TITLE: Extraction of coffee antioxidants: impact of brewing time and method

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ABSTRACT

The aim of this work was to study the extraction behavior of the main coffee antioxidants (caffeoylquinic acids, melanoidins and caffeine) and the antioxidant capacity, during brewing time in the most widely consumed coffee brew methods (filter and espresso) in coffee. Antioxidant capacity by colorimetric assays (Folin-Ciocalteau, ABTS and DPPH) and electron spin resonance spectroscopy techniques (Fremy's salt and TEMPO) were analyzed. In espresso coffee, more than 70% of the antioxidants (except dicaffeoylquinic acids, diCQA) of a coffee brew were extracted during the first 8 s. In filter coffee, a U-shape antioxidants extraction profile was observed, starting later (after 75s) in Vietnam coffee than in Guatemala one, probably due to different wettability. Other technological parameters, such as turbulences and a longer contact time between water and ground coffee in filter coffeemaker, increased extraction efficiency, mainly in less polar antioxidant compounds as diCQA. In conclusion, these technological factors should be considered to optimize coffee antioxidants extraction that can be used as ingredients for functional foods.

KEYWORDS: Antioxidants, brewing time, coffee, Maillard reaction products, phenolics.

1. INTRODUCTION

Several chronic diseases, such as cancer, cardiovascular, inflammatory, and neurogenerative pathologies are associated with oxidative stress (Aruoma, 1999; Beal, 1995; Dorea & da Costa, 2005). Beside fruits and vegetables, plant beverages such as coffee brew have been proposed as an important source of antioxidants in human diet (Pulido, Hernandez Garcia, & Saura Calixto, 2003; Svilaas, Sakhi, Andersen, Svilaas, Strom, & Jacobs, 2004). The antioxidant capacity of coffee brew is attributed to both antioxidants originally present in coffee beans, like phenolic compounds, and roasting-induced antioxidants, like melanoidins and other Maillard Reaction Products (MRP) (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002; Crozier, Jaganath, & Clifford, 2009; del Castillo, Ames, & Gordon, 2002).

The most abundant phenolic compounds of coffee are chlorogenic acids (CGA). CGA are known for their contribution to the final acidity, astringency, and bitterness of the coffee brew, but also for their potent antioxidant properties (Moreira, Monteiro, Ribeiro-Alves, Donangelo, & Trugo, 2005; Natella, Nardini, Giannetti, Dattilo, & Scaccini, 2002; Trugo & Macrae, 1984; Variyar, Ahmad, Bhat, Niyas, & Sharma, 2003). During roasting, CGA are partially degraded and at least partly incorporated in coffee melanoidins through non-covalent or covalent bounds (Bekedam, Schols, van Boekel, & Smit, 2008; Nunes & Coimbra, 2010). Melanoidins are generally defined as the browned-colored, high-molecular-weight, nitrogenous end products of the Maillard reaction. They are formed during roasting process of coffee. Beside its contribution to flavor and color, one of the important functional properties of melanoidins is its antioxidant activity (Caemmerer & Kroh, 2006; C. Delgado-Andrade & Morales, 2005; López-Galilea, Andueza, Leonardo, de Peña, & Cid, 2006; Rufián-Henares & Morales, 2007). Although there is still a discussion about their bioavailability, it is clear that at

least they may act as prebiotic or even antimicrobial depending on their nature and concentration (Borrelli & Fogliano, 2005; Rufián-Henares & de la Cueva, 2009). Also caffeine or its metabolites in humans have been proposed as antioxidant compounds against lipid peroxidation induced by reactive oxygen species (Devasagayam, Kamat, Mohan, & Kesavan, 1996; Lee, 2000). However, although caffeine has been extensively studied from the pharmacological point of view, less attention has been paid to its potential antioxidant activity that may be overshadowed by phenolic compounds and MRP.

Brewing process is essential for the antioxidant composition and health properties of a coffee brew, because the contact of water with roasted coffee grounds is the crucial step for extraction of coffee compounds. Other factors, such as origin or variety of coffee beans, blending, roasting degree and grinding also play a key role in coffee composition. Among the several brewing techniques, filter coffee (drip filter) is the most widely used coffee brew obtained by infusion method, whereas espresso coffee is the most appreciated coffee brew produced by pressure method. In drip filtration methods, water at 92-96 ºC flows through a hardly compressed ground coffee bed and the extract drips from the brewing chamber into the pot. Turbulence in the brewing chamber prevents water from becoming saturated (Lingle, 1996). In pressure methods, water at approximately 9 bars and 88-92ºC is forced to go through coffee grounds compacted in a small brewing chamber (coffee cake). Also rapid brewing time and fine particle size are necessary (Lingle, 1996). Many chemical species identified in roasted coffee, including antioxidants, exhibit different extraction rates that may also be influenced by the choice of brewing technique and conditions (Peters, 1991; Petracco, 2001; Petracco, 2005).

Even though the brewing time is given by the coffee brewing technique, the knowledge of extraction behavior of the main coffee antioxidants during this time might induce to know the technological factors with major impact on antioxidants extraction. Thus, it could be possible to obtain not only coffee brews with higher antioxidant capacity, but also coffee extracts with health properties that can be used as ingredients in functional foods. For these reasons, the aim of this work was to study the extraction behavior of the main coffee antioxidants and the antioxidant capacity, during brewing time in the most widely consumed coffee brew procedures (filter and espresso).

2. MATERIALS AND METHODS

2.1. Chemicals and reagents. The methanol (spectrophotometric and HPLC grade) and Folin-Ciocalteau reagent were from Panreac (Barcelona, Spain). ABTS (2,2'-Azino-bi(3-ethylbenzo-thiazonile-6-sulfonic acid) diammonium salt), potassium persulfate, $DPPH^{\cdot}$ (2,2-Diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, Fremy's salt (potassium nitrosodisulfonate) and TEMPO (2,2,6,6-tetramethyl-1-piperidin-1-oxyl) were purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid was from Fluka (Madrid, Spain). Pure reference standards of 5-caffeoylquinic acid (5-CQA) and caffeine were obtained from Sigma-Aldrich (Steinheim, Germany) and pure reference standards of 3,4-, 3,5- and 4,5-dicaffeoylquinic acids were purchased from Phytolab (Vestenbergsgreuth, Germany). A mixture of 3-CQA, 4-CQA, and 5-CQA was prepared from 5-CQA using the isomerization method of Trugo and Macrae (1984), also described in Farah et al. (2005).

2.2. Coffee brew samples. Roasted coffee from Guatemala (*Coffea arabica*, 3.03 % water content, L* = 25.40 ± 0.69, roasted at 219 ºC for 905 s) and Vietnam (*Coffea*

canephora var. robusta, 1.59 % water content, L* = 24.92 ± 0.01, roasted at 228 ºC for 859 s) was provided by a local factory. The L* value was analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant and CIE 1931 standard observer. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in an l cm Petri plate, and the L* value was measured in triplicate on the CIELab scale.

Roasted coffee beans were ground to a powder in a Moulinex coffee grinder (model Super Junior "s", Paris, France) for 20 s immediately before sample preparation. Filter Coffee Brew was prepared from 36 g of ground roasted coffee for a volume of 600 mL, using a filter coffee machine (model Avantis 70 Aroma plus, Ufesa, Spain). Extraction took approx. 6 min at 90 ºC. Five fractions for filter coffee were collected sequentially every 75 s. Espresso Coffee Brew was prepared from 7 g of ground roasted coffee for a volume of 45 mL using an espresso coffee machine (model Saeco Aroma, Italy). Three fractions for espresso coffee were collected sequentially every 8 s. Coffee brews and fractions were lyophilized using a CRYODOS Telstar (Terrassa, Spain) and stored at -18ºC until sample analysis.

2.3. pH. The pH measurements of coffee brews and fractions were performed with a Crison Basic 20pH-meter.

2.4. Browned compounds (Abs 420 nm). Fifty microliters of coffee brew or fraction were diluted up to 2 mL with deionized water. Browned compounds were quantified by measuring the absorbance of the sample at 420 nm after exactly 1 min, in a 3 mL capacity cuvette (1 cm length) with a Lambda 25 UV-VIS spectrophotometer (Perkin-Elmer Instruments, Madrid, Spain) connected to a thermostatically controlled chamber (25 ºC) and equipped with UV Win-Lab software (Perkin Elmer).

2.5. Folin-Ciocalteau (FC) assay. The Folin-Ciocalteau reducing capacity of coffee or fractions was performed according to the Singleton´s method (Singleton & Rossi, 1965). For every coffee sample, 1:10 dilutions with demineralized water were prepared, and 500 µL of Folin-Ciocalteau reagent were added to 100 µL of the coffee sample solution. After 2 min delay, 1.5 mL of a 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of the sample was measured at 765 nm in a Lambda 25 UV-VIS spectrophotometer (Perkin Elmer Instruments, Madrid, Spain). Gallic acid (GA) was used as reference, and the results were expressed as milligrams of GA per mililiter of coffee brew or fraction.

2.6. Antioxidant capacity by ABTS assay. The antioxidant capacity measured with ABTS was carried out according to the method described by Re et al. (1999) with some modifications. The radicals $ABTS^{\cdot+}$ were generated by the addition of 2.45 mM potassium persulfate to an 7 mM ABTS solution prepared in phosphate-buffered saline (PBS, pH 7.4) and allowing the mixture to stand in darkness at room temperature for at least 12 h before use. The $ABTS^{\cdot+}$ stock solution was adjusted with PBS to an absorbance of 0.7 (±0.02) at 734 nm in a 1 cm cuvette at 25 ºC (Lambda 25 UV, VIS spectrophotometer, Perkin Elmer Instruments, Madrid, Spain). An aliquot of 50 µL of coffee sample diluted with demineralized water (5:1000 to 15:1000) was added to 2 mL of $ABTS^{\cdot+}$ reagent and the absorbance was monitored for 18 min at 25 ºC. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue) and total antioxidant capacity was expressed as micromoles (µmol) of Trolox per mililiter of coffee brew or fraction.

2.7. Antioxidant capacity by DPPH assay. The antioxidant capacity was measured using the DPPH decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995). A

$6.1x10^{-5}$ 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 1 cm cuvette at 25 ºC (Lambda 25 UV, VIS spectrophotometer, Perkin Elmer Instruments, Madrid, Spain). Fifty microliters of appropriate diluted coffee sample (1:100 to 3:100) was added to DPPH· solution (1.95 mL). After mixing, the absorbance was monitored at 515 nm for 18 min at 25 ºC. Calibration was performed with Trolox solution and the total antioxidant capacity was expressed as micromoles (μmol) of Trolox per mililiter of coffee brew or fraction.

2.8. Antioxidant capacity by Electro Spin Resonance (ESR) spectroscopy. The ESR spectroscopy measurements were performed with Fremy´s salt and TEMPO as stabilized radicals with the same procedure described by Roesch et al. (2003) and modified by Caemmerer & Kroh (2006). For the investigation with Fremy's salt, 100 μL of every coffee sample diluted 250-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 40 s for 30 min. For the investigation with TEMPO, aliquots of 300 μL of coffee sample were allowed to react with 100 μL of 1 mM TEMPO solution. ESR spectra were obtained after 120 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 (μmol) of Trolox per mililiter of coffee brew or fraction.

2.9. Chlorogenic acids (CGA) and caffeine. Extract preparation and cleanup were carried out according to Bicchi et al. (1995). The compounds were analyzed by HPLC following the method described by Farah et al. (2005), with some modifications. HPLC

analysis was achieved with an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump and an automated sample injector. A reversed-phase Hypersil-ODS (5 µm particle size, 250 x 4.6 mm) column was used at 25 ºC. The sample injection volume was 100 µL. The chromatographic separation was performed using a gradient of methanol (solvent A) and Milli-Q water acidulated with phosphoric acid (pH 3.0, solvent B) at a constant flow of 0.8 mL/min starting with 20% solvent A. Then solvent A was increased to 50% within 15 min to be maintained at 50% for 9 min and, finally, to return to initial conditions (20% solvent A) in 3 min. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 325 nm for CGA and 276 nm for Caffeine. Identification of CGA and caffeine was performed by comparing the retention time and the photodiode array spectra with those of their reference compounds. Quantification of 5-caffeoilquinic (5-CQA) and caffeine was made by comparing the peak areas with those of the standards. Quantification of the other chlorogenic acids (CGA) was performed using the area of 5-CQA standard combined with molar extinction coefficients of the respective CGA as reported by Trugo and Macrae (1984) and Farah et al. (2005).

2.10. Statistical analysis. Each parameter was analyzed in triplicate. Results are shown as means ± standard deviations. Student's t-test was applied for each antioxidant capacity assay to know whether there were differences between both coffees in each coffee brew. One-way analysis of variance (ANOVA) was applied for each parameter to compare antioxidants extraction among fractions in each coffee brew sample. A T-Tukey test was applied as a test a posteriori with a level of significance of 95%. All statistical analyses were performed using the SPSS v.15.0 software package.

3. RESULTS AND DISCUSSION

3.1. Coffee fractions Volumes

The volumes of the coffee brews and fractions obtained by espresso and filter coffeemakers are shown in Table 1**.** The volumes of the three espresso coffee fractions were quite similar, ranging from 14 to 17 mL. In contrast, the volumes of the filter coffee fractions increased from F1 (76-80 mL) up to F3 (160-186 mL) and then decreased to F5 (26-54 mL), showing an inverted U-shape profile.

To extract coffee compounds during the brewing process, the dry coffee grounds must first absorb water. Once the water has completely surrounded a coffee particle, both inside and out, the coffee extractable material begins to move out of the bean's cellular structure and into the surrounding water. Because espresso coffeemaker applies constant pressure that forces water through the coffee grounds with a constant flow, the coffee fraction volumes were similar among each other. However, in filter coffee no mechanical forces are applied, and the brew volume dripping out from the extraction chamber depends on the water amount, and consequently on the water pressure in the extraction chamber of the coffeemaker according to Darcy's law (Petracco, 2005). Furthermore, at the beginning of the filter extraction process, part of the water is absorbed by coffee grounds. In an espresso coffeemaker, water is forced to go through the coffee cake, but, in a filter coffeemaker, during wettability, 1 g of coffee will absorb 2 mL of water as a general rule (Lingle, 1996). This fact explains the low volume obtained for F1 (0-75 s). With time, water fills the extraction chamber increasing the pressure and favoring that water passes through the coffee bed, which leads to higher volumes in the middle fractions. At the end of the brewing procedure, pressure decreases when the water reservoir depletes, giving the lowest volume in the last fraction (F5).

3.2. Antioxidant capacity of coffee fractions

The antioxidant capacity of the coffee brews and fractions obtained by espresso and filter coffeemakers was measured by means of three colorimetric assays (Folin-Ciocalteau, ABTS and DPPH) and two electron spin resonance (ESR) spectroscopy techniques (Fremy´s salt and TEMPO) and the results are shown in Figure 1 to 5.

The Folin-Ciocalteau assay is based on an electron-transfer reaction. Although this is the most popular method to evaluate the total phenolic compounds, the Folin-Ciocalteau reagent can be reduced by many electron-donors, not only phenolic compounds (Huang, Ou, & Prior, 2005). Two different stable radicals ($ABTS^{\cdot+}$ and DPPH·) were chosen to assess the radical scavenging activity in coffee fractions. These radicals react energetically with hydrogen-donors, such as phenolic compounds, being DPPH· likely more selective in the reaction with H-donors than $ABTS^{\cdot+}$ (Huang et al., 2005). In these three colorimetric assays, Vietnam coffee brews showed significantly ($p<0.01$) higher antioxidant capacity than Guatemala ones. The results were similar to those reported by other authors in espresso and filter coffee brews (Pérez-Martinez, Caemmerer, De Peña, Cid, & Kroh, 2010; Sánchez González, Jiménez Escrig, & Saura Calixto, 2005).

Espresso coffee fractions from both coffees showed a remarkable decrease in antioxidant capacity with brewing time. More than 70% of the overall antioxidant capacity of an espresso coffee brew was found in F1 (0-8 s), whereas F3 accounted for less than 12 %. These results demonstrate that the compounds responsible for the antioxidant activity of an espresso coffee brew are mainly extracted at the beginning of the brewing process and, afterwards, are diluted. Similar results were found by Alves et al (2010) for DPPH antioxidant activity in espresso coffees with different brew lengths (“short” to “long”). These authors also observed that the antiradical or reducing activity of espresso coffee brew is not only dependent on total phenolic amounts measured by Folin-Ciocalteau assay. This may be due to the fact that the Folin-Ciocalteau assay not

only evaluates phenolic compounds, but also because it is well known that roasting-induced antioxidants like Maillard reaction products (MRP), contribute to the overall antioxidant capacity of coffee (Delgado-Andrade, Rufián-Henares, & Morales, 2005; Pérez-Martinez et al., 2010).

To go deeper into the influence of brewing time on antioxidant capacity due to phenolics or MRP, Electron spin resonance (ESR) spectroscopy was applied using Fremy's salt and TEMPO radicals. Mainly phenolic compounds can be detected when Fremy's salt is used as the stabilized radical, whereas TEMPO is mainly scavenged by Maillard reaction products (MRP), such as melanoidins (Caemmerer & Kroh, 2006). The results obtained with ESR spectroscopy (Figure 4 and 5) showed that Fremy´s salt scavenging capacity was almost four times higher than TEMPO. Similar results were reported by other authors who proposed that the phenolic antioxidants evaluated by Fremy´s salt dominate the overall antioxidant capacity of coffee brews, whereas the contribution of roasting-induced antioxidants is rather limited (Bekedam, Schols, Cämmerer, Kroh, van Boekel, & Smit, 2008; Pérez-Martinez et al., 2010).

The ESR antioxidant capacity of espresso coffee fractions showed that F1 (0-8 s) accounted for 75-81 % and for 86-89 % of the Fremy´s salt and TEMPO scavenging capacity of an espresso coffee brew, respectively. Although antioxidant capacity due to phenolics and measured by Fremy's salt assay was the highest in the first fraction, 20-25 % of the scavenging capacity was still found in F2 and F3. This could be due to a slower extraction of those phenolics retained in the inner coffee particles and those bound to melanoidins that need more time and water pressure to be released. The highest percentages observed for TEMPO scavenging capacity in F1 indicate that MRP antioxidants were mainly extracted during the first 8 seconds, whereas the last fraction (16-24 s) only accounted for 1-2 %. These results agree with the significantly highest

values of Browned compounds (Abs 420 nm) showed in the first fraction (Table 2) that clearly decreased in the next ones (F2 and F3).

Filter coffee fractions showed different antioxidant capacity extraction behaviors, being also different in the two coffee samples in comparison to espresso coffee. In Guatemala filter coffee, all antioxidant capacity assays showed a U-shape profile with the highest concentration in F1 (0-75 s) and F5 (300-375 s) and the lowest in F3 (150-225 s). However, in Vietnam coffee the U-shape antioxidant capacity extraction started after 75 s, showing F1 the lowest values. This could be due to a higher water absorption in Vietnam coffee that leads to a longer wetting stage. The wettability depends on the particle shape and size that may be different depending on factors like grinding that is also influenced by coffee origin or variety and roasting degree (Lingle, 1996). In this work, taking into account that roasting degree and grinding conditions were controlled to be the same, different wettability may be due to the different brittleness of the coffee beans. The increase of antioxidant capacity in the last fractions (F4 and F5) of filter coffee brews could be due to the water pressure decrease that induces a lower flow and a longer contact time between water and ground coffee. In fact, because the last fraction (F5) had the lowest volumes (26 mL and 54 mL for Guatemala and Vietnam coffees, respectively), their contribution to the antioxidant capacity of the overall coffee brew was rather limited (~9 % and ~14 %, respectively).

The results of the antioxidant capacity due to phenolics and MRP, measured by ESR spectroscopy in filter coffee fractions using Fremy's salt and TEMPO as stabilized radicals (Figures 4 and 5), also corroborate that the antioxidants extraction seems to be delayed in Vietnam filter coffee. This was more pronounced in TEMPO antioxidant capacity that mainly evaluates the scavenging activity of melanoidins which are polymeric compounds with more difficult to be released without water pressure. In fact,

the Absorbance at 420 nm of Vietnam filter F1 fraction was significantly the lowest as shown in Table 3. Moreover, taking into account the brew volume, only ~3 % of TEMPO antioxidant capacity of the overall Vietnam filter coffee brew was extracted during the first 75 seconds (F1), whereas ~37 % was found in F2 (75-150 s). So that, the contribution of the first two fractions of Vietnam filter coffee to the overall TEMPO antioxidant capacity was similar to the ~40 % found in Guatemala filter coffee F1.

3.3. Antioxidant compounds extraction

The antioxidant capacity of coffee brew is attributed to both, natural antioxidants, like phenolic compounds, and roasting-induced antioxidants, like melanoidins and other MRPs. To know the influence of brewing time on the main antioxidant compounds, browned compounds (Abs 420 nm), caffeine and caffeoylquinic acids in coffee brews fractions were quantified and the results are shown in Table 2 and 3. Browned compounds, as previously discussed, were mainly extracted in those coffee fractions with high TEMPO antioxidant capacity showing a high correlation ($r=0.969$, $p<0.001$). Also caffeine has been proposed as an antioxidant compound against lipid peroxidation induced by reactive oxygen species (Lee, 2000). Caffeine was in significantly higher concentration in Vietnam espresso and filter coffee brews and fractions. It is very well known that Robusta coffees are richer in caffeine than Arabica ones (Belitz, Grosch, & Schieberle, 2009). Thus, caffeine might partially explain the higher antioxidant capacity of Vietnam coffee brews that could not be attributed to the main chlorogenic acids that were found in lower amounts in these coffee brews, as will be discussed later. Traditionally, the higher antioxidant capacity of Robusta coffee brews has been attributed to higher total phenolic compounds (usually measured by Folin Ciocalteau technique), and then to chlorogenic acids because 5-CQA is the most abundant phenolic in coffee. However, other authors (López-Galilea, de Peña, & Cid, 2007; Vignoli,

Bassoli, & Benassi, 2011) also observed higher antioxidant capacity but lower 5-CQA amounts in brews prepared with Robusta coffee or torrefacto blends. These authors reported high correlations between antioxidant capacity of coffee brews and caffeine, suggesting that caffeine might be a good contributor to the antioxidant capacity or reducing power of coffee brews. In the present work, also high correlations have been found between antioxidant capacity assays and caffeine (r values ranging from 0.906 for Fremy's salt assay to 0.968 for DPPH).

Chlorogenic acids (CGA) are water soluble esters formed between *trans*-cinnamic acids, such as caffeic acid, and quinic acid. They may be subdivided according to the nature, number and position of the cinnamic substituents (Clifford, 1999). Caffeoylquinic acid (CQA) is the most abundant chlorogenic acid class accounting for 76-84% of the total CGA in green coffee (Perrone, Farah, Donangelo, de Paulis, & Martin, 2008). Although during roasting CGA are lost up to 95%, CQA still are the predominant CGA in roasted coffee (Trugo & Macrae, 1984). Monocaffeoylquinic acids (3-CQA, 4-CQA, 5-CQA) and dicaffeoylquinic acids (3,4-diCQA, 3,5-diCQA, 4,5-diCQA) were identified and quantified by HPLC-DAD in each fraction and coffee brew, and the results are shown in Tables 4 and 5. 5-CQA was the major compound among CQAs in all samples, followed by 4-CQA and 3-CQA. The diCQAs were in lower concentration than CQAs. The abundance of 3,4-diCQA and 4,5-diCQA was similar in every coffee fractions or brews, whereas 3,5-diCQA was the least abundant isomer. These results are in agreement with those reported by other authors in roasted coffee (Perrone et al., 2008) and in coffee brew (Alves et al., 2010). Higher amounts of CQA in Robusta coffees than in Arabica ones have been extensively reported (Farah et al., 2005). However, in this study less amounts of CQA were found in Vietnam coffee than in Guatemala ones. Also Vignoli et al. (2011) observed higher amount of 5-CQA

in Arabica soluble coffee. This could be due to several factors, such as the origin of coffee and the higher loss of chlorogenic acids in Robusta coffee during roasting process (Clifford, 1997; Perrone, Donangelo, Donangelo, & Farah, 2010).

Fractions obtained from espresso coffeemaker showed in both coffees a steep decrease with extraction time in all three CQA isomers (3-, 4-, and 5-CQA). F1 (0-8s) accounted for about 70 %, F2 (8-16 s) for 17 % and F3 (16-24 s) for less than 14 % of the total CQA amounts found in an espresso coffee brew. The CQA extraction behavior was similar to that of the antioxidant capacity measured by colorimetric assays and Fremy´s salt, showing high correlations (r values ranging from 0.727 for 5-CQA and DPPH to 0.903 for 4-CQA and Fremy's salt, $p<0.001$), maybe because monocaffeoylquinic acids are the most abundant phenolic compounds in coffee. In contrast, diCQAs were extracted more slowly, accounting F1 for ~50 %, F2 for ~30 % and F3 still for ~20 %, showing correlations coefficients lower than 0.700 (except for 3,4-diCQA with r values ranging from 0.906 for Fremy's salt to 0.968 for DPPH). The esterification of an additional caffeic acid moiety in diCQA increases the number of hydroxyl groups and might favor the retention of these compounds by interaction with melanoidins or other polymeric compounds (Bekedam, Schols, van Boekel et al., 2008; Kroll, Rawel, & Rohn, 2003), reducing the release of diCQA. In fact, the hydrogen bonding between hydroxyl groups of the phenolic compounds and the amide carbonyls of the peptide bond were found to be a common non-covalent interaction between phenolics and melanoidins (Nunes & Coimbra, 2010). Also the weaker polarity of the diCQA compared to the CQA might explain the slower release of these compounds during extraction with water (Kroll et al., 2003). Blumberg et al. (2010) studied the influence of hot water percolation on the concentration of monocaffeoylquinic acids and

chlorogenic acid lactones and reported that dicaffeoylquinic lactones were extracted rather slowly in comparison to monocaffeoylquinic ones.

Caffeoylquinic acids extraction behavior was different in filter coffee, as can be seen in Table 5. Different extraction profiles were also found for the two coffee samples. In Guatemala filter coffee, CQAs and diCQAs extraction showed a U-shape profile with the highest concentration in F1 (0-75 s) and F5 (300-375 s) and the lowest in F3 (150-225 s), similar to that observed for antioxidant capacity according to the correlations showed before. However, in Vietnam filter coffee the U-shape extraction of caffeoylquinic acids started after 75 s, and F1 exhibited the significantly lowest caffeoylquinic acids concentration. The delay in caffeoylquinic acids extraction might be attributed to the longer wetting stage observed in Vietnam coffee, as described above. On the other hand, the increased extraction of caffeoylquinic acids in the last stage of the brewing process, mainly observed in F5 in both coffee samples, could be due to the water pressure decrease that induces a lower flow and a longer contact time between water and ground coffee. This might facilitate the hydrolysis of caffeoylquinic acids bound to melanoidins inducing their release during advanced stages of filter coffee brewing (Lingle, 1996). However, when the lowest volumes of these fractions are taking into account, it could be observed that caffeoylquinic acids only accounted for ~8 % and ~11 % of the total in Guatemala and Vietnam filter coffee brews, respectively. Unlike in espresso coffee, similar extraction percentages among CQAs and diCQAs in each coffee fraction along the filter brewing process were observed. Moreover, when the concentration of antioxidants is calculated per gram of coffee taking into account the different fractions volumes, higher extraction of these phenolic compounds per gram of coffee was obtained in filter coffee brews than in espresso ones, in agreement with Pérez-Martinez et al. (2010). This may be due to the technological differences between

espresso and filter coffeemaker. Although the high water pressure applied in espresso coffeemaker favors the extraction process, the short contact time between water and coffee grounds, the high coffee/water ratio and the limited space in coffee cake does not allow equilibrium to be reached (Petracco, 2005). In contrast, longer time and turbulences in the extraction chamber of the filter coffeemaker allow the water in immediate contact with the coffee to extract additional compounds when it has not become so saturated with dissolved material. Thus, both technological factors might favor the extraction of both CQAs and diCQAs, free and bound with melanoidins. In fact, turbulences are considered, after time and temperature, the third most important factor in filter coffee brewing (Lingle, 1996). Less turbulences during sequential coffee percolation could also be the reason why Blumberg et al. (2010) found that monocaffeoylquinic acids and monocaffeoyl and dicaffeoyl quinides extraction behaviors were more similar to those of our espresso coffee fractions than filter ones, i.e. higher extraction in the first fractions and slower release of dicaffeoyl quinides.

In conclusion, brewing time plays a key role in antioxidants extraction of coffee. To optimize their extraction in order to obtain antioxidants that can be used as ingredients for functional foods, several technological factors should be taken into account. Thus, higher water pressure increases antioxidants extraction speed like in the first fraction of espresso coffee. Nevertheless, parameters like turbulence and longer contact time, typically of a filter coffeemaker, should be considered in order to increase extraction efficiency, mainly in less polar antioxidant compounds as diCQA. Moreover, extraction conditions should also be adjusted for each coffee because cellular structure of coffee beans may also influence. Further research in the influence of technological parameters on chemical composition of coffee brew fractions, as well as their sensory properties, should be needed before to industrial development.

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Table 1. Volumes of coffee brews and fractions obtained by espresso and filter coffeemakers.

	Espresso		Filter	
	$t_{extraction}$ (s)	V (mL)	$t_{extraction}$ (s)	V (mL)
Guatemala				
Coffee brew	24	47	375	532
F1	0-8	16	0-75	80
F2	8-16	14	75-150	146
F3	16-24	17	150-225	186
F4	-	-	225-300	94
F5	-	-	300-375	26
Vietnam				
Coffee brew	24	46	375	520
F1	0-8	17	0-75	74
F2	8-16	14	75-150	120
F3	16-24	15	150-225	160
F4	-	-	225-300	112
F5	-	-	300-375	54

Table 2. pH, browned compounds and caffeine in espresso coffee brews and fractions. All values are shown as mean ± standard deviation (n=3). Different letters indicate significant differences ($p<0.05$) among different coffee fractions in each coffee.

	pH	Browned compounds [Abs_{420}]	Caffeine [mg/100mL]
Guatemala			
Coffee brew	4.01 ± 0.01	0.391 ± 0.013	141.4 ± 2.4
F1	4.83 ± 0.01 a	0.903 ± 0.008 c	296.8 ± 1.6 c
F2	4.90 ± 0.01 b	0.253 ± 0.005 b	82.66 ± 0.7 b
F3	5.09 ± 0.01 c	0.128 ± 0.004 a	39.6 ± 0.4 a
Vietnam			
Coffee brew	5.76 ± 0.00	0.458 ± 0.011	253.3 ± 2.0
F1	5.57 ± 0.01 a	1.172 ± 0.008 c	575.4 ± 3.9 c
F2	6.08 ± 0.01 b	0.297 ± 0.004 b	159.2 ± 0.1 b
F3	6.38 ± 0.00 c	0.133 ± 0.007 a	74.7 ± 0.2 a

Table 3. pH, browned compounds and caffeine in filter coffee brews and fractions. All values are shown as mean ± standard deviation (n=3). Different letters indicate significant differences ($p<0.05$) among different coffee fractions in each coffee.

	pH	Browned compounds [Abs_{420}]	Caffeine [mg/100mL]
Guatemala			
Coffee brew	5.29 ± 0.01	0.200 ± 0.003	57.1 ± 0.1
F1	5.12 ± 0.01 a	0.275 ± 0.002 c	106.8 ± 0.1 e
F2	5.25 ± 0.01 b	0.200 ± 0.002 b	57.1 ± 0.2 c
F3	5.39 ± 0.01 c	0.160 ± 0.005 a	35.7 ± 0.0 a
F4	5.37 ± 0.02 c	0.171 ± 0.005 a	48.6 ± 0.4 b
F5	5.12 ± 0.00 a	0.266 ± 0.007 c	89.0 ± 0.6 d
Vietnam			
Coffee brew	6.07 ± 0.01	0.205 ± 0.001	115.3 ± 0.4
F1	6.14 ± 0.01 c	0.132 ± 0.005 a	65.9 ± 0.6 a
F2	5.93 ± 0.01 a	0.298 ± 0.010 c	158.1 ± 0.3 e
F3	6.06 ± 0.01 b	0.210 ± 0.012 b	112.9 ± 0.2 c
F4	6.19 ± 0.01 d	0.193 ± 0.007 b	104.4 ± 0.8 b
F5	6.08 ± 0.01 b	0.273 ± 0.015 c	117.6 ± 0.9 d

Table 4. Chlorogenic acids in espresso coffee brews and fractions. All values are shown as mean ± standard deviation (n=3). Different letters indicate significant differences ($p<0.05$) among different coffee fractions in each coffee.

	3-CQA [mg/100mL]	4-CQA [mg/100mL]	5-CQA [mg/100mL]	3,4-diCQA [mg/100mL]	3,5-diCQA [mg/100mL]	4,5-diCQA [mg/100mL]
Guatemala						
Coffee brew	43.2 ± 0.1	55.6 ± 0.7	96.7 ± 1.8	5.1 ± 0.1	2.8 ± 0.2	5.0 ± 0.1
F1	91.3 ± 1.3 c	114.6 ± 0.6 c	201.1 ± 1.6 c	9.8 ± 0.2 c	4.2 ± 0.1 c	9.6 ± 0.4 c
F2	26.4 ± 0.4 b	33.8 ± 0.1 b	56.3 ± 0.6 b	6.5 ± 0.4 b	3.1 ± 0.1 b	5.7 ± 0.1 b
F3	15.0 ± 0.0 a	22.4 ± 0.1 a	29.8 ± 0.1 a	3.2 ± 0.0 a	1.8 ± 0.0 a	2.9 ± 0.0 a
Vietnam						
Coffee brew	25.8 ± 1.2	35.0 ± 0.2	52.9 ± 2.0	4.1 ± 0.0	2.0 ± 0.0	3.8 ± 0.1
F1	49.3 ± 0.6 c	70.4 ± 0.5 c	108.0 ± 2.9 c	7.8 ± 0.2 c	2.7 ± 0.2 c	5.4 ± 0.1 c
F2	16.0 ± 0.1 b	20.5 ± 0.1 b	30.8 ± 0.2 b	4.6 ± 0.0 b	1.7 ± 0.1 b	3.8 ± 0.1 b
F3	9.4 ± 0.3 a	13.0 ± 0.2 a	16.0 ± 0.4 a	2.1 ± 0.0 a	0.8 ± 0.0 a	1.7 ± 0.1 a

Table 5. Chlorogenic acids in filter coffee brews and fractions. All values are shown as mean ± standard deviation (n=3). Different letters indicate significant differences ($p<0.05$) among different coffee fractions in each coffee.

	3-CQA [mg/100mL]	4-CQA [mg/100mL]	5-CQA [mg/100mL]	3,4-diCQA [mg/100mL]	3,5-diCQA [mg/100mL]	4,5-diCQA [mg/100mL]
Guatemala						
Coffee brew	17.0 ± 0.1	25.3 ± 0.0	38.7 ± 0.1	3.8 ± 0.0	2.0 ± 0.0	3.2 ± 0.0
F1	31.0 ± 0.3 e	40.9 ± 0.1 d	70.1 ± 0.3 e	6.1 ± 0.3 d	2.9 ± 0.1 c	6.0 ± 0.0 d
F2	16.8 ± 0.4 c	26.2 ± 0.2 c	38.2 ± 0.7 c	3.8 ± 0.1 b	2.0 ± 0.1 b	3.9 ± 0.1 b
F3	11.0 ± 0.1 a	16.8 ± 0.0 a	24.8 ± 0.2 a	2.7 ± 0.1 a	1.4 ± 0.0 a	2.7 ± 0.0 a
F4	14.7 ± 0.2 b	23.0 ± 0.2 b	34.1 ± 0.4 b	3.8 ± 0.2 b	2.1 ± 0.1 b	3.7 ± 0.0 b
F5	24.5 ± 0.8 d	42.0 ± 0.9 d	61.3 ± 1.0 d	4.4 ± 0.0 c	3.0 ± 0.1 c	4.3 ± 0.1 c
Vietnam						
Coffee brew	15.0 ± 0.1	19.4 ± 0.0	21.8 ± 0.2	3.1 ± 0.0	0.7 ± 0.0	1.2 ± 0.0
F1	10.6 ± 0.2 a	13.1 ± 0.1 a	14.3 ± 0.2 a	2.0 ± 0.0 a	0.3 ± 0.0 a	0.7 ± 0.0 a
F2	18.5 ± 0.2 d	24.4 ± 0.0 e	28.3 ± 0.4 d	4.2 ± 0.1 e	0.9 ± 0.0 d	1.6 ± 0.0 d
F3	14.6 ± 0.1 bc	19.1 ± 0.0 c	21.2 ± 0.2 b	2.9 ± 0.0 c	0.7 ± 0.0 b	1.2 ± 0.0 b
F4	14.5 ± 0.3 b	18.3 ± 0.0 b	20.4 ± 0.5 b	2.8 ± 0.0 b	0.7 ± 0.0 b	1.2 ± 0.0 b
F5	15.2 ± 0.4 c	20.4 ± 0.1 d	22.6 ± 0.6 c	3.6 ± 0.0 d	0.8 ± 0.0 c	1.5 ± 0.0 c

FIGURE CAPTIONS

Figure 1. Antioxidant capacity (Folin-Ciocalteau method) of coffee brews and fractions obtained by espresso (**a)** and filter coffeemaker (**b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

Figure 2. Antioxidant capacity (ABTS method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

Figure 3. Antioxidant capacity (DPPH method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

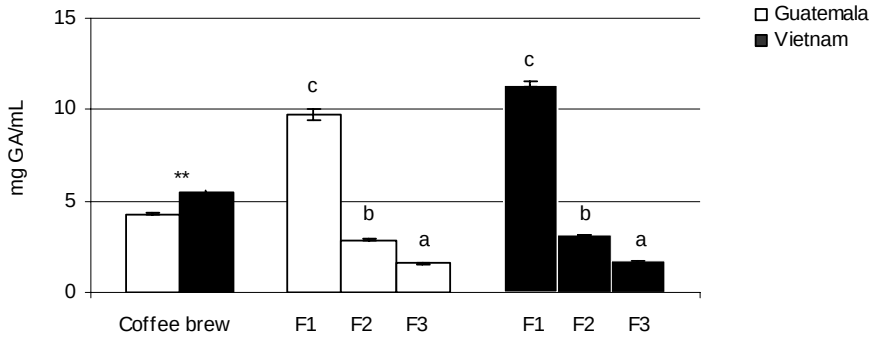
Figure 4. Antioxidant capacity (Fremy's Salt method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) and ns nonsignificant differences ($p>0.05$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

Figure 5. Antioxidant capacity (TEMPO method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) and ns

nonsignificant differences ($p>0.05$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

Figure 1. Antioxidant capacity (Folin-Ciocalteau method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

a) Espresso coffeemaker



b) Filter coffeemaker

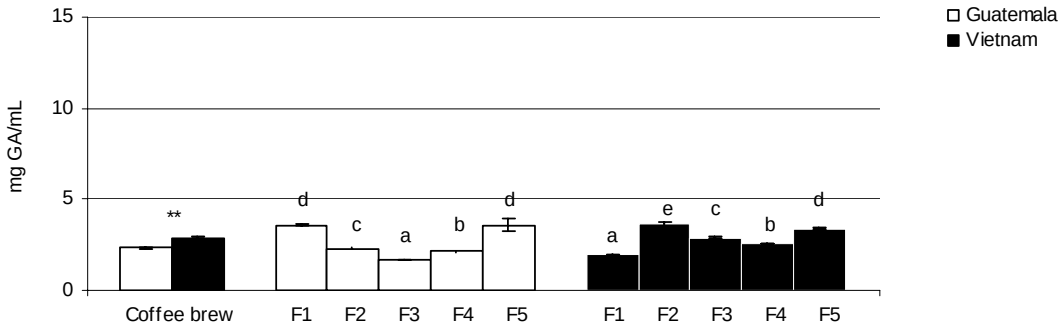
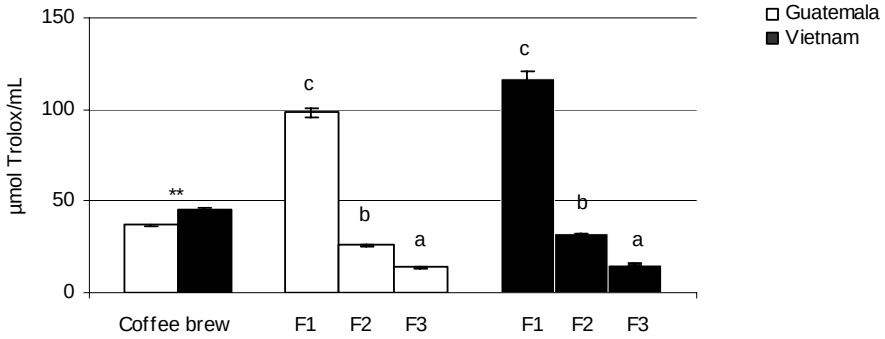


Figure 2. Antioxidant capacity (ABTS method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

a) Espresso coffeemaker



b) Filter coffeemaker

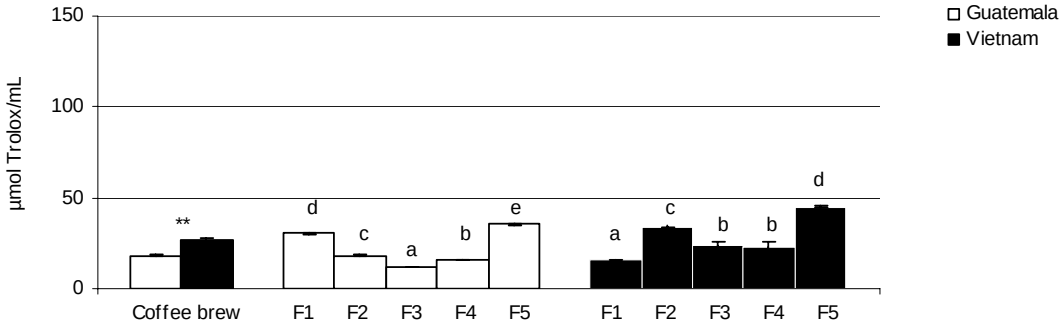
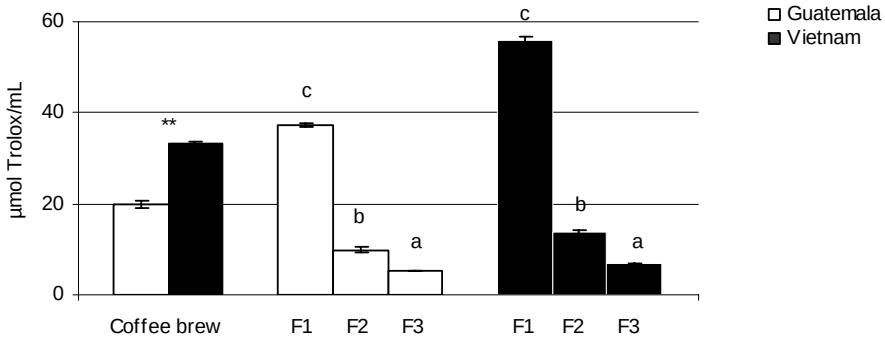


Figure 3. Antioxidant capacity (DPPH method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

a) Espresso coffeemaker



b) Filter coffeemaker

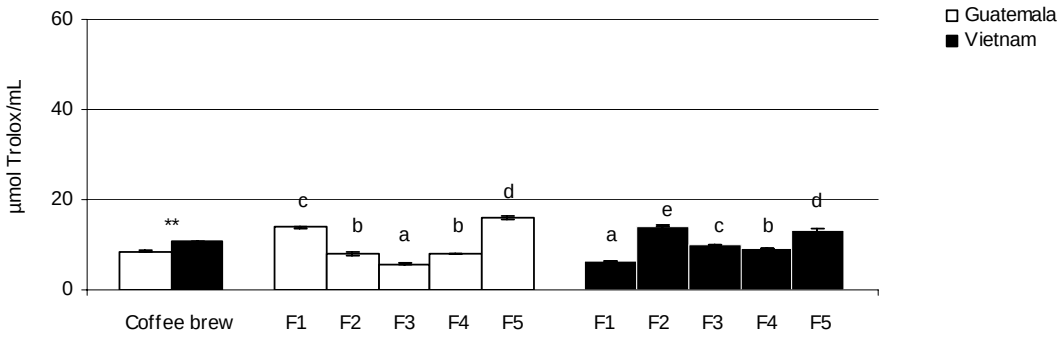
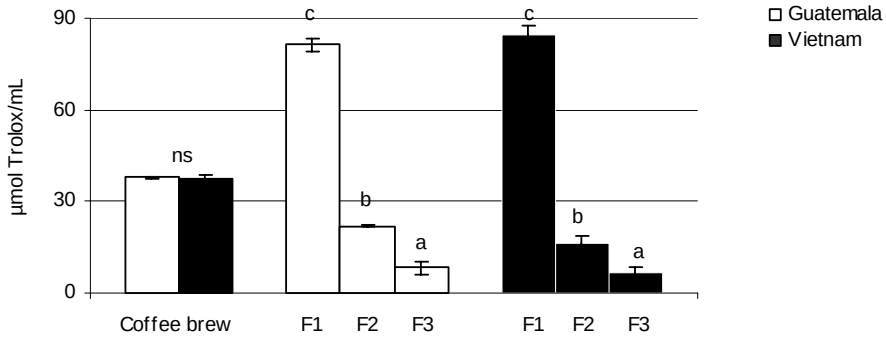


Figure 4. Antioxidant capacity (Fremy's Salt method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) and ns nonsignificant differences ($p>0.05$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

a) Espresso coffeemaker



b) Filter coffeemaker

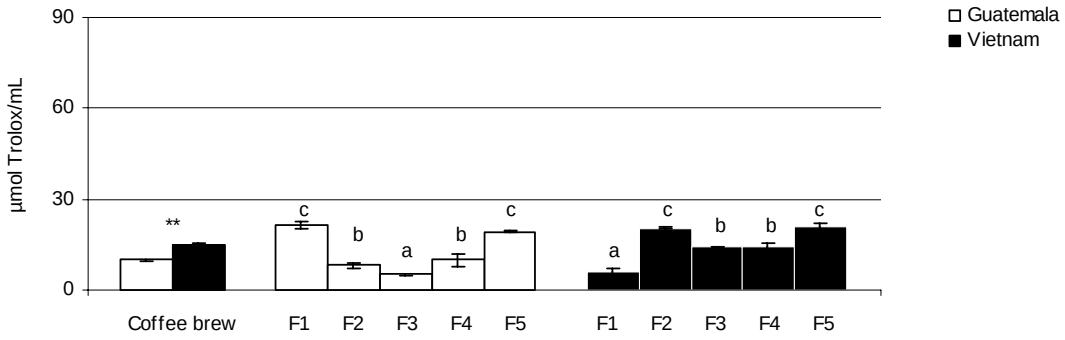
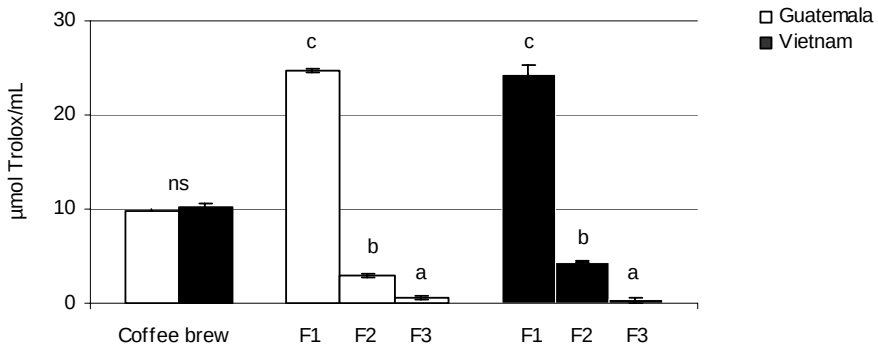


Figure 5. Antioxidant capacity (TEMPO method) of coffee brews and fractions obtained by espresso **(a)** and filter coffeemaker **(b)**. All values are shown as mean ± standard deviation (n=3). ** indicates highly significant differences ($p<0.01$) and ns nonsignificant differences ($p>0.05$) between coffee brews. Different letters indicate significant differences ($p<0.05$) among coffee fractions in each coffee.

a) Espresso coffeemaker



b) Filter coffeemaker