Investigation on the extractability of melanoidins in portioned espresso coffee

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ABSTRACT

Coffee melanoidins have attracted interest as a result of its potential health benefits. This investigation aims to elucidate the extraction behavior of melanoidins and their populations during the preparation of portioned espresso coffee and its relationship with the antioxidant activity of the coffee brew. Filter-paper pods, FAP capsule, and clone capsule containing light roasted coffee have been investigated. An accumulative fractionation approach has applied to model the extraction kinetics of melanoidins, melanoidin populations, browning, chlorogenic acids (CGA), and antioxidant activity. Melanoidins were very efficiently extracted in clone capsules since less than 9 s was necessary to extract the 50% of the melanoidin content as compared with pods and FAP capsules, and the kinetic of extraction is slower than CGA. The extraction profile of melanoidins and browning fitted better with the antioxidant capacity than CGA and total solids profile. Melanoidin populations were obtained according to ethanol solubility. Total melanoidin content and the ratio between melanoidin populations did not change during extraction volume for espresso coffee. Melanoidin populations soluble at 75% ethanol showed the highest antioxidant activity. However, melanoidins with higher antioxidant activity are extracted at higher volumes. This investigation could make possible the adjustment of the technological requirements of espresso coffeemakers to produce an espresso coffee with high levels of beneficial compounds.

KEYWORDS: Espresso coffee, Portioned coffee, Pods, Melanoidins, Antioxidant capacity, Extraction, Kinetics.

INTRODUCTION

In addition to the stimulatory properties, coffee consumption is related to certain health benefits, such as anti-inflammatory and antimutagenic effects, reduction of cardiovascular risk, rheumatologic diseases, endometrial tumors, Parkinson's disease, Alzheimer's disease, and the regulation of insulin or body weight, among other (Bichler et al., 2007; Cavin et al., 2008; Lindsay et al., 2002; Morales, Somoza, & Fogliano, 2012; Ranheim & Halvorsen, 2005; Salazar-Martinez et al., 2004; Wang, Qian, & Yao, 2011). Coffee brew is a source of caffeine, hydroxycinnamates, particularly chlorogenic acids (CGA), antioxidants and indigestible fiber (Díaz-Rubio & Saura-Calixto, 2007; Farah & Donangelo, 2006; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Some of the positive physiological effects of coffee consumption have been explained by the antioxidant properties of its constituents, such as the presence of phenolic compounds and Maillard reaction products (Borrelli, Visconti, Mennella, Anese, & Fogliano, 2002; Daglia, Papetti, Gregotti, Bertè, & Gazzani, 2000; Delgado-Andrade, Rufián-Henares, & Morales, 2005). Human trials considering the Italian consumer average coffee intake (5 cups of espresso per day) found that coffee was able to significantly increase plasma glutathione concentration (Esposito et al., 2003). The contribution of coffee consumption to the overall intake of antioxidants may reach up to 70% in Western diets (Torres & Farah, 2010) but also represents a significant portion of daily intake of fiber (Díaz-Rubio & Saura-Calixto, 2007).

During the roasting of coffee green beans chemical and structural changes take place as a result of the Maillard reaction, caramelization and pyrolysis. The first consequence of roasting is the loss of thermolabile compounds such as trigonelline and chlorogenic acids in a different extent according to the roasting degree and also the formation of new compounds such as melanoidins (Farah, de Paulis, Trugo, & Martin, 2005; Illy & Viani, 2005). Melanoidins are high molecular weight (HMW) Maillard reaction end-products, which are a

heterogeneous mixture of brown colored, nitrogen containing polymers, formed through the reaction of reducing sugars with proteins/amino acids (Moreira, Nunes, Domingues, & Coimbra, 2012). Coffee polysaccharides, galactomannan-like and arabinogalactan-like carbohydrates, proteins, and phenolic compounds, mainly hydroxycinnamates, contribute to the formation of coffee melanoidins (Bekedam, Schols, van Boekel, & Smit, 2008; Moreira et al., 2012; Nunes & Coimbra, 2007). Coffee brew is one of the main sources of melanoidins in the human diet and the intake of coffee melanoidins estimated to range between 0.5 and 2.0 g per day for moderate and heavy consumers, respectively (Fogliano & Morales, 2011). The impact of melanoidins to coffee brew is not limited to their color contribution since these molecules are involved in the modulation of flavor release, antioxidant and metal-chelating properties, and dietary fiber behavior (Hofmann, Czerny, Calligaris, & Schieberle, 2001; Morales et al., 2012; Moreira et al., 2012; Reichardt, Gniewchitz, Steinhart, Bunzel, & Blaut, 2009). Therefore, melanoidins (including also melanoproteins from bakery products) may contribute 20.2% to the daily antioxidant capacity intake, more than 50% corresponding directly to coffee melanoidins (Pastoriza & Rufián-Henares, 2014). Silván, Morales, and Saura-Calixto (2010) suggested a new definition of coffee melanoidins as a maillardized fiber with potential beneficial effects on gastrointestinal health by combining the antioxidant activity of melanoidins and the low transit of these polymers (Borrelli, Esposito, Napolitano, Ritieni, & Fogliano, 2004). Indeed, condensation of phenolic compounds with indigestible polysaccharides and melanoidins present in coffee could explain the association between coffee consumption and the antioxidant capacity of feces from healthy subjects (Garsetti, Pellegrini, Baggio, & Brighenti, 2000).

There are many different devices and methods to produce coffee brew, which are generally characterized by extraction pressure, extraction process, extract/cup volume, and solid content (Parenti et al., 2014; Petracco, 2005). There is not established an official definition for espresso coffee but it could be described as the brew obtained by percolation of hot water under pressure through compacted cake of roasted ground coffee, where the energy of the water pressure is spent within the cake (Illy & Viani, 2005). During espresso brewing, water at approximately 9 bars and 88–92 °C is forced to go through coffee grounds compacted in a small brewing chamber (coffee cake) (Albanese, Di Matteo, Poiana, & Spagnamusso, 2009). Temperature, pressure, brewing time, coarse grind and ground coffee portion, cake shape and moistening, and water quality, among others, have an influence on the physical and sensorial properties of the espresso coffee and the effectiveness of the extraction process (Albanese et al., 2009; Petracco, 2005). The overall quality of espresso coffee depends largely on the quality of the roasted coffee, but also on the extraction procedure.

Extensive studies have been focused on the effect of different physical variables during the percolation process on the extraction of caffeine, CGA, key aroma compounds and sensory analysis (Andueza, de Peña, & Cid, 2003; Andueza et al., 2002; Gloess et al., 2013; Illy & Viani, 2005; López-Galilea, de Peña, & Cid, 2007; López-Galilea, Fournier, Cid, & Guichard, 2006; Maeztu et al., 2001). Ludwig et al. (2012) investigated 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. This investigation concluded that more than 70% of the antioxidants (except dicaffeoylquinic acid) of an espresso coffee were extracted during the first 8 s.

Nowadays, portioned coffee is being well accepted by consumer and a number of commercial brands of single-dose and formats are already present in the market. Recently, Mestdagh, Davidek, Chaumonteuil, Folmer, and Blank (2014) reported the kinetics of coffee aroma extraction during espresso coffee preparation by applying an accumulative approach for fraction collection. To our knowledge, investigation on the extraction of melanoidins in filtered and espresso coffee is scarce, and they were considered as the browning rate and samples were sequentially collected during coffee brewing (Ludwig et al., 2012). Then a complete understanding of the kinetic of melanoidins extraction during preparation of espresso coffee is missing. Since melanoidins have attracted huge interest as a result of claims for potential health benefits, antioxidant activity and sources of dietary fiber, this investigation aims to elucidate the extraction behavior of melanoidins and their populations during the preparation of portioned espresso coffee. In addition, the antioxidant capacity related to the melanoidins extraction, browning and CGA content is also investigated.

MATERIALS AND METHODS

Materials

Four portioned coffee samples were supplied by three Spanish coffee companies. Single-dose samples were freshly produced, commercially available and identified as gourmet coffees roasted under light medium conditions. Sample F was filter-paper pods hermetically sealed between a two fine layers of filter paper and individually packaged in a protected atmosphere. Sample B was FAP capsule packaged into a biodegradable plastic capsule in a protected atmosphere, which preserves the quality of the ground coffee by protecting it against moisture and oxidation processes. Samples NC and NB were clone capsules closed with aluminum foil (Nespresso® compatible). Pure Arabica coffee (*Coffea arabica*) from Colombia was used for samples F, B, and NC. An Arabica and Robusta coffee (*Coffea canephora*) blend from Vietnam, Guatemala, and Brazil at percentage 35:65 was used for sample NB. Samples were stored at 12–16 °C and kept 1 h at ambient temperature before use. According to the coffee companies, the grinding grade of the samples was the most adequate to each type of container and coffee machine. Technical characteristics of the espresso coffeemakers, containers, and coffee are summarized in Table I.

Preparation of coffee brew

Extraction was performed with three commercial espresso coffee machines and customized to filter-paper pods (F), FAP capsules (B), and clone capsules (N) as described in Table I. Mineral water (Solan de Cabras, Cuenca, Spain) was used with next characteristics (mg/L); 260 dry matter, 284 bicarbonate, 5.1 sodium, 1.1 potassium, 7.4 chloride, 21.3 sulfate, 59.4 calcium, and 7.2 silica. Coffee brew fractions F1, F2, F3, and F4 were collected with final volumes of 5, 9, 16, and 46 mL for coffee samples F, B, NC, and NB. Samples were collected in marked containers for volume. In addition, a fifth fraction of 115 mL was collected for samples F and B, and 85 mL for coffee samples NB and NC. Coffee machine N has a preselected volume of 46 mL for espresso coffee and 96 mL for Longo coffee. Coffeemakers F and B had a variable control of the volume. Accumulative fraction collection (each fraction contains the previous ones plus certain additional volume) was chosen for higher precision than the sequential collection. Fraction F4 and F5 are also identified as conventional espresso coffee and Longo coffee, respectively. Percolation times ranged from 3 to 80 s regarding the espresso machine and fraction collected. The manufacturer instructions for brewing and adequate machine settings, including preheating, were followed before each coffee preparation. At least an average of 10 extractions were performed and pooled for each fraction. Coffee fractions were lyophilized (VirTis Benchtop-6 KB, SP-Scientific, Ipswich, UK) and stored at –18 °C until use. In other case, coffee brew was freeze from three different extractions. The temperature profiles (n=3) were registered by digital thermometer (Model HD2107.1, Delta OHMSRL, Italy) linked to two thermocouples K type ($\varnothing = 0.5$ mm) located at the exit and at bottom of the cup.

Basic determinations

pH was determined using an electronic pH meter (Schottmodel CG-837, Mainz, Germany). Total solids content in coffee brew and fractions were determined gravimetrically after sample lyophilization of coffee brew. Additionally, total solid content in fraction F4 and F5 was calculated by oven drying 25 mL of coffee brew at 105 °C until constant weight (18 h). The rate of extraction was defined as the percentage of total solids with respect to ground roasted coffee dosage in the container. The maximum rate of extraction in pods and capsules was calculated after manually withdrawing of ground coffee in the container (n = 5) and manually extracting the ground coffee (7 g) with 100 mL of water at 80 °C under stirring for 5 min. The resulting coffee brew was filtered (Whatman Filter Paper no 40, Whatman, U.K.), cooled-down, and oven dried. Results are expressed as gram of solids extracted per 100 g of coffee. Concentration was defined as

the ratio between total solid content and the volume of the coffee brew. The total carbohydrate content, calculated as the sum of anhydro sugars, was determined using the phenol-sulfuric acid method as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Standard calibration curves were obtained using mannose and arabinose, respectively.

Determination of chlorogenic acids and caffeine

Caffeine and total chlorogenic acids (CGA) were determined according the method described by Farah et al. (2005) and Fujioka and Shibamoto (2008). One milliliter of coffee brew was mixed with 7mL of a 40% methanolic solution. After treatment with Carrez-I and Carrez-II solutions, supernatant was filtered through a 0.45 μm filter and the samples were then analyzed with HPLC. Quantification was conducted with a Shimadzu HPLC system (Kyoto, Japan) equipped with an LC-20 AD pump, an SIL-10ADvp autosampler, a CTO-10ASVP oven, and an SPD-M20A diodearray detector. The chromatographic separation was carried out on a Mediterranean Sea ODS-2 column (250 $\times$ 4.0 mm, 5 μm , Tecknokroma, Barcelona, Spain). The flow rate was 1.0 mL/min, and the injection volume was 10 μL . The mobile phase consisted of 0.5% acetic acid in water (v/v) (solvent A) and methanol (solvent B), and the gradient program was as follows: 0 min, 15% B; 3 min, 15% B; 35 min, 35% B; 40 min, 60% B; 48 min, 80% B; 50 min, 15% B; and 60 min, 15% B. The total running time was 60 min, and chromatograms were further analyzed at 276 nm (for caffeine determination) and 325 nm (for CGA determination). CGAs content in coffee was determined as the sum of caffeoylquinic acids, feruoylquinic acids, and dicaffeoylquinic acids by using 5-caffeoylquinic acid as an external standard for calibration. The limit of quantification was set at 45.5 mg/100 mL and 4.1 mg/100 mL for caffeine and CGA analysis, respectively.

Determination of browning

Coffee brew fractions (1 mL) were diluted 20-fold with water and 200 μL placed in a 96-wells plate. Browning at 420 nm was rapidly measured at room temperature using a BioTek SynergyTM HT-multimode microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA).

Determination of antioxidant activity

The antioxidant activity was estimated in terms of ABTS radical scavenging activity as described by Delgado-Andrade et al. (2005). Briefly, ABTS radical was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Coffee brew fractions and melanoidins fractions were properly diluted in water, and 40 μL of the diluted samples ($n = 4$) was added with 200 μL of water and 60 μL of ABTS solution at 37 °C in dark. Water was used as blank. Absorbance at 734 nm was taken after 10 min using a BioTek SynergyTM HT-multimode microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). Trolox (16 to 500 $\mu\text{mol/L}$) was used for calibration and results were expressed as μmol Trolox equivalent antioxidant capacity (TEAC) /L of coffee brew or μmol TEAC/g of coffee.

Determination of coffee melanoidin content by spectrophotometry

Melanoidins from fractions F1, F2, F3, F4, and F5 were obtained after ultrafiltration (10 kDa cutoff) as described by Delgado-Andrade and Morales (2005). Coffee fractions were diluted to a solid content between 0.8 and 1.7mg/mL. Then samples were subjected to ultrafiltration using a MicroconYM-10 regenerated cellulose 10 kDa (Millipore, Bedford, MA) at 12000g for 10 min. The retentate containing the high molecular weight (HMW) material, corresponding to coffee melanoidins, were recovered by inverting the Microcon device and centrifuged at 1000 g for 10 min. Finally, the retentate was dissolved in 0.45 mL of distilled water and measured at 405 nm as described by Bekedam, Schols, Van Boekel, and Smit (2006) but

adapted to a microplate reader (Synergy-HT, BioTEK Instruments, Winooski, VT, USA). Coffee melanoidins were quantified using the external standard method within the range of 0.1–1 mg/mL with a reference coffee melanoidin. Reference coffee melanoidin was prepared from medium roasted coffee beans (*C. arabica*) as described elsewhere (Silván et al., 2010). Sample reporting levels outside the calibration range were additionally diluted 10-fold. Melanoidins content ($n = 3$) was expressed as mg/mL coffee brew fraction or mg/g ground coffee.

Fractionation and isolation of melanoidin populations

Melanoidins corresponding to the highmolecular weight (HMW) material of espresso coffee were isolated from coffee fractions F1, F3, and F4 of coffee sample F based on the procedure described by Delgado-Andrade and Morales (2005). Lyophilized coffee fractions (1 g) were dissolved in 200 mL of water and ultrafiltrated using an Amicon ultrafiltration cell (Model 8400, Amicon, Weymouth, MA, USA) with a nominal molecular weight cutoff of 10 kDa. The retentate corresponding to melanoidins was filled to 200 mL with water and ultrafiltered again at least three times, and then retentate (approximately 50 mL) was freeze-dried and stored. The HMW material was separated into four fractions (V1, Et50, Et75, and Sn75) according to the procedure described by Nunes and Coimbra (2007). Galactomannans and melanoagalactomannans can be obtained by precipitation in 50% ethanol solutions (Et50), arabinogalactans, and melanoarabinogalactans can be obtained by precipitation in 75% ethanol solutions (Et75), and a second melanoarabinogalactan fraction and the melanoidins of low complexity in carbohydrates remain soluble in 75% ethanol solutions (EtSn). Freeze-dried HMW material (300 mg) was dissolved in 20 mL of water and kept at 4 °C for 1 h. The residue (V1) was removed by centrifugation (5000 rpm, 10 min, 4 °C). The supernatant was added drop wise with 20 mL of absolute ethanol during 15 min of constant stirring. The resulting solution containing 50% ethanol was kept at 4 °C for 1 h. The residue (Et50) was removed by centrifugation. The supernatant was extended with absolute ethanol to reach a 75% ethanolic solution. The residue (Et75) was removed by centrifugation (5000 rpm, 10 min, 4 °C), and the supernatant (Sn75) was separated. Ethanol was removed by flushing nitrogen and rotary evaporation at 40 °C, and then samples were lyophilized and the yield was calculated.

Curve fitting and parameter estimation

SigmaPlot 12.5 (Systat Software, Chicago, IL) was used to fit 2-parameter hyperbolic single rectangular curves to describe the in-cup extraction kinetics of each coffee constituent. Data were adjusted to $f(y) = a \cdot x / (b + x)$. Data set at zero was also included considering an extraction rate of 0 for 0 mL. Graphs were constructed from 2000 iterations. Reduced chi-square was used to compute standards error in weighted regression. Shapiro–Wilk test was used for normality test at a significance level 0.05. Parameters V50, V95, and Vmax meant the milliliters necessary to reach the 50%, 95%, and maximum rate of the parameter measured (melanoidins, browning, antioxidant activity, and total CGA content) as normalized to fraction F5. Additionally, results for 46 mL of extraction volume corresponding to fraction F4 are also calculated from the non-linear regression model. Representations were normalized to fraction F5 (large coffee brew) for comparison purposes. Statistical analyses were performed using the Statgraphics Centurion XV statistical program (Herndon, VA). Data were expressed as the mean value $\pm$ SD. Analysis of variance (ANOVA) and the Duncan test were applied to determine differences between means. Differences were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Description of coffeemakers, single-dose units, and ground coffee

Nowadays, there are many devices and methods to produce espresso coffee, although the use of single-dose units (pods and capsule systems) is growing among consumers and gaining market share because they are

user-friendly. The overall quality of a cup of coffee depends on the raw material but also on the extractability of a myriad of volatile and non-volatile compounds that makes the taste unique (Petracco, 2005). It is well known that percolation is a very complex phenomenon to model but it depends on the ground coffee portion, particle size distribution, cake porosity, cake shape, water quality, temperature, pressure, percolation time, cake moistening, machine cleanliness, etc. (Parenti et al., 2014; Petracco, 2005).

In this investigation, three commercial available espresso coffeemakers for single-dose units were selected and their technical characteristics are described in Table 1. Three samples (F, B, and NC) were from the coffee specie most commercialized worldwide *C. arabica*. Sample NB is a coffee commercial product that consists of blend of *C. Arabica* and *C. canephora*. Espresso coffeemakers have common features such as the application of constant pressure that forces water through the coffee grounds with a constant flow. The filter-paper pods (sample F) used for machine F has the largest contact area and lower extraction flow as compared with FAP capsules (sample B) used in machine B or clone capsules (samples NC, and NB) used in machine N. The espresso coffeemaker using the capsules with a clone format has the highest ratio water/coffee for an espresso coffee (46 mL), followed by filter-paper pods, and FAP capsules. Clone capsules have the lowest ground coffee content but the highest ratio water to coffee. The maximum extraction rate was not significantly different among the samples, and it reached an average about 27% (Table 1). This fact is important since the final extraction rate obtained at fraction F5 will be due to the efficacy of each coffeemaker and the type of single-dose container.

Ground coffee was partly characterized for the total carbohydrate, caffeine, CGA, and melanoidin content as summarized in Table 2. Total carbohydrate content (expressed as mannose equivalents), caffeine, and total CGA ranged from 33 to 46 g/100 g, from 1.1 to 1.8 g/100 g, and from 0.79 to 1.6 g/100 g, respectively. Carbohydrates, caffeine, and CGA contents are in line with literature (Farah, 2012). As expected higher content of CGA, carbohydrates and caffeine was obtained in the Arabica/Robusta blend (sample NB) as compared with Arabica (sample NC). Sample F showed the higher melanoidin content (11 g/100 g coffee) as compared to capsules (about 7.5 g /100 g coffee). All the samples showed a melanoidin content close to mild roasting conditions where their formation is still low (Silván et al., 2010).

It is well known that the concentration of coffee constituents in the brew is affected by the type and origin of ground coffee, roasting degree, and brewing procedure (Andueza et al., 2003; Petracco, 2005). Extraction for pods and capsules was further investigated by applying an accumulative fractionation approach during the brewing process as described by Mestdagh et al. (2014). Five consecutive different fractions were collected. For instance, fraction F3 contains fraction F2 plus and additional volume. Fig. 1 describes the temperature-volume profile for the three espresso coffeemakers using filter-paper pods, FAP capsules and clone capsules. Dotted lines represent the volume of the five fractions collected. Coffee brew volume for fractions F1, F2, F3, and F4 (corresponding to espresso coffee) was the same for all samples. However, fraction F5 (corresponding to Longo coffee) was 115 mL for pods and FAP capsules and 85 mL for sample clone capsules. Traditionally, espresso coffee is brewed in short volumes (20 mL to 50 mL), and Longo is a less intense beverage characterized by a larger cup size (100 mL up to 250 mL), where size depends on culture habits, lifestyle, social and working environment, everyday habits and financial considerations, and flavor preference (Parenti et al., 2014; Petracco, 2005). Percolation time for fraction F5 was 80 s, 70 s for pods and FAP capsules, and 44 s for clone capsules. The lowest extraction flow was for coffeemaker for pods with 1.5 mL/s (Table 1). The temperature at the exit was $87.13\text{ }^{\circ}\text{C} \pm 5.83$, $85.6\text{ }^{\circ}\text{C} \pm 0.35$, and $90.85\text{ }^{\circ}\text{C} \pm 0.92$ for coffeemakers F, B, and N, respectively. Thermal profiles were registered during the extraction process.

After a phase of instability at the beginning, temperatures stayed around a mean temperature (Albanese et al., 2009).

Extraction rate

It is expected that the extraction efficiency of the coffee constituents was driven by their solubility in water (Gloess et al., 2013; Parenti et al., 2014; Petracco, 2005). Higher volume of water during extraction, as for Longo coffee in fraction F5, should lead to higher extraction efficiency (Ludwig et al., 2012; Mestdagh et al., 2014; Parenti et al., 2014). The extraction rate for each coffeemaker and single-dose container is depicted in Fig. 2. Extraction rate increases very rapidly in all samples with the extraction volume although conditions for the espresso coffee (fraction F4) are already close to the maximum rate achieved in Longo coffee (fraction F5). After that, an increment in the percolation time did not reflect a significant increment in the extraction rate. It is noteworthy that each sample reached a different final extraction rate varying from 20.4% to 27.1%. If compared the final extraction rate with the maximum extraction rate described in Table 1, single-dose pod behaved very efficiently since nearly 100% of the maximum extraction rate was reached. The lowest extraction rates were obtained for B, NC, and NB samples, 78.5%, 93.5%, and 85%, respectively, as compared with the maximum extractability. In terms of solids content, fraction F5 contains 16.2 ± 0.42 , 13.9 ± 1.58 , 15.1 ± 1.00 , and 14.2 ± 0.53 mg/mL of coffee brew for F, B, NC, and NB, respectively. For comparative purposes, data for density, pH, and concentration of fraction F4 (espresso coffee) is summarized in Table 1.

Different coffeemakers, single-dose units (pods, and capsule), and ground coffee with different origin and likely roasting degree are used in this investigation. There are many physical parameters governing the extraction such as temperature inside the cake, the pressure above and under the cake, percolation time, dose, particle size and shape, compacting of the bed, etc (Albanese et al., 2009; Andueza et al., 2002; Andueza et al., 2003; Illy & Viani, 2005; Petracco, 2005). Table 2 summarizes the chemical characterization of fraction F5 for samples F, B, NB, and NC in terms of total carbohydrate, caffeine, CGA, melanoidins, total solids content, browning, and antioxidant capacity. Since every sample has a different composition, it is not reliable a direct comparison among samples without a normalization of the process. The kinetic for the extraction was normalized to fraction F5 (corresponding to Longo coffee) since it contains every previous fractions.

Extractability of melanoidins

The kinetic of the melanoidins extraction for sample F, B, and NC is depicted in Fig. 3a. The extraction profile for NB (arabica:robusta blend) is not displayed to maintain clearer the graphical representation. Table 3 summarizes the parameters and adjustment of the fit, including calculation of the extraction volume necessary to reach the 50% (V50), 90% (V90), and maximum (Vmax) melanoidin content. The extractability of the melanoidins behaves significantly different according to the type of single-dose unit used. Melanoidins were efficiently extracted in NC samples since less than 9 s was necessary to extract the 50% of the melanoidin content. In contrast, melanoidins in sample B were slowly extracted during brewing, and 18 s was necessary to reach the 50% of the melanoidin content in fraction F5. Extrapolating the model for espresso coffee (fraction F4, 46mL), coffee brewed from clone capsules allows significantly a faster extraction of melanoidins (94.3 % of the total) than filter-paper pods (84.8 %) and FAP capsules (77.4 %), regardless the initial melanoidin content in the Arabica ground coffee.

Melanoidins are defined as polymeric brown-color material. Indeed, previous investigation used the browning rate as an approximation of the melanoidin content (Ludwig et al., 2012). Browning during the

brewing of coffee from pods and capsules was also modeled. Fig. 3b depicts the browning of the different coffee fractions and the model is normalized to fraction F5. In contrast to the melanoidins profile, sample F and B did not show significant differences during the extraction. However, the extractability of brown colored compounds in fraction F4 was significantly higher for sample NC (93.8 %) as compared with samples F (81.8%) and B (82.2%) (Table 3).

Extractability of browning rate, antioxidant compounds and CGA

Coffee melanoidins have different functional properties apart from their contribution to color and technological properties, being able to bind flavors, exerting antioxidant capacity to foods and suppressing oxidative stress in cells, metal-chelating properties, antimicrobial activity, antihypertensive, suppressing *Helicobacter pylori* adhesion, modulating chemopreventive enzymes, and prebiotic activity, among others (Fogliano & Morales, 2011; Wang et al., 2011). Perhaps the most relevant characteristic of coffee melanoidins is their antioxidant capacity. Literature suggests that the main compounds responsible for the antioxidant activity in roasted coffee are several phenolic compounds naturally present in green coffee beans and Maillard reaction products formed in the roasting process (Borrelli et al., 2002; Farah & Donangelo, 2006). Antioxidant capacity of the fractions collected during coffee brewing was investigated as depicted in Fig. 3c, and adjustment of the fit is described in Table 3. Again, espresso coffeemakers using clone capsules extracted significantly faster the compounds accounting to the overall antioxidant capacity than filter-paper pods and FAP capsules. Nearly 90% of the total antioxidant capacity of coffee brew in fraction F4 was already extracted in clone capsules, 83% for filter-paper pods, and 79% for FAP capsules.

Despite to melanoidins and low molecular weight (LMW) Maillard reaction products, CGA are the main contributor to the antioxidant capacity of the coffee brew. Fig. 3d depicts the extraction kinetics for CGA and parameters for the fit are summarized in Table 3. Both filter paper pods and clone capsules extracted very efficiently (N90% from the total) the CGA in espresso coffee. There is no significant differences in the extraction profile for filter-paper pods and clone capsules (N 94%), but significant lower extraction rate was reached for FAP capsules (87%) in espresso coffee.

According to literature, CGA are considered to be the major contributors to the antioxidant capacity of coffee brew, followed by melanoidins, and the LMW compounds formed during coffee roasting (Daglia et al., 2000; Delgado-Andrade et al., 2005; Vignoli, Bassoli, & Benassi, 2011). Ludwig et al. (2012) described that espresso machine increases extraction speed of browning and antioxidants in coffee brew as compared with filter coffee. Fig. 4 depicts the relationship between the antioxidant capacity for each single-dose unit and browning rate, melanoidins, solids, and CGA content. The extraction profile of all these parameters are directly related to the antioxidant capacity in each fraction. However, extraction profile of melanoidins and browning fitted better with the antioxidant capacity for all the fractions than CGA and total solids profile. These results are in line with Coelho et al. (2014), who found a significant correlation between the amount of phenolic compounds incorporated to melanoidins and the amount of protein and color characteristics of the different melanoidin populations being observed. In fractions F1, F2, or F3 for pods (Fig. 4a), FAP capsule (Fig. 4b), and clone capsules with blend (Fig. 4d), the higher extraction of CGA and total solids did not reflect a significant increment in the antioxidant capacity.

Extractability and antioxidant capacity of melanoidin populations

The contribution of the different populations of melanoidins to the total antioxidant capacity of coffee brew and their kinetic of extraction was further investigated in filter-paper pods. The HMW material of coffee brew is composed by a mixture of thermally transformed galactomannans and arabinogalactans and by a

diversity of melanoidin populations presenting different physical properties and chemical composition (Moreira et al., 2012). Fractions F1, F3, and F4 of pods were included in the investigation. The total solids content in fractions F1, F3, and F4 were 109.9, 80.7, and 38.2 mg/mL, respectively. After cell stirring ultrafiltration with a membrane cutoff of 10 kDa, the content in HMW material correspond to 33.7, 23.0, and 12.4 mg/mL for fraction F1, F3, and F4, respectively. It is noteworthy to highlight that this result matched with the calculation of melanoidin content reported above by using the spectrophotometric method. HMW material was further fractionated according to solubility in ethanol as described by Nunes and

Coimbra (2007). Four fractions were obtained corresponding to the water insoluble fraction (WI), insoluble at ethanol 50% (Et50), insoluble at ethanol 75% (Et75), and soluble at ethanol 75% (Sn75). The average proportions of each population of melanoidins were 10% (8.1–12.3), 27% (25.5–31.5), 15% (10.2–16.4), and 48% (47.5–50.4) for fractions WI, Et50, Et75, and Sn75, respectively. The predominant population of melanoidins in all the coffee fractions was Sn75 that accounted for nearly 50% of the total HMW material in weight. These results are in line with Coelho et al. (2014), who reported an HMW material content of 3.73 g/100 g in filter coffee (total soluble solids contents of 14.9 g/100 g of coffee), whereas 32.7%, 16.8, and 50.5% correspond to the fractions Et50, Et75, and Sn75. The melanoidin population soluble in ethanol contained the majority of the phenolic compounds incorporated into the HMW material during roasting by either ester linkages or condensed forms (non-ester covalent linkages (Delgado-Andrade & Morales, 2005; Bekedam et al., 2008; Nunes, Cruz, & Coimbra, 2012; Perrone, Farah, & Donangelo, 2012).

Literature describes that melanoidins accounted for 26–38% of the overall antioxidant capacity the coffee brew, depending on the assay applied (Perrone et al., 2012; Rufián-Henares & Morales, 2007). Fig. 5 describes the antioxidant capacity of the HMW material and LMW material isolated from fractions F1, F3, and F4. Results are expressed per gram of sample to allow the direct comparison between activities of the fractions. Regardless the fraction investigated, the antioxidant capacity of melanoidins was always higher than their respective LMW weight material in terms of absolute value of mass. In other words, same amount of HMW material exerted higher antioxidant capacity of the LMW fraction. However, it is important to keep in mind that content of LMW material in coffee brew is higher, nearly 70%, than the HMW material, as described above. There were not significant differences in the contribution of melanoidins to the antioxidant capacity in fractions F1, F3, and F4. However, LMW material extracted in fraction F1 had significantly higher antioxidant capacity than those extracted in fractions F3 and F4. This result is expected since CGA is extracted faster than melanoidins and browning rate (Table 3). In the first 68.3 mL, all the CGA is extracted, but more than 100 mL is necessary to extract all the melanoidins and browning. Results also showed that melanoidin population Sn75 had the highest antioxidant capacity as compared with Et50 and Et75, which could be explained by the higher presence of phenolic compounds linked to their structure and lower carbohydrate complexity (Coelho et al., 2014).

Many investigations pointed out that melanoidins may have beneficial *in vivo* effects, such as antioxidant activity, and phase I and phase II enzyme-modulating activity, including to be beneficial to human health, displaying *in vivo* antioxidant, antimicrobial, and prebiotic activity in the intestine because certain melanoidin populations behaves as an antioxidant indigestible fiber (Borrelli et al., 2004; Daglia et al., 2000; Morales et al., 2012; Reichardt et al., 2009; Silván et al., 2010; Wang et al., 2011). However, melanoidins have also technological effects since their presence is directly associated with the reduction of the intensity of the sulfury-roasty odor quality of the coffee brew (Hofmann et al., 2001). To better implement the health benefits of melanoidins to consumers, it is necessary to understand their kinetic of extraction during

preparation of coffee brew. This investigation will allow the optimization of the espresso coffeemakers to enhance the proper extraction of melanoidins in the cup.

CONCLUSIONS

This investigation pointed out that the type of portioned coffee and espresso coffeemaker is critical for the extractability of melanoidins regardless the coffee origin. Melanoidins are rapidly extracted ere nearly 50% is already extracted within first 20mL regardless the type of single dose unit used. However, the efficacy of the melanoidins extraction was higher in clone capsules, as compared with pods and lately to FAP capsules. In addition, no significant differences were observed in the extractability of the populations of melanoidins for espresso coffee with pods, accounting for nearly the 10%, 27%, 15%, and 48% of the total melanoidin content for fraction W1, Et50, Et75, and Sn75, respectively. However, the antioxidant capacity of the melanoidin populations increases with the extraction volume, and their structure is very likely not the same since their antioxidant activity is different. Melanoidin population related to Sn75 showed the highest antioxidant activity and increased significantly from fraction F1 to fraction F3 or F4. It could be plausible that more complex phenolic-rich structures with higher antioxidant capacity need longer extraction volumes. This statement should be confirmed with further experiments that involves chemical characterization of the melanoidin populations. In addition, extraction kinetics of browning, antioxidant capacity, and melanoidins are not always correlated, and it will likely depend on the type of the ground coffee and roasting conditions. Investigation on the extractability of melanoidins could make possible the adjustment of the technological requirements of coffee machines and extraction techniques to produce a coffee with high levels of beneficial compounds.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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FIGURES AND TABLES

Table 1. Identification of samples, description of technical parameters for espresso coffee machines, types of single-dose containers, and characterization of espresso coffee.

	F	B	NC	NB
Coffeemaker	Basic (Ascaso Factory, ES)	Cappuccino (Espressos del Capitano, IT)	Inissia (DeLonghi, HU)	Inissia (DeLonghi, HU)
Pressure (bar)	16	20	19	19
Water reservoir (mL)	2000	1000	700	700
Extraction flow (mL/s)*	5.2	3.2	6.0	6.0
in-cup temp. (°C)*	68.3 ± 1.8a	77.8 ± 0.5b	75.2 ± 1.2c	75.2 ± 1.2c
Coffee container	Filter-paper pod	Biodegradable polypropylene FAP capsule	Plastic with aluminum foil clone capsule	Plastic with aluminum foil clone capsule
Container size (mm)	60 × 45 × 10	40 × 34 × 20	38 × 20 × 25	39 × 20 × 25
Coffee origin	Colombia	Colombia	Colombia	Vietnam, Guatemala, Brazil
Coffee blend	Arabica 100%	Arabica 100%	Arabica 100%	Arabica 35% Robusta 65%
Coffee (g) per container	6.866 ± 0.141b	7.853 ± 0.053c	5.224 ± 0.058a	5.211 ± 0.027a
Particle size (µm)	200–400	300–800	300–600	300–700
Extraction flow (mL/s)	1.5	2.0	2.1	2.1
In-cup temperature (°C)	75.0 ± 2.9a	82.7 ± 4.3b	79.8 ± 3.0ab	79.7 ± 3.3ab
Max. extraction rate (%)	27.8 ± 0.75a	26.3 ± 0.82a	26.7 ± 1.01a	27.9 ± 0.74a
Water/coffee (mL/g)**	6.7	5.9	8.8	8.8
Density at 20 °C (g/mL)**	0.941 ± 0.014b	1.037 ± 0.031c	0.744 ± 0.027a	0.705 ± 0.066a
pH**	5.0 ± 0.1a	5.0 ± 0.3a	5.0 ± 0.1a	5.2 ± 0.1a
Concentration (mg/mL)**	38.2 ± 0.28c	31.1 ± 0.27b	28.2 ± 1.75b	24.6 ± 0.70a

Different letters in a same row identify significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation. *Extraction without pods or capsule. **Calculated for a volume of 46 mL, corresponding to espresso coffee.

Table 2. Carbohydrate, caffeine, total chlorogenic acids (CGA), melanoidins, total solid content, total solids on filtrate, browning, and antioxidant capacity in ground coffee and their respective fraction F5.

	F	B	NC	NB
<i>Ground coffee (g/100 g)</i>				
Carbohydrate (as mannose eq.)	38.3 ± 1.0ab	33.3 ± 6.2a	39.5 ± 4.4ab	46.3 ± 6.0b
Carbohydrate (as arabinose eq.)	27.2 ± 7.0ab	24.0 ± 0.1a	29.0 ± 7.5b	34.6 ± 8.8b
Caffeine	1.15 ± 0.12a	1.10 ± 0.11a	1.17 ± 0.01a	1.76 ± 0.03b
Total CGA	1.12 ± 0.15b	1.18 ± 0.16b	0.79 ± 0.09a	1.59 ± 0.33c
Melanoidins	11.0 ± 1.6b	7.7 ± 1.0a	7.8 ± 0.1a	7.4 ± 0.2a
<i>Fraction F5 (mg/mL)</i>				
Carbohydrate (as mannose eq.)	7.3 ± 1.2a	6.8 ± 0.7a	6.1 ± 0.7a	6.2 ± 1.1a
Carbohydrate (as arabinose eq.)	5.2 ± 0.4b	4.7 ± 0.8ab	4.1 ± 0.8a	4.2 ± 0.5a
Caffeine	0.79 ± 0.16ab	0.80 ± 0.23ab	0.77 ± 0.18a	1.08 ± 0.26b
Total CGA	0.72 ± 0.24ab	0.85 ± 0.33b	0.52 ± 0.19a	0.65 ± 0.20a
Melanoidins	6.3 ± 0.7b	3.8 ± 0.3a	4.9 ± 0.5ab	4.5 ± 0.2a
Total solid content	16.2 ± 0.4b	13.9 ± 1.6a	15.1 ± 1.0ab	14.2 ± 0.5a
Total solids on filtrate	15.3 ± 0.6d	12.6 ± 0.1a	14.8 ± 0.3c	13.6 ± 0.6b
Browning (A_{495}/mL)	7.6 ± 0.3c	5.3 ± 0.4a	6.0 ± 0.4b	5.4 ± 0.3ab
Antiox. capacity (TEAC mmol/L)	17.7 ± 0.1b	15.0 ± 0.7a	15.1 ± 0.6a	17.3 ± 0.3b

Volume for fraction F5 is 115 mL for F and B samples, and 85 mL for NC and NB samples. Different letters in a same row identify significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation. Eq: equivalent; Antiox: antioxidant; TEAC: Trolox equivalent antioxidant capacity.

Table 3. Curve fit parameters obtained from the model $f(y)=(a \cdot x) / (b + x)$ applied to extraction of melanoidins, browning, antioxidant capacity, and chlorogenic acids (CGA) in samples from filter-paper pods (F), FAP capsule (B), and clone capsule (NC, and NB).

	F	B	NC	NB
<i>Melanoidins</i>				
a parameter	115.5 ± 1.71	115.6 ± 6.32	119.3 ± 4.8	115.7 ± 2.48
b parameter	16.7 ± 0.73	22.7 ± 3.40	12.2 ± 1.5	13.9 ± 0.89
r ²	0.999	0.991	0.993	0.998
P	<0.0001	<0.0001	<0.0001	<0.0001
V ₅₀ (mL)	12.6 ± 0.19c	18.0 ± 0.8d	8.97 ± 0.36a	10.63 ± 0.23b
V ₉₅ (mL)	77.3 ± 1.44c	104.7 ± 5.72d	47.8 ± 1.92a	63.8 ± 1.37b
V _{max} (mL)	107.8 ± 1.60c	115.0 ± 6.29c	63.1 ± 2.53a	85.0 ± 1.82b
Espresso (F4, %)	84.8 ± 0.26b	77.4 ± 0.38a	94.3 ± 1.27d	88.9 ± 0.56c
<i>Browning</i>				
a parameter	120.5 ± 2.80	126.7 ± 8.67	114.1 ± 3.93	114.8 ± 4.59
b parameter	21.7 ± 1.40	24.9 ± 4.57	9.9 ± 1.14	9.0 ± 1.24
r ²	0.998	0.988	0.994	0.991
P	<0.0001	<0.001	<0.0001	<0.0001
V ₅₀ (mL)	15.3 ± 0.36c	16.2 ± 1.11c	7.6 ± 0.26a	7.0 ± 0.28b
V ₉₅ (mL)	80.9 ± 1.88c	74.6 ± 5.10c	49.5 ± 1.71b	43.2 ± 1.73a
V _{max} (mL)	106.5 ± 2.48c	93.0 ± 6.36c	70.4 ± 2.43b	60.8 ± 2.43a
Espresso (F4, %)	81.8 ± 0.21a	82.2 ± 0.31b	93.8 ± 1.29c	96.0 ± 1.64d
<i>Antioxidant activity</i>				
a parameter	119.7 ± 3.96	120.5 ± 2.91	114.2 ± 2.83	115.6 ± 1.37
b parameter	20.1 ± 1.88	24.6 ± 1.60	11.9 ± 0.93	12.8 ± 0.47
r ²	0.996	0.998	0.997	0.999
P	<0.0001	<0.0001	<0.0001	<0.0001
V ₅₀ (mL)	14.4 ± 0.48b	17.1 ± 0.41c	9.3 ± 0.23a	9.6 ± 0.11a
V ₉₅ (mL)	77.3 ± 2.55b	91.6 ± 2.21c	59.1 ± 1.47a	61.1 ± 0.72a
V _{max} (mL)	102.0 ± 3.37b	115c	84.0 ± 2.08a	82.3 ± 0.98a
Espresso (F4, %)	83.3 ± 0.38b	78.5 ± 0.12a	90.7 ± 0.78c	90.4 ± 0.35c
<i>CGAs</i>				
a parameter	116.1 ± 7.19	119.04 ± 5.58	112.0 ± 4.20	115.3 ± 5.10
b parameter	11.0 ± 2.42	4.7 ± 2.35	7.7 ± 1.05	8.9 ± 1.36
r ²	0.981	0.992	0.992	0.989
P	<0.001	<0.001	<0.001	<0.001
V ₅₀ (mL)	8.1 ± 0.50c	12.6 ± 0.60d	6.3 ± 0.24a	7.0 ± 0.31b
V ₉₅ (mL)	49.4 ± 3.06a	67.8 ± 3.18b	42.8 ± 1.61a	41.8 ± 1.85a
V _{max} (mL)	68.3 ± 4.23a	90.3 ± 3.18b	64.1 ± 2.40a	58.4 ± 2.58a
Espresso (F4, %)	93.7 ± 2.41b	86.8 ± 0.69a	96.0 ± 2.17b	96.6 ± 2.24b

V₅₀ (volume needed to extract the 50%), V₉₅ (volume needed to extract the 95%), V_{max} (volume needed to extract the maximum), and percentage extracted for espresso (fraction F4, 46 mL). Different letters in a same row identify significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation.

Figure 1. Temperature-volume ($^{\circ}\text{C}/\text{mL}$) profile at the exit and in-cup of the espresso coffeemakers used for filter-paper pods (a), FAP capsule (b), and clone capsule (c). Dotted lines represent the volumes for sampling. F1 (5mL), F2 (9mL), F3 (16mL), F4 (46mL), and F5 (115 mL for a and b) and F5* (85 mL for c).

Figure 2. Extraction rate (%) for espresso coffeemaker and single-dose container. Filter-paper pods (F), FAP capsule (B), clone capsule (NC), and clone capsule containing a blend of Robusta and Arabica ground coffee (NB).

Figure 3. Extraction kinetics of melanoidins (a), browning (b), antioxidant capacity (c), and chlorogenic acids (d) normalized to fraction F5 for paper-filter pods (F), FAP capsules (B), and clone capsules (NC). Lines correspond to non-linear model adjustment.

Figure 4. Relationship between antioxidant capacity and solids, melanoidins, browning, and chlorogenic acids (CGA) normalized to fraction F5 for paper-filter pods (F; a), FAP capsules (B; b), and clone capsules (NC; c, and NB; d). Data are expressed in percentage of the specific parameter to its total content (solids, melanoidins, and chlorogenic acids), activity (antioxidant), or rate (browning).

Figure 5. Antioxidant capacity (TEAC expressed as $\mu\text{mol Trolox/g}$) for high molecular weight (HMW), low molecular weight (LMW) material, and melanoidin populations (Et50, Et75, and Sn75) in fractions F1, F3, and F4 obtained from filter-paper pods. Different letters in a same sample identify significant differences ($p < 0.05$). Et50: insoluble at ethanol 50%, Et75: insoluble at ethanol 75%, and Sn75: soluble at ethanol 75%.