ≥ mocha ≥ capsule ≥ espresso. These results are in agreement with those obtained by [16] when the antioxidant activity measured by ABTS is calculated per gram of coffee taking into account the coffee/water ratio. Other authors also reported higher ABTS antioxidant capacity in filter and mocha coffees than in espresso [17–19]. None of the studies referred above investigated the antioxidant capacity of coffee samples by ORAC assay, and no measurement of overall antioxidant activity in capsule coffee beverages has been published so far. Our results obtained in antioxidant capacity measured by ORAC are in line with those of [38], presenting similar values performed with the same coffee/water ratio of our espresso beverage. [39] found slightly higher values ranging from 1430 to 2250 μmol TE/g of coffee for a filtered coffee from different origins. It should be noted that single-dose coffee capsules showed lower antioxidant capacity than the traditional coffee extraction methods, in some cases two/threefold less.

The total phenolic contents in foods are widely performed by Folin–Ciocalteu method. This reagent can be reduced by the transfer of electrons from phenolic compounds in alkaline medium [37]. Once again, filter-brewed coffee exhibited the highest TPC, in line to those results reported by other authors [16, 17] which are in discrepancy with those observed by [19]. They described that the major phenolic amount was in espresso coffee compared to filter and mocha. ORAC and TPC data showed the equal trend in each coffee sample and the relationship between these two assays was
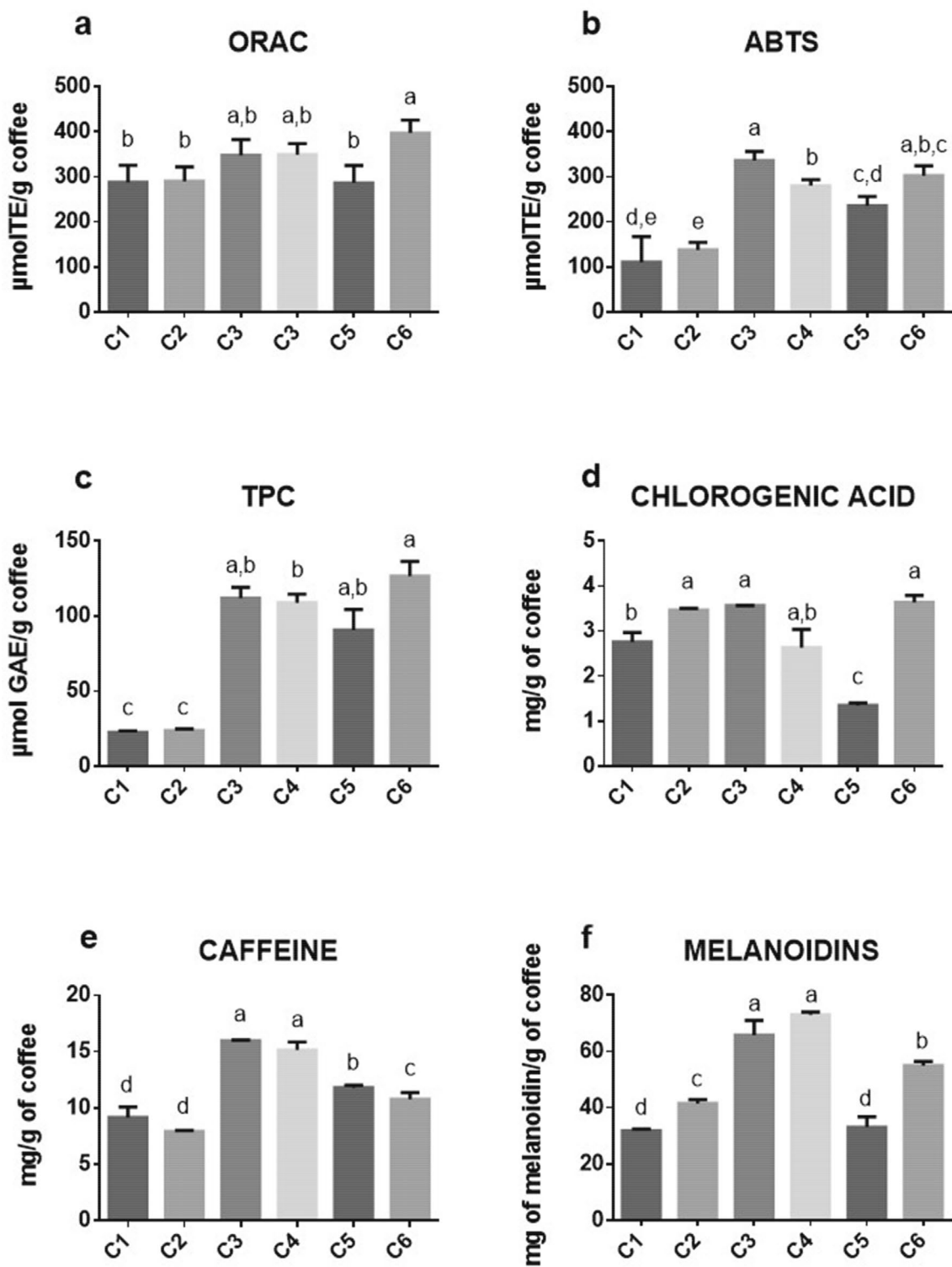
Fig. 5 Evaluation of overall antioxidant capacity by ORAC (**a**) and ABTS (**b**) total phenolic content (**c**), chlorogenic acid (**d**), caffeine (**e**), and melanoidins (**f**) of beverages obtained from six commercial coffee capsules. Data are expressed as mean ± SD ($n = 3$). Differ-ent letters mean that values are significantly different (Tukey test. $p < 0.05$). *Abs* Absorbance, *GAE* Gallic Acid Equivalent, *TE* Trolox Equivalent, *TPC* total phenolic content

Table 1 Aluminum content and pH values in caffeinated coffee (CC) and decaffeinated coffee (DC) obtained by mocha, filter, and capsule methods

Sample	Extraction method	Al (μg/L)	pH
CC	Mocha	[a]	5.20 ± 0.03
	Filter	15.6 ± 0.01	5.15 ± 0.08
	Capsule IML	13.9 ± 2.5	5.35 ± 0.01
	Capsule PBT	[a]	5.19 ± 0.02
DC	Mocha	[a]	5.57 ± 0.23
	Filter	29.2 ± 12.7	5.19 ± 0.01
	Capsule IML	205.6 ± 43.7	5.51 ± 0.04
	Capsule PBT	160.9 ± 20.3	5.01 ± 0.01

Each value is the mean ± SD (n = 3)

Al aluminum, *IML* In Mold Labeling, *PBT* polybutylene terephthalate

[a]Less than the LOQ (9.6 μg/L)

previously described [40]. It has been reported that in filter coffee, the extraction was slower and most efficient in the beginning and at the end of the extraction process [16] and our results could be related to the longer contact time of this coffeemaker method. In line with antioxidant capacity, single-dose coffee capsules showed lower TPC compared with traditional coffee extraction methodologies.

Chlorogenic acid and caffeine are the major phenolic and alkaloid compounds in coffee beverage. Several studies have marked the beneficial effects of these coffee components in human health. Both together in the coffee matrix contribute lowering the risk of chronic related diseases such as type 2 diabetes mellitus, Parkinson's disease, and liver disease [41]. However, as mentioned in the Introduction, the concentration of these components in coffee may vary depending on several factors as bean variety, roasting degree, and brewing method [42]. The values obtained in our study corroborated that brewing method plays a crucial role in chlorogenic acid and caffeine content in coffee beverages. Filtered coffee

showed the highest extraction of both components, followed by mocha and capsules, being espresso the one containing the lowest amount. Other similar studies confirm a higher extraction efficiency of coffee compounds for filter coffee and mocha extraction, respectively, than for espresso or capsules [16, 19, 23]. Our results suggest that consumption of a cup of filter coffee contributes to higher intake of bioactive compounds, chlorogenic acids, and caffeine, by consumers followed by mocha, espresso, and, finally, single-dose coffee capsules.

Melanoidins are defined as high-molecular-weight nitrogenous and brown-colored Maillard reaction products. They are formed during roasting process of coffee. Besides its contribution to flavor and color, one of the important functional properties of melanoidins is their antioxidant activity [43]. Unlike the other assays, in this case, espresso machine shows the highest extraction of melanoidins (MW > 10 KDa) followed by filtered coffee, mocha, and capsules; this particular results contrast with those obtained by [15] whose article reported the highest extraction yield of melanoidins in mocha pot. This fact could be the reason which coffee espresso exhibited more antioxidant capacity and TPC than capsule coffee, although its chlorogenic content was the lowest one. As described before, the degree of antioxidant effectiveness by ORAC is determined by the ability of a solution to scavenge peroxyl oxygen radicals. It has been reported that melanoidins have the ability to scavenge free peroxyl radicals among others [44]. This effect might be caused due to high pressure and temperature applied by espresso machine, which emulsifies lipidic melanoidins in the final beverage [45]. According to the results obtained, single-dose capsules exhibit in most cases the lowest extraction of bioactive compounds that affect to the overall antioxidant capacity in the final beverage.

As consumption of coffee capsule is increasing, various commercial capsules were assayed to go deeper into the antioxidant capacity and concentration of main phytochemicals

Table 2 Aluminum content and pH values in brewed-coffee samples CC, DCSW, and DCSF obtained by mocha, filter, and capsule methods and different decaffeinated process

Sample	Decaffeination process/solvent used	Extraction method	Al (μg/L)	pH
DC	Organic solvents/methylene	Mocha	[a]	5.57 ± 0.23
		Filter	29.2 ± 12.7	5.19 ± 0.01
		Capsule IML	205.6 ± 43.7	5.51 ± 0.04
DCSW	Swiss Water®/water	Mocha	126.2 ± 27.9	5.33 ± 0.32
		Filter	126.1 ± 11.5	4.87 ± 0.01
		Capsule IML	1916.9 ± 39.1	4.85 ± 0
DCSF	Supercritical fluid/carbon dioxide	Mocha	12.0 ± 4.3	5.30 ± 0.05
		Filter	11.6 ± 4.4	5.19 ± 0.03
		Capsule IML	n.a	n.a

Each value is the mean ± SD (n = 3)

Al aluminum, *IML* In Mold Labeling, *n.a* not available

[a]Less than the LOQ (9.6 μg/L)

in coffee. Indeed, the analysis of commercial coffee capsules beverage composition is of interest, because they are representative of real samples and contribute to gain information of bioactive compounds intake by consumers through them. Although similar ORAC values were observed in commercial capsules samples, even though the values were low compared with the traditional extraction methods, the differences in antioxidant capacity and TPC may be caused for several factors, since different commercial brands have distinct origin, roasting, and grinding degree [13].

According to the information reported by EFSA [8], the major route of exposure to aluminum for the general population is through food and exposures which may arise from the use of aluminum compounds in pharmaceuticals and consumer products. Since single-dose capsule formats have aluminum in their packaging, it is important to investigate whether migration of aluminum capsules into the beverages is produced and whether the content of aluminum in coffee capsules is higher than in the coffee obtained by other extraction methods. Besides, to the best of our knowledge, aluminum content in single-dose coffee beverages has not been reported yet. The aluminum content found in the present study in coffee beverages was between 0.009 and 0.2 mg/L which is in the range of those described in the literature [46, 47]. The major aluminum content into coffee brew was observed in the decaffeinated samples performed with capsule coffeemaker. As a result, we decided to investigate aluminum content from non-caffeinated coffees obtained by different decaffeination methodologies, such as organic solvents, Swiss Water® extraction, and supercritical carbon dioxide. The decaffeinated process by organic solvents consists in extracting most of the caffeine in the green coffee beans using organic solvents as ethyl acetate and methylene chloride [48]. The Swiss Water® methodology involves soaking of the green coffee bean in hot water, and then, caffeine and flavor from the beans are dissolved into the water. In a second step, water with dissolved compounds passes through an activated carbon filter that removes the caffeine [49]. The extraction with supercritical fluid uses liquid carbon dioxide that is pumped at 300 atm and 65 °C flowing across the extractor and dissolves caffeine [49]. Decaffeinated method by water extraction exhibited a major content in aluminum. In addition, the increased aluminum content in brewed-decaffeinated coffees was notably higher in decaffeinated Swiss Water® coffee prepared by capsule system. As reported by [50], aluminum is found in drinking and bottled water and water is considered one of the main routes of exposure to aluminum in humans. Since the Swiss Water® decaffeinated method uses water extraction to remove caffeine, it was not surprising to find that aluminum content was especially high in these coffee samples. Generally, solubility of aluminum increases under more acidic environment [50], and it is well known that the speciation of aluminum depends on the pH and the chemical environment of the solution. The toxic effect of the different forms of aluminum (speciation) increases in the following order: $Al(OH)^4$, $Al(OH)^3$, $Al(OH)^{2+}$, $Al(OH)^{2+}$, Al^{3+}. At a low pH (about 4.3), trivalent aluminum (Al^{3+}) is the most abundant form and also the most toxic for living organisms [51, 52]. In our study, Swiss Water® coffee samples prepared by capsule system exhibited the lowest pH value (4.85), and this could be related to the high aluminum concentration and the potential toxic effects of this metal in these samples. Thus, the combination of coffee decaffeination by Swiss Water® and preparation by capsule machine increases the concentration of aluminum in the coffee beverage up to 80-fold. During the pre-soak, in the Swiss Water® process, the green coffee bean is maintained in water to change the cellular structure of coffee bean and remove the caffeine. In this step, the green coffee bean might be taking the aluminum from the water increasing its content. However, the aluminum content found in this coffee beverage is at least twofold lower than the aluminum content described to tea infusion or wine [49].

In conclusion, the new coffee-preparation method, single-dose capsule, showed the lowest antioxidant capacity when compared to the conventional coffee-preparation methods, showing three-to-four times lower than filtered coffee method. The lowest antioxidant activity of coffee capsules was related to the low extraction of bioactive compounds mainly by chlorogenic acid due to the shorter contact time water/coffee of this coffeemaker. These results suggest that worldwide population is reducing the intake of health-promoting bioactive compounds from coffee per cup, due to the increase of coffee capsule consumption. Moreover, despite the fact that the coffee is considered to be a poor source of aluminum for humans, when the sample is decaffeinated, especially by Swiss Water® method and prepared by capsule machine, its aluminum content notably increases. The results from this investigation might be used to improve the bioactive compounds consumption by coffee beverages, and thus, it has impact in human health. Although single-dose capsules appear to have less bioactive compounds in the composition of the final beverage as shown in this study, the increasing consumption of this type of new coffee format may balance the daily ingestion of bioactive compounds in consumers. Further studies including human intervention studies using new capsules coffee-preparation method to explore the beneficial effects and the influence of decaffeination method in the aluminum coffee content are needed.

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Compliance with ethical standards

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