Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition

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A B S T R A C T
Coffee is one of the most popular beverages in the world, prepared and consumed in many different ways. Taste, aroma and composition of the coffee brew vary depending on the preparation method. Therefore, this study investigates the effect of different brewing methods on the polyphenol and methylxanthine composition and antioxidant capacity of thirteen different coffee brews. The content of total phenols and flavonoids was determined spectrophotometrically and the content of chlorogenic acid derivates (3-CQA, 4-CQA and 5-CQA) and caffeine using the high performance liquid chromatography (HPLC-PDA). Antioxidant capacity of coffee brews was evaluated by using the ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) and FRAP (ferric-reducing antioxidant power) assays. Instant coffee brews showed the highest values in content of total phenols, chlorogenic acid derivates, caffeine and antioxidant capacity, which significantly decreased by milk addition. The antioxidant capacity of coffee brews was in compliance with the total phenol content and content of chlorogenic acid derivates.

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1. Introduction
Desirable sensory properties, as well as the stimulant effects of caffeine, make coffee one of the most often consumed and most popular beverages in the world (Rojo Camargo, Toledo, & Farah, 1999). It is estimated that 80–90% of adults are regular consumers of caffeine-containing brews (coffee, tea, cocoa, cola brews and energy drinks), making caffeine the most widely consumed psychoactive substance (McCusker, Goldberger, & Cone, 2006). Due to its popularity, coffee presents a very important food commodity, with strong economic, social and cultural impact.

A large number of previous studies has reported adverse effects of coffee consumption (coronary heart disease, insomnia, anxiety, osteoporosis, anemia, hypertension, depression, iron and zinc mal absorption during pregnancy, adverse effect on fetus, newborn and nursing infant) (Nawrot et al., 2003; Smith, Smith, Miners, McNeil, & Proudfoot, 2000). However, newer epidemiological and experimental studies have shown many positive effects of regular coffee intake on psychoactive responses (alertness, mood change), neurological conditions (Parkinson’s disease), metabolic disorders (e.g. diabetes) and developing gallstone disease, as well as positive effect of coffee on gonad and liver function (Dorea & daCosta, 2005). Low caffeine doses (60–200 mg per day – usually consumed during the day) enhance alertness, increase perception and levels of concentration on simple tasks. These positive effects of coffee on human health are attributed to the presence of bioactive compounds with strong antioxidant and radical scavenging activities (Cämmerer & Kroh, 2006; Parras, Martinez-Tome, Jimenez, & Murcia, 2007), especially polyphenols (Pojjana, Ames, & del Castillo, 2002). Among these compounds chlorogenic acids (quinol esters of hydroxycinnamic acids) (Olthof, Zock, & Katn, 2001), caffeic, ferulic, p-coumaric acid (Richelle, Travazzi, & Offord, 2001) and proanthocyanidins (Arts, van de Putte, & Hollman, 2000) are the most important ones. Chlorogenic acids, ranging from 6% to 10% of dry weight of coffee, includes three main groups: caffeoylquinic acids (CQAs), with 5-O-caffeoylquinic acid being by far the most abundant, feruloylquinic acids (FQAs) and di-caffeoylquinic acids conjugated with tyrosine, tryptophan or phenylalanine (cinnamoyl-amides depending on origins) (Clifford & Knight, 2004).

The preparation technique has a significant influence on the taste, aroma and composition of coffee brews. Recently, three different extraction methodologies (decoction, infusion and pressure methods) have been developed. Each of these methods is related to specific granulation (coffee grind) of coffee powder, water/coffee proportion, temperature and brewing time. Filter or drip coffee and instant coffee brews are obtained by infusion method. The most appreciated coffee brew, traditionally centered in Italy, is

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espresso, which is prepared by pressure method. In order to meliorate taste or abate caffeine effect, coffee is often consumed with milk. The preferred forms of coffee consumption are macchiato and latte brews prepared with the addition of different amounts of milk, which alters the bioactive composition and antioxidant capacity of coffee brew. Yusel, Avci, and Erdem (2010) studied the ability of polyphenols to interact with dietary proteins (including caseins and whey proteins) indicating that these compounds may form polyphenol–protein complexes which affects the bioactive composition of coffee.

In Croatia and the south-eastern European region, coffee brews are prepared in different ways including espresso, Turkish/Greek, instant and filter, but they are very often consumed with the addition of milk in macchiato and latte coffee brew forms. Therefore, the aim of this study was to determine the content of caffeine and chlorogenic acid derivates in differently prepared coffee brews (espresso, Turkish/Greek, instant and filter – plain, macchiato and latte), decaffeinated and instant cappuccino brews, and to assess the effect of milk addition on the content of polyphenols and antioxidant capacity of these brews.

2. Materials and methods

2.1. Chemicals

Analytical grade of Folin–Ciocalteu, formic acid, sodium carbonate, formaldehyde, ferric chloride hexahydrate, ferrous sulfate heptahydrate and hydrochloric acid were supplied by Kemika (Zagreb, Croatia) and sodium acetate trihydrate was supplied by Alkaloid (Skopje, FYR Macedonia). Methanol (HPLC grade) was supplied by J.T.Baker (Deventer, Netherlands). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid diammonium salt) and caffeine were purchased from Fluka (Switzerland) and chlorogenic (3-ethylbenzthiazo line-6-sulfonic acid diammonium salt) and tetramethylchromane-2-carboxylic acid), ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid diammonium salt) and caffeine were purchased from Fluka (Switzerland) and chlorogenic acid was supplied by Sigma–Aldrich (Steinheim, Germany).

2.2. Coffee samples

Thirteen different coffee samples (in bean or in grounded form) and four different instant cappuccino samples (Tables 1 and 2) were obtained by the leading coffee manufacturer in Croatia. Samples containing blends of different coffees (classic, bonus, jubilar exclusive, gold), as well as ready-to-use espresso dosages (containing 7 g grounded coffee blend) prepared for specially designed espresso coffee machines were also analyzed.

2.3. Preparation of coffee brews

Bean coffee samples were ground in a standard domestic grinder prior to brew preparation. In order to simulate the standard coffee brew preparation, 7 g of coffee sample was used for the preparation of 50 ml of all coffee brews (using distilled water heated to 95–97 °C), including instant and espresso. Different amounts of homogenized and sterilized milk (2.6% milk fat) were added to the prepared coffee brews; 30 ml for the preparation of macchiato coffee brew (total amount of macchiato coffee brew is 80 ml) or 50 ml for obtaining latte coffee brews (total amount of latte coffee brew is 100 ml). For the preparation of instant cappuccino brews, 7 g of sample was prepared with 50 ml of hot tap water or hot milk (95–97 °C).

2.4. Determination of total phenol and flavonoid content

Total phenol content (TPC) of coffee brews was determined spectrophotometrically according to a modified method of Lachman, Hosnedl, Pivec, and Orsak (1998). To determine the content of total flavonoids (TFC), these compounds were precipitated using formaldehyde, which reacts with C-6 or C-8 atoms of 5, 7-dihydroxy flavonoids to form methyl derivates that further react with other flavonoid compounds also at C-6 and C-8 positions. The condensed products of these reactions were removed by filtration and the remaining non-flavonoid phenols were determined as previously described. Flavonoid content was calculated as the difference between total phenol and non-flavonoid phenols content. Gallic acid was used as the standard and the results were expressed as mg/L gallic acid equivalents (GAE). All measurements were performed in triplicate.

2.5. HPLC analysis of phenolic compounds and caffeine

The samples were filtered through a 0.45 μm filter (Nylon Membranes, Supelco, Bellefonte, USA) before HPLC analysis. 20 μl of each sample was injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) by using a reversed-phase column Pinnacle II C-18 column (Restek, USA) (250 × 4.6 mm, 5 μm i.d.). The solvents consisted of 3% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 ml/min. The elution was performed with a gradient starting at 2% B to reach 32% B at 20 min, 40% B at 30 min and 95% B at 40 min, and becoming isocratic for 5 min (Komes, Horžič, Belščak, Kovačevič Ganić, & Vulić, 2010). Chromatograms were recorded at 278 nm. Detection was performed with a Photodiode Array Detector by scanning between 200 and 400 nm, with a resolution of 1.2 nm. Phenolic compounds and caffeine were identified by comparing the retention times and

<table>
<thead>
<tr>
<th>Preparation way</th>
<th>Water (50 mL)</th>
<th>Milk (50 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic</td>
<td>IC1w</td>
<td>IC1m</td>
</tr>
<tr>
<td>Chocolate</td>
<td>IC2w</td>
<td>IC2m</td>
</tr>
<tr>
<td>Vanilla</td>
<td>IC3w</td>
<td>IC3m</td>
</tr>
<tr>
<td>Irish cream</td>
<td>IC4w</td>
<td>IC4m</td>
</tr>
</tbody>
</table>

| Table 2 | General description of instant cappuccino brews analyzed in this study. |
spectral data with those of standards. The data acquisition and treatment were conducted using Star Chromatography Workstation Version 5 software. All analyses were repeated three times.

2.6. Antioxidant capacity determination of coffee brews

2.6.1. Ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) assay was carried out according to a standard procedure by Benzie and Strain (1996). All measurements were performed in triplicate. Aqueous solutions of FeSO$_4$ × 7H$_2$O (100–1000 μM) were used for the calibration curve and the results are expressed as mM Fe (II).

2.6.2. Radical scavenging capacity assay

The Trolox equivalent antioxidant capacity (TEAC) of coffee brews was estimated by the ABTS radical cation decolourisation assay (Re et al., 1999). The results, obtained from triplicate analyses, were expressed as Trolox equivalents, and derived from a calibration curve determined for Trolox (100–1000 μM).

2.6.3. Statistical analysis

All measurements and analyses were carried out in triplicate. The results were analyzed statistically using the Statistica 7.0 program to determine the average value and standard deviation. Variance analysis, with a significance level of α = 0.05%, was performed to determine the differences in the phenolic content due to different brewing methods. Correlation analysis was also run with the same statistical package.

3. Results and discussion

In this paper the effect of different brewing methods on the content of total polyphenolics, caffeine and chlorogenic acid derivatives, as well as antioxidant capacity of most often consumed coffee brews was analyzed. In order to examine the effect of milk on the content of bioactive constituents of coffee brews and their antioxidant properties, plain coffee brew, macchiato and latte were analyzed. Instant cappuccino brews were prepared as water and milk based brews.

3.1. Total phenol and flavonoid content of coffee and instant cappuccino brews

Total phenol content (TPC) and total flavonoid content (TFC) of 13 different coffee brews and 4 instant cappuccino brews is shown in Fig. 1. As can be seen, all tested samples present a rich source of phenolic compounds. Rusak, Komes, Liki, Horžič, and Kovač (2008) reported the TPC of different types of teas, which ranged from 2141 mg/L in white tea to 2377 mg/L GAE in green tea. Among the analyzed coffee brews, the highest TPC and TFC were detected in instant classic coffee (11 – 17,307 mg GAE/L and 8460 mg GAE/L, respectively), while the lowest TPC and TFC were detected in the filter coffee brew (F – 2967 mg GAE/L and 1633 mg GAE/L, respectively). The results obtained in the present study indicate that instant coffee brews exhibit up to 7.3-fold higher polyphenol content compared to green teas. Sánchez-Gonzalez, Jamenez-Escrig, and Saura-Calixto (2005) also studied the polyphenol content and antioxidant capacity of differently prepared coffee brews (Italian, espresso and filter), and found 2.1–12.1 g of total phenols/100 g dry matter (2940–16,940 mg GAE/L), which is comparable to the results obtained in our study (2967–17,307 mg GAE/L).

The polyphenolic content of different coffee brews was significantly (p < 0.05) affected by the brewing method, which is in part attributed to the processing conditions during coffee production. Namely, instant coffee is produced by water extraction and liquid separation under high temperatures (180 °C) followed by concentration using dehydration (freeze-drying or spray drying) (Noguiera & Trugo, 2003). In that way, the specific steps in the production of this type of coffee allow for concentrating of water-soluble or dispersible compounds, including polyphenols and result with higher content of these substances when compared to other types of coffee.

Lower TPC and TFC of filter coffee could be caused by the filter used during the brew preparation. Mišik et al. (2010) studied the effect of paper filtered coffee on oxidative DNA-damage and found that filtration of coffee brews lead to removal of some bioactive compounds According to their results, previously determined contents of kahweol and cafestol (90 and 182 mg/L, respectively) decreased after filtration to 0.2–0.3 mg/L. The results of our study are in agreement with the ones mentioned above, indicating that filter coffee, due to the employed brewing technique, provides the lowest content of beneficial polyphenolic compounds.

Decaffeinated Turkish/Greek and instant coffee brews showed lower TPC than their regular coffee counterparts, confirming previously published results by Fujioka and Shibamoto (2008).

Our results revealed a higher content of TPC in two decaffeinated espresso coffee brews when compared to their regular coffee counterparts. This observation could be attributed to the employed decaffeination technique, however further research needs to be performed in order to determine the exact effect of decaffeination on specific bioactive compounds.

As can be seen on Fig. 2a and b, in comparison to plain brews prepared only with water, the addition of milk significantly (p < 0.05) decreases the TPC of coffee and decaffeinated coffee brews, as opposed to instant cappuccino brews prepared with milk, which exhibit higher TPC when compared to plain water-made brews (Fig. 2c). Milk containing food products represent a very complex matrix where strong polyphenol–protein interactions are well known to occur (Siebert, Troukhanover, & Lynn, 1996) and can directly interfere with accurate polyphenols determination by significantly reducing analytical recovery, which may be the reason for the overestimation of milk-prepared cappuccino brews.

Among both macchiato and latte brews, the highest TPC and TFC were determined in instant coffee brews and the lowest in filter coffee brews. Both macchiato and latte brews showed significantly (p < 0.05) lower TPC and TFC when compared to plain brews. This decrease could be the attributed to the inclination of polyphenols to create polyphenol–protein complexes, as reported for tea samples (Dubeau, Samson, & Tajmir-Riahi, 2010; Sharma, Kumar, & Rao, 2008; Yuksel et al., 2010), blueberry fruit (Serafini et al., 2009), chocolate (Belšak, Komes, Horžič, Garic–Kovacevic, & Karlovic, 2009) and coffee (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006). Similar trend was also observed for the TPC and TFC of decaffeinated coffee brews. Among decaffeinated coffee brews (Fig. 2b), instant coffee brew exhibits the highest TPC and TFC (D11 – 16,220 mg GAE/L, 7000 mg GAE/L, respectively) followed by macchiato decaffeinated instant coffee brew (D11M – 12,513 mg GAE/L, 6253 mg GAE/L, respectively) and latte decaffeinated instant coffee brew (D11L – 10,447 mg GAE/L, 5320 mg GAE/L, respectively).

Beside coffee, instant cappuccino brews are also very often consumed and were therefore analyzed in this study. Instant cappuccino brews can be prepared either by brewing with water or milk, depending on the consumer preferences, so TPC and TFC were determined in both brew types. Significant differences (p < 0.05) in the TPC and TFC of water-prepared instant cappuccino brews were observed. The highest TPC and TFC were determined in Cappuccino Classic (IC1 – 3480 mg GAE/L, 1467 mg GAE/L, respectively), which is comprised of 17% of instant coffee among other ingredients. The
lowest TPC and TFC were detected in Irish Cream Cappuccino (IC4 – 1400 GAE/L, 793 GAE/L, respectively), containing only 4% of instant coffee. Contrary to the results obtained for coffee brews prepared with the addition of milk, instant cappuccino brews made with milk exhibited higher TPC and TFC than those made with water. The highest TPC and TFC were detected in Cappuccino Chocolate (IC2m – 5827 mg GAE/L, 4387 mg GAE/L, respectively) containing 13% of instant coffee and 12% of cocoa powder. Irish Cream Cappuccino (IC4m – 4173 mg GAE/L, 2660 mg GAE/L, respectively), with only 4% of instant coffee in its composition once again exhibited the lowest TPC and TFC. The higher content of milk prepared cappuccino brews can be attributed to the previously mentioned presence of milk derived compounds and reduced analytical accuracy, as well as the lack of selectivity of Folin–Ciocalteu reagent which reacts not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugars and vitamin C. Since apart from instant coffee, the composition of instant cappuccino includes milk powder, powdered whey proteins, lactose, sugar, vegetable fat, salt, aroma and E99 (calcium lactobionate) all these ingredients can interfere with the determination of phenolic compounds in this assay, thus contributing to the inconsistency of the results.

3.2. High performance liquid chromatography of coffee and cappuccino extracts

It has been well established that chlorogenic acid (CGA) derivatives are the predominant antioxidants in coffee brews. Chlorogenic acids are a family of esters formed between trans-cinnamic acids and quinic acid. The most usual and widespread individual chlorogenic acid is formed between caffeic acid and quinic acid and the most abundant CGAs in coffee are caffeic acid including 5-caffeoylquinic acid (5-CQA) and together with two major positional isomers, 4-CQA and 3-CQA (Farah & Donangelo, 2006). The content of these three isomers was determined in all studied coffee and instant cappuccino brews and the results are displayed in Table 3.

Isomer 5-CQA was determined in the highest content, followed by 4-CQA and finally 3-CQA. These results are comparable with the ones obtained by Fujioka and Shibamoto (2008) who studied the content of chlorogenic acid in various commercial coffee brews, and detected CGA isomers in the same decreasing order (5-CQA>4-CQA>3-CQA). According to the results of HPLC analysis performed in our study, instant coffee brews are the richest source of CGA (Table 3), while the lowest content of CGA was determined in filter coffee brew. Decaffeinated coffee brews contained higher total content of chlorogenic acids than their regular coffee counterparts, which is in compliance with the results of Fujioka and Shibamoto (2008), who also found an inconsistency in the CGA content of regular and decaffeinated coffee brews. Steinhart, Luger, and Piotz (2001) found that during steam decaffeination, the CGAs content is reduced, which results with a 10–20% decrease of antioxidant capacity of decaffeinated brews.

During domestic coffee preparation, the prepared Turkish/Greek coffee brews are often reheated, so the effect of reheating on the content of chlorogenic acid and caffeine was also examined in our study. Reheating of the sample to the boiling state significantly (p < 0.05) increased the content of CGA and caffeine when compared to the initially prepared brews. In the reheated sample the content of 3-CQA increased for 116.25 mg/L (464.28 mg/L), the content of 4-CQA for 136.11 mg/L (534.62 mg/L) and the content of 5-CQA for 217.91 mg/L (1013.29 mg/L). The content of caffeine also increased after reheating from 1942.38 mg/L to 2551.56 mg/L. The obtained results may point to a contradictory outcome caused by reheating of coffee brew. Although reheating the prepared brew increased the content of beneficial bioactive compound – CGA it has also increased the content of caffeine which is often avoided by consumers due to its stimulative and physiological effects.

As it can be seen on Fig. 3, instant coffee brew I1 – 4716.28 mg/L contained the highest content of caffeine and espresso brew the lowest E4 – 977.12 mg/L. The caffeine content of regular coffee brews ranged from 6.98 to 33.69 mg/g of ground coffee, which is in agreement with the results of Fujioka and Shibamoto (2008) (10.9–16.5 mg/g of ground coffee). Caffeine was determined in all decaffeinated coffee brews. The presence of caffeine in decaffeinated coffee brews was also confirmed by Fujioka and Shibamoto (2008) who obtained 0.34–0.47 mg/g ground coffee, which is in compliance with the content of caffeine determined in the present study (0.25–1.69 mg/g ground coffee).

Because of the presence of instant coffee in the instant cappuccino composition, instant cappuccino brews also contain caffeine and were therefore also analyzed for their caffeine content. Cappuccino Classic (17% instant coffee powder) brew exhibited the
Fig. 2. Total flavonoid and nonflavonoid contents of (a) coffee brews, (b) decaffeinated coffee brews (prepared with water, and as macchiato and latte brews) and (c) instant cappuccino brews (prepared with water or milk). Results are expressed as mg GAE/L ± SD.
Table 3
Chlorogenic acids (3CQA, 4-CQA and 5-CQA) content (mg/L) of coffee, decaffeinated coffee and instant cappuccino brews prepared only with water.

<table>
<thead>
<tr>
<th>Samples</th>
<th>3-CQA</th>
<th>4-CQA</th>
<th>5-CQA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plain coffee brews</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>200.44 ± 2.07*</td>
<td>239.56 ± 6.34*</td>
<td>486.52 ± 21.73*</td>
<td>926.52</td>
</tr>
<tr>
<td>E2</td>
<td>136.70 ± 5.56*</td>
<td>152.33 ± 7.69*</td>
<td>261.96 ± 8.94*</td>
<td>550.99</td>
</tr>
<tr>
<td>E3</td>
<td>178.84 ± 11.48*</td>
<td>211.10 ± 7.06*</td>
<td>427.77 ± 23.61*</td>
<td>817.71</td>
</tr>
<tr>
<td>E4</td>
<td>120.02 ± 5.13*</td>
<td>143.13 ± 5.14*</td>
<td>232.41 ± 9.36*</td>
<td>495.56</td>
</tr>
<tr>
<td>TG1</td>
<td>348.28 ± 0.73*</td>
<td>398.51 ± 12.15*</td>
<td>795.38 ± 3.43*</td>
<td>1542.17*</td>
</tr>
<tr>
<td>TG1a</td>
<td>464.53 ± 0.10*</td>
<td>534.62 ± 0.10*</td>
<td>1013.29 ± 0.10*</td>
<td>2012.44</td>
</tr>
<tr>
<td>TG2</td>
<td>362.04 ± 12.59*</td>
<td>407.84 ± 11.51*</td>
<td>825.83 ± 0.30*</td>
<td>1595.73*</td>
</tr>
<tr>
<td>I1</td>
<td>786.69 ± 13.83*</td>
<td>768.52 ± 3.49*</td>
<td>960.07 ± 22.30*</td>
<td>2515.28</td>
</tr>
<tr>
<td>I2</td>
<td>744.10 ± 10.10*</td>
<td>677.28 ± 0.10*</td>
<td>879.39 ± 0.10*</td>
<td>2300.77</td>
</tr>
<tr>
<td>F</td>
<td>277.41 ± 49.72*</td>
<td>323.83 ± 22.26*</td>
<td>634.15 ± 67.50*</td>
<td>1235.39</td>
</tr>
<tr>
<td><strong>Decaffeinated coffee brews</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE1</td>
<td>225.73 ± 7.74*</td>
<td>263.97 ± 2.94*</td>
<td>496.03 ± 7.66*</td>
<td>985.73</td>
</tr>
<tr>
<td>DE2</td>
<td>161.76 ± 20.87*</td>
<td>168.80 ± 8.65*</td>
<td>339.07 ± 4.44*</td>
<td>669.63</td>
</tr>
<tr>
<td>DTG1</td>
<td>369.48 ± 3.06*</td>
<td>431.10 ± 3.75*</td>
<td>823.26 ± 12.05*</td>
<td>1623.84*</td>
</tr>
<tr>
<td>DI1</td>
<td>1262.36 ± 0.10*</td>
<td>1157.48 ± 0.10*</td>
<td>1614.57 ± 0.10*</td>
<td>4034.41</td>
</tr>
<tr>
<td><strong>Instant cappuccino brews</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC1w</td>
<td>26.49 ± 0.10*</td>
<td>26.92 ± 0.10*</td>
<td>28.37 ± 0.10*</td>
<td>81.78*</td>
</tr>
<tr>
<td>IC2w</td>
<td>30.50 ± 9.33*</td>
<td>35.68 ± 10.26*</td>
<td>38.47 ± 3.37*</td>
<td>104.65*</td>
</tr>
<tr>
<td>IC3w</td>
<td>18.15 ± 0.10*</td>
<td>15.91 ± 0.10*</td>
<td>26.83 ± 0.10*</td>
<td>60.89*</td>
</tr>
<tr>
<td>IC4w</td>
<td>n.d.</td>
<td>9.12 ± 0.10*</td>
<td>6.77 ± 0.10*</td>
<td>15.89*</td>
</tr>
</tbody>
</table>

The same letters (a–j) denote the content of chlorogenic acid derivates, which are not significantly (p > 0.05) affected by the coffee brew preparation method.

Table 3 The results of both assays confirmed the significant antioxidant capacity of analyzed brews (Table 4). Instant coffee brew was characterized with the strongest antioxidant properties (11 - 49.34 ± 0.05 mmol/L Trolox, 244.82 ± 0.86 mmol/L Fe²⁺), while the lowest antioxidant capacity was exhibited by filter coffee brew (F - 14.37 mmol/L Trolox, 36.56 mmol/L Fe²⁺). The antioxidant capacities of coffee brews obtained by both ABTS and FRAP assays were in accordance with the TPC and TFC and, as it can be expected, it decreases with addition of milk (Table 4). Significant linear correlation was confirmed between the TPC and the antioxidant capacity of all coffee brews determined by both ABTS (r = 0.82–0.99) and FRAP (r = 0.82–0.99) assays. A significant (p < 0.05) difference in the antioxidant capacity of plain water made coffee brews, macchiato and latte brews was observed. Sánchez-González et al. (2005) obtained similar results, and reported that antioxidant capacity of differently prepared coffee brews (Italian, espresso and filter) has significantly decreased with the addition of different amounts of milk. This can be explained by the fact that up to 1/3 of the quantity of chlorogenic acid, as the main antioxidant compound, interacts with milk proteins in coffee (Dupas et al., 2006). Binding affinity of polyphenols to proteins is dependent on their molecular size (De Freitas & Mateus, 2001) and it increases with increasing of their molecular size. Liang and Xu (2003) found that polyphenols such as catechins can form insoluble complexes by interacting with proline-rich proteins such as β-casein, the most abundant milk protein. Some authors proposed that the formation of polyphenol–milk protein complexes may decrease the bioavailability and the antioxidant potential of polyphenols in vivo (Arts et al., 2002). However, other similar studies (Dubeau et al., 2010; Sharma et al., 2008; Yuksel et al., 2010) showed no relevant effect of milk on the bioavailability or antioxidant capacity of tea polyphenols. The inhibitory effect of milk on the antioxidant capacity of polyphenols has been reported by Dubeau et al. (2010) who found that milk decreased the antioxidant capacity of green, Drajeening and English breakfast tea. Serafini et al. (2009) found that addition of milk to blueberry extracts resulted in precipitation of blueberry antioxidants, leading to a decrease in total antioxidant capacity of the supernatants. This inhibitory effect can be related to the binding of polyphenols to the milk caseins via covalent and non-covalent interactions. These interactions can be either multi-site (many polyphenols bound to one protein), or multidentate

3.3. Antioxidant capacity of coffee and instant cappuccino brews

In order to obtain reliable information regarding the antioxidant capacity of coffee and instant cappuccino brews, two different widely used antioxidant assays (ABTS and FRAP) were applied.
These instant coffee brews exhibited the highest antioxidant capacity than their regular coffee counterparts. Decaffeinated brews.

Decaffeination between the bound polyphenols and the free oxidants in solution and therefore restrict the interaction through diffusion of many proteins (Sharma et al., 2008). Both types lead to protein interactions (one polyphenol bound on multiple sites of one or more proteins) (Sharma et al., 2008). The same letters (a–d) denote the antioxidant capacity, which are not significantly (p > 0.05) affected by the coffee brew preparation method.

4. Conclusions

Coffee brews represent a remarkable source of antioxidants whose content is comparable with the bioactive compounds content of tea and wine. However, polyphenol content and antioxidant capacity of coffee brews greatly depend on the preparation method. Instant coffee brews posses the highest TPC and TFC, as well as the highest antioxidant capacity, while filter coffee brews showed the lowest content of polyphenols and the lowest antioxidant capacity. As the predominant antioxidants, chlorogenic acid derivatives are present in all coffee brews, in the following decreasing order: 5-CQA>4-CQA>3-CQA. The addition of milk decreases both the polyphenolic content and antioxidant capacity of brews, which points to potential interactions between polyphenols and milk proteins.

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References


