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EVOLUTION OF ANTIOXIDANT ACTIVITY IN HEATED COFFEE BREW

EVOLUCIÓN DE LA ACTIVIDAD ANTIOXIDANTE EN CAFÉ PERCOLADO Y CALENTADO

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Abstract
Coffee brew (CB) was prepared using a standard filter coffeemaker (24 g of ground roasted coffee, 400 mL of water). The glass flask containing CB was maintained in the coffeemaker hot-plate at 75 OC. The antioxidant activity was monitored and assayed during 8 h by means of DPPH, FRAP, total phenols content, browning index and voltammetry. Antioxidant activity showed a fast increase during the first 1.0-1.5 h. Heating times longer than about 2 h produced nearly constant values for FRAP, total phenols and caffeine contents, but DPPH and oxidative capacity by voltammetry decreased probably due to the oxidation of antioxidant compounds by oxidative stress induced by adverse environmental factors. It was postulated that Maillard reactions products were formed in the heated CB, probably due to the degradation of chlorogenic acids (CGAs) that complexed with proteins/polysaccharides, giving way to potent antioxidants compounds such as melanoidins.
Keywords: coffee brew, heating time, antioxidant activity, Maillard reactions products

Resumen
Se preparó café (CB) con una cafetera de goteo estándar (24 g de café tostado molido, 400 mL de agua). El recipiente de vidrio conteniendo CB se mantuvo sobre la placa caliente de la cafetera a una temperatura de 75 °C. Se monitoreó y determinó la actividad antioxidante durante 8 h por medio de DPPH, FRAP, contenido total de fenoles, índice de pardeamiento y voltametría. La actividad antioxidante mostró un rápido incremento durante las primeras 1.0-1.5 h. Un calentamiento de aproximadamente 2 h o más produjo valores casi constantes de FRAP, fenoles totales y cafeína, pero DPPH y la capacidad oxidativa por voltametría disminuyeron, probablemente debido a la oxidación de los compuestos antioxidantes por estrés oxidativo inducido por factores ambientales adversos. Se postuló que se formaron productos de reacciones de Maillard en el café caliente, probablemente debido a la degradación de los ácidos clorogénicos (CGAs) que se complejaron con proteínas/polisacáridos, dando origen a potentes compuestos antioxidantes tales como las melanoidinas.
Palabras clave: café percolado, tiempo de calentamiento, actividad antioxidante, productos de reacciones de Maillard.

1 Introduction

World coffee consumption was of approx. 9.08 million metric tons in 2015/2016 (ICO, 2016). Besides its sensorial properties, brewed coffee is an important source of antioxidants, which play an important role as health protecting factors (Nicoli *et al*., 1997; Brezová *et al*., 2009; Nieber, 2017). For instance, consumption of coffee brew (CB) has been linked to reduced risk of death attributed to inflammatory and cardiovascular diseases (Andersen *et al*., 2006), and to reduced incidence of diabetes and liver disease (Cano-Marquina *et al*., 2013). Coffee has a high content of phenolic acids, particularly of chlorogenic acids (CGAs), with CGAs contents ranging from 2.3 to 26 $g.kg^{-1}$ (Ramírez-Velasco *et al*., 2016). Maillard reaction products (MRPs) are the prevailing antioxidants in roasted coffee. The antioxidant action associated with roasted MRPs involved hydrogen atom transfer and single electron transfer mechanisms

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(Liu and Kitts, 2011). However, high chain-breaking and oxygen consumption activities do not increase linearly with increased roasting degree. CB obtained from medium-dark roasted ground coffee was found to have the highest antioxidant properties (Nicoli *et al*., 1997). Pérez-Martínez *et al*. (2010) studied the impact of the brewing method, finding that mocha coffeemaker produced the highest yield of antioxidant extract per gram of roasted ground coffee, although espresso coffee was richest in terms of antioxidant intake (per milliliter of CB). Ludwig *et al*. (2012) studied the impact of the brewing time on the antioxidant activity of CB for filter and expresso brew methods, finding that higher water pressure increased the antioxidant extraction speed. Recently, Moreira *et al*. (2012) reviewed critically the structure, formation mechanisms and potential health benefits of melanoidins, which are defined as high molecular weight nitrogenous and brown-colored compounds, in coffee.

CB made with filter machine is extensively consumed in all spheres of human activity, including workplaces, restaurants, homes and meetings. Commonly, after the CB is made, the glass flask containing it is heated via hot-plate (typically 65-80 °C) in order to keep it warm, for a period of time that can range from minutes to several hours. As heating time evolves, the CB develops an increasingly darker color and astringent taste. Thus, it is likely that further MRPs are formed during this process. With this in mind, the aim of this work was to evaluate the evolution of antioxidant activity, assayed by different methods, of a coffee brew obtained using a standard filter coffeemaker, and kept hot at 75 °C for 8 h.

2 Materials and methods

2.1 Materials

Vacuum-packed Colombian Arabica roasted ground coffee beans was purchased in Wal-Mart (Member's Mark, Wal; production lot 6217; expiry date may 2018; Mexico City, Mexico), and stored at room temperature until required for the coffee brew (CB) preparation. Folin-Ciocalteu´s phenol reagent, 2-2 diphenyL^{-1}-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and caffeine reference standard (molecular weight 194.19) were acquired from Sigma Aldrich (Toluca, State of Mexico, Mexico). Acetonitrile, ferric chloride, acetate buffer solution (pH 3.6), and ethanol were purchased from J.T. Baker (Naucalpan, State of Mexico, Mexico). All reagents used were ACS reagent grade. All the water used in the experiments was deionized.

2.2 Coffee brew preparation

To avoid oxidative damage, the coffee bag was opened immediately before the preparation of the CB. Roasted ground coffee (24g, put into Melita paper basket filter) and 400 mL of water were put into a filter coffee machine (DCM1100B, Black and Dekker, Mexico City, Mexico). The extraction process took about 7 min at 90 oC. The glass flasks (8 in total) containing the CB were covered with a high-temperature resistant plastic bag to avoid water evaporation and for maintaining temperature at 75.0±3.3 °C. A CB sample was withdrawn every hour from a different glass flask, in order to minimize different heating conditions due to CB diminished volume. CB samples were then cooled down in an ice-bath to 3±0.2 °C for subsequent analysis. The pH of the CB samples was measured with a pH meter (Hanna Instruments, model HI 98240, Smithfield, RI, USA) at 25 °C.

2.3 Color determination

The color of CB samples was assessed by using a Minolta colorimeter (CR-300, Konica Minolta, Osaka, Japan). The equipment was calibrated with a standard white tile (L = 97.43, a = 0.01 and b = 1.64) and set for illuminant D65, and a 2° observer angle for all samples. The CB samples (10 mL) were placed in Petri dishes (5 cm in diameter) and their color was evaluated at room temperature. The Hunter L, a, and b values correspond to lightness, greenness ($-a$) or redness ($+a$), and blueness ($-b$) or yellowness ($+b$). Total color difference $\Delta E = (L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2$, was also computed. The subscript '0' indicates initial values of the L, a, and b parameters.

Additionally, the browning index of the CB samples was recorded using a spectrophotometer (Spectronics Genesys 5 UV/Vis, Spectronic Unicam, Rochester, NY, USA) at 420 nm (Liu and Kitts, 2011; Lee *et al*., 2016). To this end, the CB samples (5 mL) were diluted with deionized water (100 mL). Deionized water was used as blank.

2.4 DPPH antioxidant assay

The free radical scavenging activity of the CB was measured using DPPH assay (Luna-Ramírez *et al*., 2017). An aliquot of 250 μL of CB sample was mixed with 250 μL water and 3.5 mL of DPPH ethanol solution (23 mg DPPH.L^{-1}). Absorbance was determined at 517 nm (Spectronics Genesys 5 UV/Vis, Spectronic Unicam, Rochester, NY, USA) until the reaction kinetics reached a plateau. Antioxidant activity was expressed as the inhibition percentage (% I) of the free radical DPPH, calculated with the following equation:

$$\%I = \left(\frac{Abs_b - Abs_s}{Abs_b}\right) \times 100 \quad (1)$$

where ABS_b and ABS_s are the absorbance of control reaction (without the sample) and of the sample, respectively. A standard curve of Trolox concentration (18-0.4 μg.μL^{-1} ethanol) versus absorbance values was obtained using DPPH as described above.

2.5 Total antioxidant capacity (TAC)

The TAC was determined by means of the ferric reducing-antioxidant power (FRAP) method as reported by Benzie and Strain (1999). The samples were prepared by mixing 1800 μL of the FRAP solution with 100 μL of the sample and water were added for achieving a final volume of 4 mL. The calibration standard curve of trolox was determined from 55-0.85 μg.μL^{-1} ethanol versus absorbance. Absorbance was determined at 595 nm. The results were expressed mg.mL^{-1} of trolox equivalent.

2.6 Total phenolic content determination

Total phenolic content was determined with the Folin-Ciocalteu reagent (Domínguez-Hernández *et al*., 2016). Briefly, a CB sample (200 μL) was mixed with 250 μL de Folin-Ciocalteu reagent (1 N). In a second step, 1250 μL of a sodium carbonate (20%) and water were added for achieving a final volume of 4 mL. The mixture was incubated for 30 minutes at room temperature (about 20 °C). The change in absorbance was measured at 760 nm. To this end, trolox was used as a standard. The total phenolic content was expressed mg of trolox equivalent.mL^{-1}.

2.7 Caffeine content

The caffeine content of samples was characterized using high-resolution HPLC analysis (Naegele, 2013). To this end an Agilent HPLC (model HP 1100, with autosampler model 1200 mca, Agilent Technologies, Germany) with a Nucleosil 100-5 column (Nautilus, Macherey, England) was used. A gradient procedure was used for the composition analysis with a mixture of water and acetonitrile (75:25). The flow was set up to 0.8 mL.min^{-1}, 76 bar, 5 min and was monitored at a 273 nm with diode arrangement. 20 μL was injected and caffeine (1 mg.mL^{-1}) was detected by comparing the retention time with respect to pure standards. Experiments were carried out at 25 °C.

2.8 Voltammetry

Samples were loaded into a PARSTAT Potentiostat/Galvanostat (Princeton Applied Research, Oak Ridge, TN, USA) model 2273 cell (volume = 50 mL) equipped with a Faraday cage. The arrangement setup consisted of a typical cell (volume = 50 mL) with three-electrodes: (1) a saturated sulfate reference electrode ($Hg/Hg_2SO_4/K_2SO_4$), SSE, (E = 0.645V/SHE, XR200 Radiometer), (2) a platinum foil as counter-electrode, and (3) a platinum plate as working electrode. Prior to each experiment, the surface of the working electrode was polished using Buehler alumina powder (final grain size $\leq$ 0.05 μm) to a mirror finish. The electrode was then rinsed with deionized water and placed in an ultrasonic bath for 5 min. The reaction cell was equipped with a temperature control system to maintain the system at 20°C. Voltammograms were obtained by applying a potential scan at 100 mV.s^{-1}, from 1.0 to -1.0 V, in the anodic direction.

2.9 ATR-FTIR spectroscopy

CB samples were dried overnight at 37 °C (Convection oven RIOSSA, HCF-41D, Mexico City, Mexico). Fourier Transform Infrared (FTIR) spectra of the powders were obtained using a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) equipped with a crystal diamond universal ATR sampling accessory. Each spectrum represented an average of three scans.

2.10 Data analysis

The data were expressed as means ± S.D. Statistical analysis of the coffee brew assays results were subjected to analysis of variance using the Statgraphics 7 statistical analysis system (Statistical Graphics Corp. Manugistics Inc., Cambridge, MA).

When it was pertinent, significant differences ($p \leq 0.05$) between means were detected with Tukey's test. All the experiments were done by triplicate.

3 Results and discussion

Table 1 shows the measured Hunter color parameters (L, a, and b) and the total color change, ΔE, of CB were affected negatively by heating time. Within the first hour of heating time, the L, a, and b parameters showed non-significant values variations, but as heating time continued up to 8 h, all of them showed a continuous significant decrease with time. These results are indicative that heating induced a degrading color quality in CB. The decreases in L, a, and b were manifested in a significantly higher ΔE increase with heating time. These color changes might be best described by the browning index, BI (Table 1). The BI showed a continuous significant increase with heating time. The above results suggest that browning of the CB could be due to Maillard reaction which give rise to particularly complex mix of various compounds of different molecular weights (Maillard reaction products), which include not only aldehydes, ketones, dicarbonyls, acryl amides, and heterocyclic amines, but also melanoidins and advanced glycation end products, which are polymeric products formed at the advanced steps of MR. All these compounds contribute to increase the browning index of CB with heating time (Wang *et al*., 2011; Echavarría *et al*., 2012).

Table 2 shows that the pH decreased with time, indicating a greater activity of H^+ ions. Increased acidity is a characteristic of matured CB. The evolution of the antioxidant properties of CB are also presented in Table 2. The different antioxidant activity assays are strongly dependent on the conditions used and the substrates or products monitored. As a consequence, the methods might lead to differing results. DPPH method analyzes the antiradical activity developed in methanolic media, whereas FRAP estimates the ferric reducing ability of samples. DPPH showed a fast increase in the first hour and remained nearly constant in the subsequent hours. In contrast, FRAP remained nearly constant during the first 4 h, and then showed a fast increase of about 25% during the final 3 h. Total phenols also increased with time, following a linear pattern (R^2=0.92) to achieve an increase of about 8% after 8 h. Caffeine content increased during the first 4 h, achieving a nearly constant value of about 0.15 mg.mL^{-1} during subsequent hours. These results indicate that the browning and astringency of mature CB was caused by an increase of antioxidant compounds. However, the antioxidant activity assayed using different methods showed dissimilar increasing patterns. In general, the development of the browning color is linked to an increase of the antioxidant properties. The prevalent mechanism giving rise to compounds possessing antioxidant activity in coffee brew is due to Maillard reactions (Nicoli *et al*., 2004). Interestingly, caffeine content also increased by about 30% after 7-8 hours.

Figure 1.a presents voltammogram of CB at three different times. The voltammogram of water was used as blank. The redox activity reflected by the voltammetry measurements was significantly higher for CB than for water. CB displayed a broad oxidant peak at about 0.5-1.0 volts. The oxidation activity was weakened after reversing the potential direction until a zero-current value at 0.7-0.75 volts was achieved.

Table 1. Evolution of the color parameters of the coffee brew with heating time.

Time (h)	L	a	b	ΔE	Abs (A.U.)
0	25.59±0.01a	5.70±0.17[a]	7.76±0.02a	0±0.00i	0.21±0.03j
0.5	25.63±0.01a	5.63±0.17[a]	7.72±0.02a	0.09±0.01i	0.24±0.01j
1	25.97±0.02a	5.65±0.11a,	7.71±0.01a	0.39±0.02h	0.41±0.02i
2	25.43±0.01a,b	5.52±0.18b	7.23±0.01b	0.58±0.03g	0.46±0.03h
3	24.33±0.01b,c	5.46±0.16b	6.53±0.42c	1.78±0.03f	0.52±0.01g
4	23.57±0.01c	5.35±0.19c	5.36±0.46d	3.16±0.03e	0.58±0.02f
5	22.80±0.01d	5.21±0.13c,d	4.50±0.01e	4.32±0.04d	0.60±0.02d
6	21.78±0.01d,e	4.75±0.12e	3.75±0.02f	5.61±0.04c	0.63±0.01c
7	20.78±0.02f	3.25±0.11f	1.78±0.01g	8.06±0.04b	0.66±0.01b
8	19.45±0.01f,g	2.92±0.13g	1.26±0.01h	9.36±0.05a	0.70±0.01a

Values are means ± standard error, of three replicates. Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).

Table 2. Evolution of antioxidant properties of coffee brew with heating time.

Time (h)	pH	DPPH (%I)	FRAP$\times 10^{-3}$ (mg/mL ET)	TPh$\times 10^{-3}$ (mg/mL ET)	Caffeine (mg/mL)
0	5.88±0.03a	14.88±0.17d	1.29±0.02c	1.07±0.01a	0.11±0.01c
0.5	5.82±0.02a	15.21±0.09c	1.28±0.01c	1.09±0.02a	0.11±0.01b,c
1	5.72±0.03a,b	16.30±0.11c	1.30±0.01b	1.09±0.01b	0.10±0.01b
2	5.51±0.04c	16.77±0.18b	1.27±0.01b,c	1.07±0.01c	0.11±0.02b
3	5.41±0.01d	16.98±0.16b	1.28±0.42b	1.10±0.01d	0.12±0.01b
4	5.32±0.03d,e	17.19±0.19a,b	1.29±0.46b	1.11±0.01c	0.15±0.01a
5	5.29±0.02e	17.61±0.13a	1.33±0.01a,b	1.12±0.01c	0.16±0.02a
6	5.23±0.04e	16.40±0.12a	1.39±0.02b	1.12±0.01c	0.15±0.01a
7	5.09±0.02f	16.23±0.11c	1.50±0.02a	1.16±0.01c	0.16±0.01a
8	5.02±0.04f,g	15.33±0.13d,e	1.53±0.01a	1.15±0.01c	0.16±0.02a

TPh: Total phenols. Values are means ± standard error, of three replicates. Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).

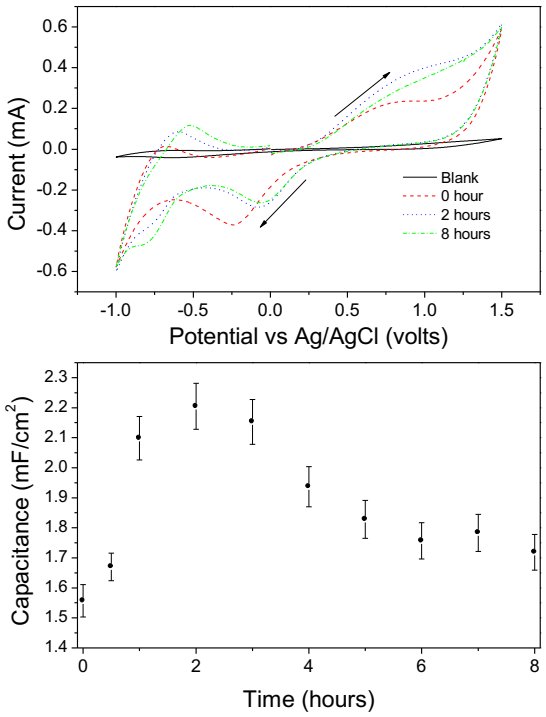

Fig. 1. (a) Voltamperograms for coffee brew for three different times. The voltamperogram of the water used in experiments is included for comparison. (b) Electrical capacitance estimated by integration in the first oxidation peak.

A reduction peak at -0.5-0.0 volts was exhibited, which might be associated to the reduction of the oxidation products obtained during the oxidation of the CB components. Note that this reduction peak was accompanied by an oxidation peak at about -0.5 volts, which might be reflecting the oxidation of adsorbates produced by the reduction occurring during the first oxidation peak and the subsequent reduction peak. The first oxidation peak reflected the reduction capacity of the CB, which in turn can be taken as a further indication of the antioxidative capacity. If one considers the CB as an antioxidants reservoir, one can quantify the antioxidant capacity as a double-layer capacitance. Numerically, this capacitance is obtained by integration of the current-potential response as follows:

$$C_E = \frac{\int_{E_{\min}}^{V_{\max}} I(V)dV}{\nu(V_{\max} - V_{\min})} \qquad (2)$$

where ν is the scanning rate, $V_{\min}$ and $V_{\max}$ are the minimum and maximum voltages, respectively. Since the reduction and oxidation peaks for negative potentials were associated to products of the first oxidation peak, the computations were restricted to positive potential values. Figure 1.b presents the variations of the capacitance with time. In line with DPPH measurements (Table 1), the capacitance showed a fast increase in the first hour. The capacitance maximum was achieved after 2 h showing a gradual decrease in subsequent hours. This decrease was displayed also by DPPH, but not by FRAP and total phenols content.

Finally, Figure 2 illustrates the FTIR patterns for three different times. The meaning of the transmittance peaks tagged in the figure is described elsewhere (Pujol *et al*., 2013). The peaks at 2855 and 1778 cm^{-1} correspond to symmetric stretching of C-H bonds in aliphatic chains and to carbonyl vibration (C=O) in aliphatic esters (Lyman *et al*., 2013).

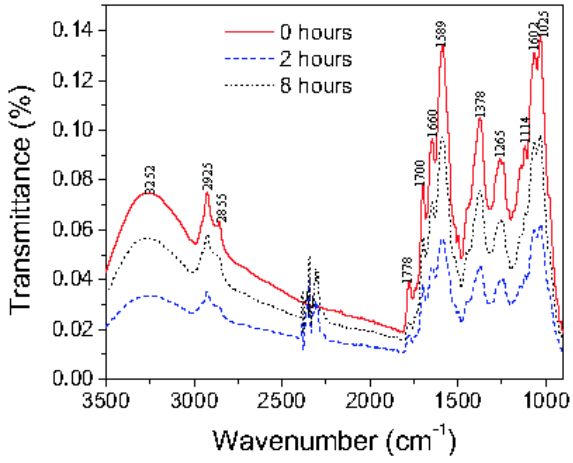

Fig. 2. FTIR for dried coffee brew corresponding to three different times.

These bands decreased with the maturation time, and can be linked to lipids in the CB. The band at 1660 cm-1 can be due to C=C vibrations of lipids and fatty acids. Maillard reactions taking place in the CB led to a decrease of the lipids detectable with FTIR measurements. The bands at 1114, 1265 and 1378 cm^{-1} could be attributed to CGAs, which are esters formed by quinic acid and certain trans-cinnamic acids (Clifford *et al*., 2008). Interestingly, the relative values of these bands decreased with time. It has been suggested that browning reactions in roasted coffee degrade chlorogenic acids to form potent antioxidants (Kamiyama *et al*., 2015). In this regard, it seems that the increase of antioxidant activity detected by DPPH, FRAP and caffeine content can be explained from the degradation of chlorogenic acids. On the other hand, the decay of the antioxidative activity detected by DPPH and voltammetry might be reflecting the oxidative degradation of the CB as it entered in contact with oxygen from the environment. In particular, voltammetry results in Figure 1.a indicated that the maximum antioxidant activity was obtained within the first 1.0-1.5 h after the CB preparation.

Conclusions

The results in this work showed that CB kept heated at 75 °C increased its antioxidant activity, probably caused by the degradation of CGAs that complexed with proteins/polysaccharides contained in the CB, forming potent antioxidants (e.g., melanoidins). However, the antioxidants formation competed with the oxidation of these compounds by oxidative stress induced by adverse environmental conditions.

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