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# THE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND ANTIOXIDANT ACTIVITY IN COFFEE DEPENDING ON BREWING METHOD

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Key words: coffee, antioxidants, brewing method, caffeine, reactive oxygen species.

Abstract

Coffee is one of the world's most popular beverages. It is rich in biologically active compounds possess antioxidant activities. The aim of the study was to determine the influence of coffee brewing method to the total antioxidant activity, the phenolic acid, flavonoid and caffeine content of coffee infusion from beans available in local markets.

Three methods of brewing coffee were evaluated: pouring hot water over coffee beans, using coffee percolator where the water is boiling through ground coffee and collecting as coffee above and preparing in an automatic coffee maker. Three species of coffee: arabica, robusta and green coffee beans infusions were analyzed.

Total antioxidant activity of the infusion was measured using 2,2-*diphenyl*-1-picrylhydrazyl radical (DPPH). Polyphenol, flavonoid and caffeine contents were determined by spectrophotometry.

The results showed that antioxidant activity of analyzed infusion was no significant changes depending on coffee species and beverage preparing method in roasted coffee beans. It has been also shown that the method of brewing unroasted coffee beans significantly affects the antioxidant potential of infusion, as well as the brewing time (first, second, third). Methods of brewing did not make a difference to the total polyphenol content. The caffeine concentration and total flavonoid content in the coffee infusion changed depending on the conditions of brewing.

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# ZAWARTOŚĆ ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W KAWIE ORAZ ICH AKTYWNOŚĆ PRZECIWUTLENIAJĄCA W ZALEŻNOŚCI OD SPOSOBU PARZENIA

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Słowa kluczowe: kawa, antyoksydanty, sposoby przygotowywania naparu, kofeina, wolne rodniki tlenowe.

Abstract

Kawa jest jednym z najpopularniejszych napojów na świecie. Charakteryzuje się dużą zawartością związków biologicznie czynnych o właściwościach antyoksydacyjnych. Celem pracy było określenie wpływu metody parzenia kawy na całkowitą aktywność antyoksydacyjną, zawartość kwasów fenolowych, flawonoidów i kofeiny w naparach kawy przygotowanych z ziaren dostępnych w lokalnych sklepach.

Oceniano trzy sposoby parzenia kawy: zalanie zmielonych ziaren kawy gorącą wodą, przygotowanie naparu z użyciem kawiarki,w której gorąca woda obmywa zmielone ziarna i do górnego zbiornika skrapla się napar, oraz przygotowanie naparu za pomocą ekspresu do kawy. Analizowano napary przygotowane z trzech gatunków kawy: arabiki, robusty i zielonych ziaren kawy.

Całkowitą aktywność przeciwutleniającą naparu oceniano, stosując rodnik 2,2-difenylo-1-pikrylhydrazylowy (DPPH). Całkowitą zawartość polifenoli, flawonoidów i kofeiny ustalono spektrofotometrycznie.

Wykazano, że aktywność antyoksydacyjna naparów z palonych ziaren kawy nie uległa istotnym statystycznie zmianom w zależności od gatunku kawy i sposobu przygotowywania napoju. Dowiedziono również, że przygotowywanie naparów z niepoddanych procesowi palenia ziaren kawy ma znaczący wpływ na jego potencjał utleniający, który zależy także od liczby zaparzeń. Metody przygotowywania naparów nie miały wpływu na całkowitą zawartość polifenoli. Stężenie kofeiny i całkowita zawartość flawonoidów w naparze kawy zmieniały się w zależności od metody przygotowywania.

## Introduction

Coffee remains one of the most commonly consumed beverages across the world (MUSSATTO et al. 2011). The homeland of coffee is Ethiopia (MIRAN 2012), from where it spread though Arabic countries, reaching Europe a few hundred years later. The most popular species are *Coffea arabica* and *Coffea canephora* var. Robusta (KLEIN 2003).

Coffee contains more than 1,000 biologically active substances. Besides caffeine (1–2%), green coffee beans comprise carbohydrates (59–61%), lipids (10–16%), proteins (10%), chlorogenic acids (7–10%), minerals (4%), aliphatic acids (2%), trigonelline (1%) and free amino acids (<1%). Roasted

coffee beans have a slightly different composition: carbohydrates (38–42%), proteins (8%), chlorogenic acids (3–4%), and free amino acids, lipids (11–17%), minerals (5%), aliphatic acids (3%), and trigonelline (1%). Some of these compounds, such as polyphenols, possess antioxidant activity (LUDWIG et al. 2014).

Although polyphenols are known for their vital antioxidant properties, the same is true for caffeine. The antioxidant activity of phenolic compounds proceeds by a variety of mechanisms of action. They are capable of reducing a substrate by donating electrons or a hydrogen atom to bind free radicals, stabilize or delocalize unpaired electrons, activating chelating enzymes that can bind metal ions catalyzing oxidation reactions, inhibiting the action of oxidase, interrupting radical chain reactions, and stabilizing free radicals through their hydrogenation or complexion (FERNANDEZ-PANCHON et al. 2008). Polyphenols can be classified into four categories: phenolic acids and flavonoids, stilbenes and lignans. The most abundant polyphenols in human diet, flavonoids are composed of two aromatic rings connected by a three-carbon bridge, making a total of 15 carbon atoms. An important group of phenolic compounds formed as secondary plant metabolites are the hydroxycinnamic acids, represented by caffeic acids (CA) and their esters, chlorogenic acids (CGA). Apart from their antioxidant activity (SATO et al. 2011), both compounds demonstrate a range of properties. For example CA and CGA inhibit the activity of $\alpha$-amylase and $\alpha$-glucosidase, key enzymes linked to type 2 diabetes (OBOH et al. 2015), and acetylcholinesterase and butyrylcholinesterase, which are linked to Alzheimer's disease (OBOHet al. 2013). It has also been reported that CA and CGA possessanticancer properties (ROCHA et al. 2012), anti-metastatic activity (TANAGORNMEATR et al. 2014) and anti-inflammatory effects (SERGENt et al. 2010). They also possess the ability to inhibit the activation of transcription factor NF-κB (MAet al. 2013) and can inhibit DNA methylation (VUCIC et al. 2008). Studies have shown that CA has greater antioxidant activitythan CGA (CHEN and HO 1997), which is positively correlated with the number and position of hydroxyl groups bound to the aromatic ring, as well as the nature of its substituents (RICE-EVANSet al. 1996).

Caffeine (1,3,7-trimethylxanthine), a natural alkaloid, is the most extensively studied compound in coffee. It is composed of two fused rings whose chemical properties are closely related to those of purines (NUHU 2014). The caffeine content in coffee beans depends on the species. Robusta has approximately twice the caffeine content as Arabica (FOX et al. 2013). In general, the daily caffeine intake for adults has been found to be approximately 3–4 mg $kg^{-1}$ body weight $day^{-1}$(BARONE and ROBERTS 1996). It

reaches maximum concentration in the blood after about 15–120 minutes and remains steady for about four hours. The physiological effect of caffeine is stimulation of central nervous system. High doses of caffeine, of up to 6 mg $kg^{-1}$ body weight $day^{-1}$ have adverse effects such as overstimulation of central nervous system and other harmful properties such as general toxicity, cardiovascular effects, effects on bone status and calcium balance, changes in adult behavior, increased incidence of cancer and impaired male fertility (NAWROt et al. 2003). In addition, it has been also reported that caffeine demonstrates antioxidant activity against lipid peroxidation caused by reactive oxygen species (LEE 2000).

Around over the world many brewing methods may be used to prepare coffee brews. Brewing techniques are classified inthree main groups:

1. Original Italian method under high pressure.
2. Infusion, by pouring hot water on ground coffee followed by a filtration.
3. Decoction or boiling method (PETRACCO 2001).

Brewing coffee by boiling was the earliest method. It is prepared by adding the water to the grinding coffee beans and bringing it to the boil for no more than an instant in a pot.

Another common brewing technique of coffee using a percolator applies both aspects of the pressure and gravity methods. The percolator has two chambers: water is placed inside the percolator's lower chamber, while coffee grounds is placed inside the filter basket within the percolator's upper chamber. The water in the chamber boils and is forced, through a tube, to the top of the percolator and drips down over the coffee grounds.

The most popular pressure brewing method is espresso. Espresso is obtained by pushing hot water, slightly under boiling temperature, with pressure, through ground coffee (CAPRIOLI 2015).

The aim of this work was to analyze the main biologically active substances in coffee with regard to brewing method. For this purpose, four commercially available types of roasted coffee and one type of green coffee were examined, together with the following methods of coffee brewing: pouring hot water over the coffee beans, making coffee using a percolator and using an automatic coffee maker. The total antioxidant activitywas measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Total polyphenol content was measured using the by Folin-Ciocalteu assay and flavonoid content by the Dowd method. The quantity of caffeine was determined by spectrophotometry following extraction from the coffee infusion using polar-nonpolar solvent extraction techniques.

## Materials and Methods

Chemicals.2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), catechin, quercetin were provided from Sigma-Aldrich (Poznan, Poland). Ethanol, Folin-Ciocalteu reagent, chloroform, nitrate (III) sulfate, aluminum chloride, sodium carbonate, potassium acetate and ferric chloride were obtained from Avantor Performance Materials Poland S.A. (Gliwice, Poland).

Coffee Material. Four roasted coffee samples, blends of Arabica and Robusta, numbered 1–4, and one sample of green coffee (Table 1) obtained from retail stores in Lodz (Poland) were analyzed. The most popular brands of pre-grounded roasted coffee and whole beans of unroasted coffee were selected for the analysis.

Table 1

Characterization of selected coffee

| Number of coffee | Species of coffee | Roasted/unroasted | Country of the beans origins |
|---|---|---|---|
| *1* | arabica | roasted (medium) | South and Central America, Brazil |
| *2* | arabica | roasted | – |
| *3* | arabica | roasted | Brazil, Colombia and Central America |
| *4* | robusta | roasted | – |
| Green coffee beans | – | unroasted (green coffee) | – |

Sign "–" means that the information has not been provi

Preparation of beverages. The five types of coffee including roasted and green beans were prepared in three different ways. In the first method, 100 ml of hot water (90°C) was poured onto 2 g of ground coffee beans and dripped through paper filter after 10 minutes. In the second one, the extract was prepared using a percolator (Domotti, model 32704 Vella, Poland): 100 ml of cold water was added to the reservoir and then 2 g of ground coffee to the basket. The percolator was placed over a heat source. After complete brewing, coffee solutions were dripped through a paper filter. The final method was to brew a 100 ml measure of coffee from 2 g ground coffee beans using a coffee machine (De'Longhi, model EC145, Italy) and drip the infusion through paper filter.

For the green coffee, the beans were ground to a powder in a coffee grinder (Siemens, model MC23200, Germany) for 30 s immediately before sample preparation. Green coffee was brewed three times, according to the

instructions on the package. The extract was filtered and the seeds were poured once more with water. Freshly prepared coffee brews were taken to analyses. All analyses were performed using a UV-Vis spectrometer (Cary 100 UV-Vis, Agilent Technologies, Santa Clara, USA).

Antioxidant activities by DPPH assay. Theantioxidant activity of coffee beverages was determined according to (BRAND-WILLIAMSet al. 1995) using the synthetic radical DPPH. The absorbance of the solution was measured at λ = 517 nm. Following this, 0.5 mM DPPH alcoholic solution stored in the dark was prepared.

The spectrophotometer was first calibrated using ethanol, before 100 ul of ethanol and 100 ul diluted coffee samples (10, 15, 25, 35, 50, 75,100, 150, 200 x dilutions) were added to 1 ml DPPH solution. After 30 minutes from the initiation of the reaction, the absorbance of the DPPH radical solution ($A_0$) and samples ($A$) were monitored. Each measurement was done in triplicate and meanvalues ($A_{mean}$) were calculated. The ability of the antioxidant to counteract the oxidation reaction were calculated by the formula (MOLYNEUX 2004):

$$\text{Inhibition [\%]} = 100\ (A_0 - A_{mean}) / A_0$$

when:

$A_{mean}$ – the meanabsorbance of test the solution containing antioxidant

$A_0$ – absorbance of DPPH radical solution.

The total phenolic content of the extract was determined by the Folin--Ciocalteu method [11]. Briefly, 200 µL of crude extract (1 mg $ml^{-1}$) were made up to 3 ml with distilled water, mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

Total polyphenol content. The content of total phenolic compounds was determined by spectrophotometry using the Folin-Ciocalteu reagent and caffeic acid as a standard (range 0–200 µg $ml^{-1}$). 100 µl of coffee extract solutions (2 g/100 ml) were placed in a 10 ml volumetric flask, then 1 ml of 0.2 M Folin-Ciocalteu reagent and 6 ml of distilled water were added and mixed. After a three minute pause, 1.5 ml 20% sodium carbonate was added and made up to 10 ml with distilled water, followed by incubation in darkness at room temperature for 120 min. Following this, the solution was mixed again and the absorbance of the samples were measured at a wavelength of 765 nm against an ethanol blank.

The total polyphenol content was calculated using a linear equation based on a calibration curve ($y = 0.11x + 0.0558$, $r = 0.9935936$) and expressed in μg of caffeic acid/ml (SINGLETON and ROSSI 1965).

Total flavonoid content. The flavonoid content was determined spectrophotometrically according to the Dowd method (KAŠKONIENIE et al. 2009), using quercetin as a standard in the range 0–200 μg $ml^{-1}$. The coffee extracts were placed in a 10 ml volumetric flask. The mixtures were made up to 5 ml with distilled water, and 0.3 ml of 5% aqueous solution of $NaNO_2$ was added. 1 ml of the samples (2 g/100 ml) were then mixed and left for five minutes before 0.6 mL of 10% hexahydrate solution of $AlCl_3$ was added and mixed again. After five minutes, 2 ml of 1 M aqueous solution of NaOH was added and made up to 10 ml with distilled water. The solution was mixed again and the absorbance of the samples wasmeasured at a wavelength of 510 nm against a ethanol blank. The total flavonoid content was calculated using linear equations based on the calibration curve ($y = 0.1125x - 0.1027$, $r = 0.998203$) and expressed in μg of quercetin/ml. (KAŠKONIENIE et al. 2009).

Caffeine content. The contentof caffeine was determined by spectrophotometry at 276 nm, after prior extraction of the caffeine by chloroform in alkaline solutions of coffee (pH 12.5–12.7). The alkaline extract solutions were transferred to a separatory funnel and the caffeine was extracted with three further portions of chloroform (10ml/5ml/5ml). The samples were mixed for one minute and the solutions were allowed to separate for five minutes at room temperature. The organic layer of the extracted caffeine was collected in a 25 ml flask. The solution was made up to 25 ml with chloroform, and the samples were dried by adding anhydrous $MgSO_4$ to the organic phase and swirl with the dual purpose of removing water and breaking any emulsion and then filtered. The absorbance was measured at a wavelength of 277 nm (maximum absorption for caffeine) against a chloroform blank. Commercially obtained caffeine at concentrations ranging from 0 to 0.2 mg $ml^{-1}$ was used as a standard solution. The caffeine content was calculated using a linear equation based on calibration curve ($y = 0.1335x - 0.1094$, $r = 0.998233$) and expressed in mg of caffeine per 1 ml (PARADKAR and IRUDAYARAJ 2002).

Statistical Analysis.The results were expressed as mean ± standard deviation (SD). Each parameter was examined in triplicate. One-way analysis of variance (ANOVA) was used to access the statistical significance of any difference between the coffee brews. A $p$-value less than 0.05 was considered as significant. All statistical calculations were performed using Statistica software (StatSoft, Inc., Tulsa, OK, USA).

## Results and Discussion

Coffee is one of the most popular beverages throughout the world. The present study focuses on the antioxidant activity, total polyphenol content, flavonoid content and caffeine content of coffee extracts prepared in three different ways (Table 2 and Table 3).

Table 2

Total polyphenols, flavonoids and caffeine contents of roasted coffee depending on the species and the method of preparing infusion

| Coffee species | Method of preparing infusion | Total polyphenols | Total flavonoids | Caffeine |
|---|---|---|---|---|
| *1* | hot water | 498±12,4 | 269±4.84 | 0.41±0.05* |
| | percolator | 540.4±35.8 | 355.8±6.67 | 0.34±0.02* |
| | coffee machine | 593.9±12.6 | 308.2±2.65 | 0.39±0.01* |
| *2* | hot water | 481±15.8 | 276±5.12 | 0.47±0.05* |
| | percolator | 533±56.7 | 355.5±5.64 | 0.42±0.04* |
| | coffee machine | 519.9±30.02 | 29.71±5.27 | 0.33±0.02* |
| *3* | hot water | 578.4±46.9 | 297.4±0.62 | 0.70±0.05* |
| | percolator | 623±58.5 | 331.6±0.62 | 0.65±0.05* |
| | coffee machine | 529.4±1.7 | 298±19.2 | 0.41±0.02* |
| *4* | hot water | 587.7±13.6 | 340.7±1.32 | 0.76±0.06* |
| | percolator | 600.3±27.2 | 403.8±13.88 | 0.69±0.03* |
| | coffee machine | 573.4±19.7 | 374.3±1.82 | 0.15±0.01* |

* Statistically significant between groups

Table 3

Total polyphenols, flavonoids and caffeine contents of green coffee depending on the species and the method of preparing infusion

| Brewing | Method of preparing infusion | Total polyphenols | Total flavonoids | Caffeine |
|---|---|---|---|---|
| I | hot water (*H*) | 380.9±6.77* | 153.8±1.37* | 0.28±0.02 |
| | percolator (*P*) | 384.9±4.46* | 212.8±6.39* | 0.13±0.02 |
| | coffee machine (*M*) | 271.7±7.15* | 86±7.05* | 0.16±0.02 |
| | statistically significant between: | *P* and *M*, *H* and *M* | *H* and *P* and *M* | *H* and *P*<br>*H* and *M* |
| II | hot water (*H*) | 138.4±7.95 | 55.5±2.54* | 0.09±0.03 |
| | percolator (*P*) | 174.2±14.4 | 102.6±0.85* | 0.05±0.02 |
| | coffee machine (*M*) | 125.2±5.9 | 32.7±4.32* | 0.09±0.00 |
| | statistically significant between: | *H* and *M*<br>*P* and *M* | *H* and *P* and *M* | – |
| III | hot water (*H*) | 55.8±8 | 26±2.65* | 0.06±0.00 |
| | percolator (*P*) | 68.1±0.1 | 36.6±4.51* | 0.02±0.01 |
| | coffee machine (*M*) | 47.6±12.6 | 5.6±2.83* | 0.05±0.00 |
| | statistically significant between: | – | *H* and *P* and *M* | *H* and *P*<br>*M* and *P* |

I – first brew, II – second brew, III – third brew

* Statistically significant between groups

The analyses of the different brewing processes are given for roasted coffee in Figure 1, and for green coffee in Figure 2.

Generally, no significant changes were found between the method of preparing the beverages and the percentage of inhibition of DPPH radicals. For the beverages from roasted coffee beans, the mean inhibition values were 64.35±3.98% for pouring hot water over the ground beans; 67.33±4.87% for the percolator and 64.4±2.35% for the coffee machine, for 120 μg $ml^{-1}$ concentration of the coffee beans. The concentration 120 μg $ml^{-1}$ was chosen as an example to illustrate no differences in the antioxidant properties of the analyzed coffee extracts depending on the method of brewing.

For the green coffee beans (120 μg $ml^{-1}$ concentration), the mean inhibition values of DPPH radical scavenged by the antioxidants included in the coffee beveragesare 36.20±0.40% by pouring hot water, 46.46±4.87% for percolator coffee and 21.93±0.66% for the coffee machine for first brewing, 10.01±0.06, 20.51±3.07, 13.63±0.72 for second brewing, 5.86±0.84, 11.91±2.01, 5.96±0.47 for third brewing, respectively.

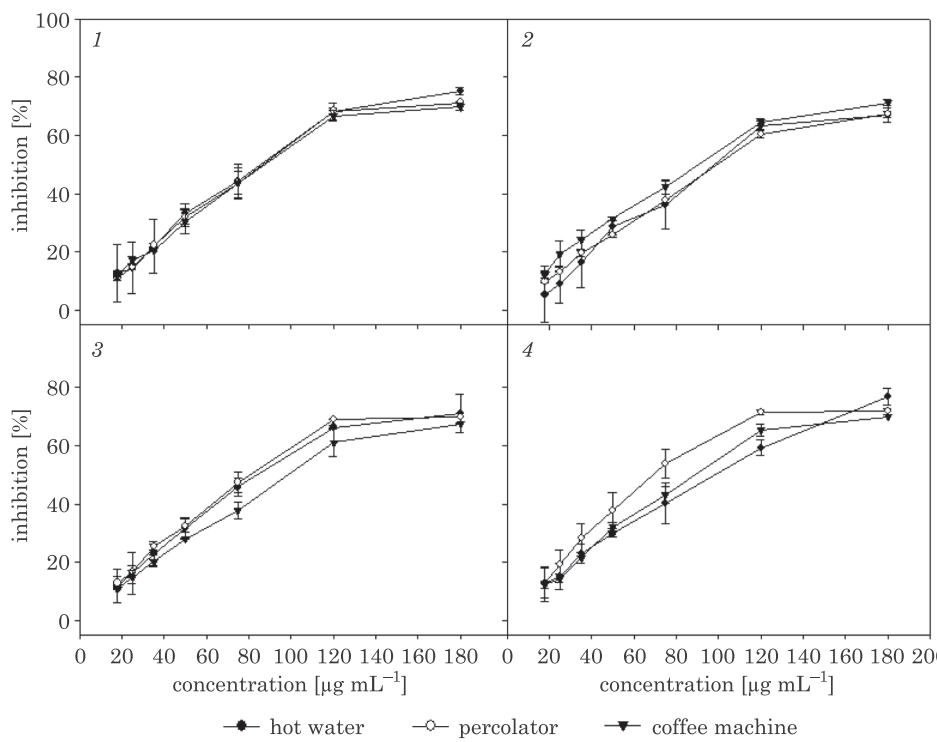


Fig. 1. Scavenging effect (percentage of remaining DPPH radical) of four roasted coffee extracts depending on three different brewing methods during the DPPH test, as measured by changes in absorbance at 517 nm. The numbers *1*, *2*, *3* and *4* represent types of coffee

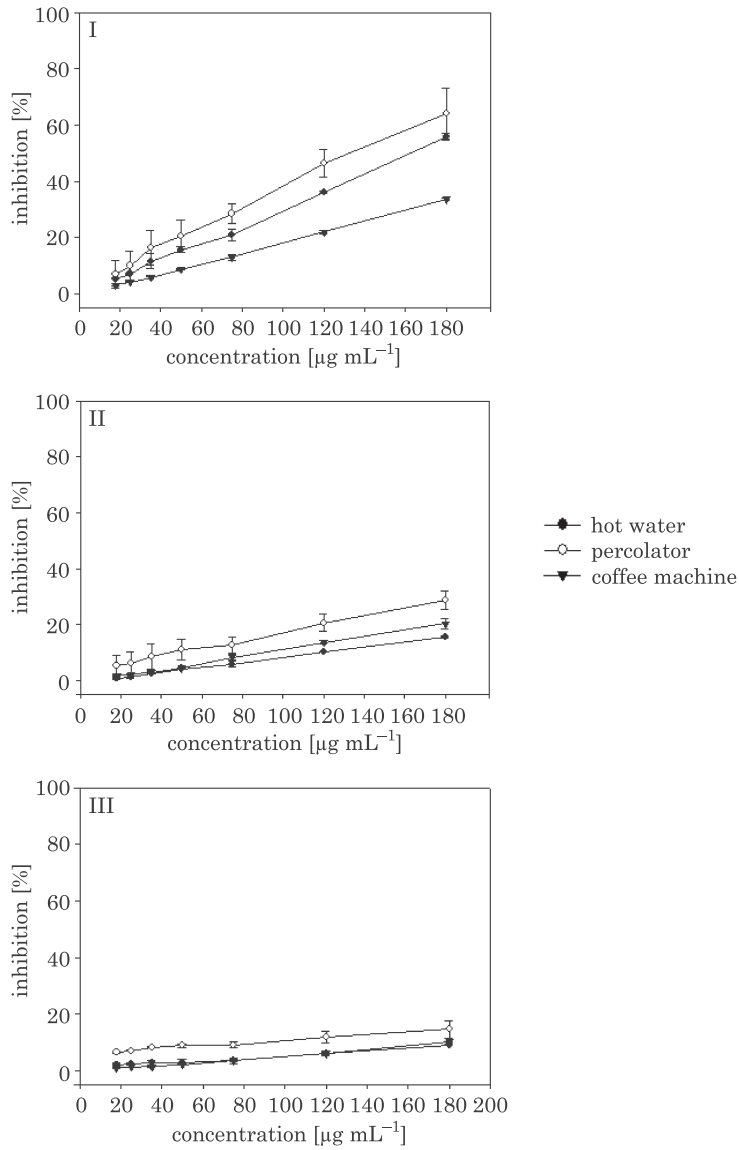


Fig. 2. Scavenging effect (percentage of remaining DPPH radicals) of green coffee extracts depending on three different brewing methods during the DPPH test, as measured by changes in absorbance at 517 nm: I – first brew, II – second brew, III – third brew

These results indicate that the best way to brew coffee to obtain the largest number of antioxidants is by using a coffee percolator, with pouring hot water over ground coffee beans being less effective, followed by using a coffee machine for coffee number one and two. For coffee number 3

and 4, the percentage values are very similar for all preparation methods. However, no statistically significant difference was found between these methods.

In addition, it is noteworthy that the studied extract of green coffee beans demonstrated significantly lower antioxidant activity than roasted coffee beans. Similar results were reported by GUNALAN et al. (2012), who note a 52% mean percentage of inhibition for two tested roasted coffee bean extracts at a concentration of 100 µg coffee $ml^{-1}$. RAMADAN-HASSANIEN (2008) reports the mean DPPH inhibition to be 33.2% for coffee prepared by pouring hot water over coffee beans, but it should be noted that this study uses half the content of coffee (1 g) and DPPH solution (0.5 ml), and a larger quantity of water (200 ml) to prepare the brew, compared to the present study. Lower antioxidant potential, ranging from 15.2% to 24.3%, were observed by DE OLIVEIRA et al. (2014) for 100 µg $ml^{-1}$ extract for selected types of coffee. Regarding the antioxidant activity of a sample of green coffee, PRIFTIS et al. (2015) report that the roasted beans exhibited greater antioxidant activity than their green counterparts in eight of 13 tested varieties, with the opposite being the case in the remaining five varieties. GORNAS (2016) demonstrated that the green coffee samples exhibited in turn the highest antioxidant capacity, decreasing with the rise in the degree of coffee bean. WOLSKA et al. (2017) show that the antioxidant activity of infusions was high and dependent on the species of coffee used and the condition of brewing. However, significant differences were found only between green coffee and arabica, and green coffee and robusta. Our research also has indicated, that, depends on brewing method, the greatest difference in antioxidant potential was found in green coffee infusions with reference to roasted coffee beans extracts.

## Total polyphenol content

The results according to brewing process, expressed as µg caffeic acid/100 µl sample of coffeeextract (2 g/100 ml), are shown in Figure 3 for the roasted coffee, and Figure 4 for the green coffee.

Generally, no significant changes were found regarding brewing method or total polyphenol content for roasted coffee. For the beverages from roasted coffee beans, the mean polyphenol content were as fallow: 536.28±16.54 µg caffeic acid $ml^{-1}$ for pouring hot water over coffee beans, 574.18±15.49 µg caffeic acid $ml^{-1}$ for percolator coffee and 554.15±11.92 µg caffeic acid $ml^{-1}$ for the coffee machine. The green coffee beans were examined after three consecutive brews. For the first brew, mean polyphenol content

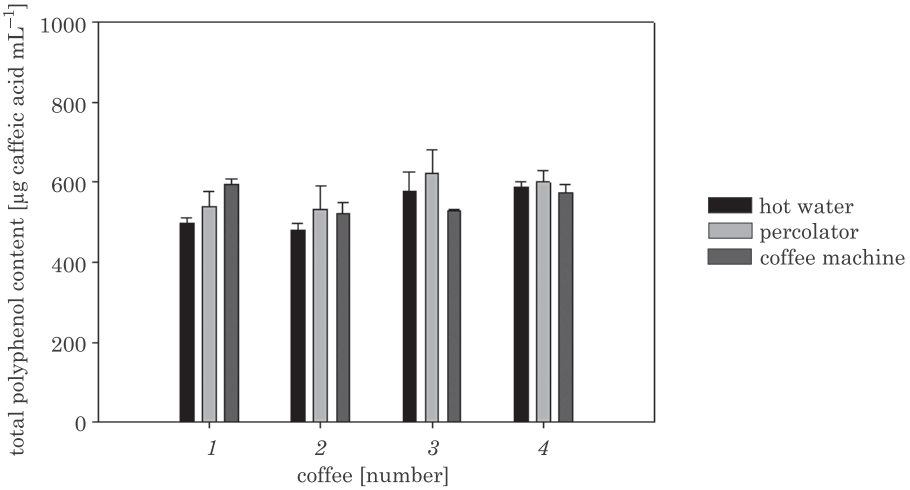


Fig. 3. Total polyphenol content in four roasted coffee beverages depending on three different methods of brewing

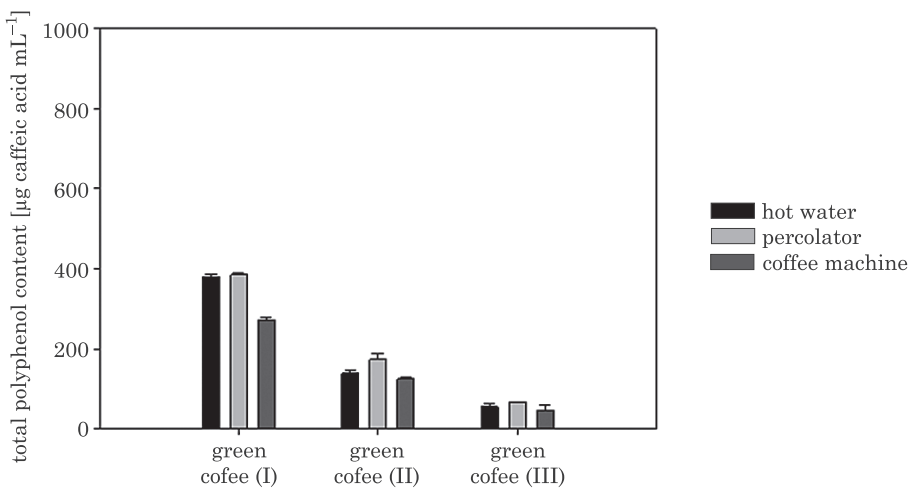


Fig. 4. Total polyphenol content in green coffee beverages depending on three different brewing methods: I – first brew; II – second brew; III – third brew

were 380.9±6.77 µg caffeic acid ml$^{-1}$ for pouring hot water over coffee beans, 384.9±4.46 µg caffeic acid ml$^{-1}$ for the percolator and 271.7±7.15 µg caffeic acid ml$^{-1}$ for the coffee machine. For the second brew, the respective values were 138.4±7.95, 174.2±14.4 and 125.2±5.9 µg caffeic acid ml$^{-1}$, according to brewing method. For the third brew, the respective values were 55.8±8, 68.1±0.1 and 47.6±12.6. Significant differences were found in first and second brew and the highest polyphenol content was in percolator

beverages. The extract of green coffee beans was found to have lower concentrations of biologically active compounds. Caffeic acid is one of the most important polyphenols in coffee beans responsible for their antioxidant activity. No differences in polyphenol content were reported in a similar study (KREICBERGS et al. 2011). However, in a study of 13 coffee varieties, green coffee beans were found to have higher amounts of polyphenols in seven varieties and the roasted beans in six varieties (PRIFTIS et al. 2015).

As reported by DEROSSI 2017 the American coffee presented higher values of total polyphenol content than espresso and Turkish coffees. In this case, different amounts of coffee powder was used for the experiments.

## Total flavonoid content

The results, based on the brewing process, expressed as percentage of quercetin $ml^{-1}$ of coffee beverage, are shown in Figure 5 for the roasted coffee beans, and in Figure 6 for the green coffee beans.

No significant differences in total flavonoid content between different species of roasted coffee were measured while using simple infusion, coffee machine and percolator (Figure 5). For the beverages from roasted coffee beans, the mean flavonoid content was found to be 295.78±2.34 µg quercetin $ml^{-1}$ for pouring hot water over coffee beans, 361.68±5.47 µg quercetin $ml^{-1}$ for the percolator coffee and 252.55±8.11µg quercetin $ml^{-1}$ for the coffee machine. For the beverages from green coffee beans, the mean flavonoid content was found to be 153.80±1.37 µg quercetin $ml^{-1}$ for hot water poured

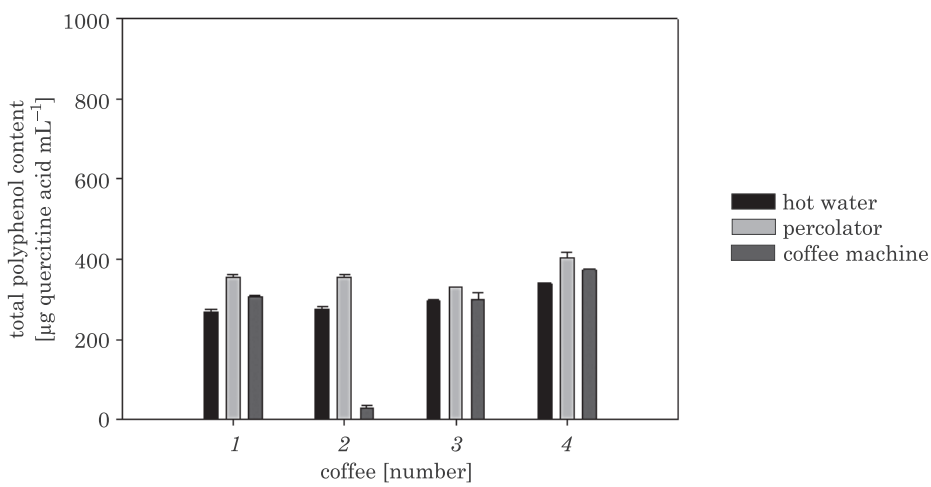


Fig. 5. Total flavonoid content in four roasted coffee beverages according to the three different brewing methods

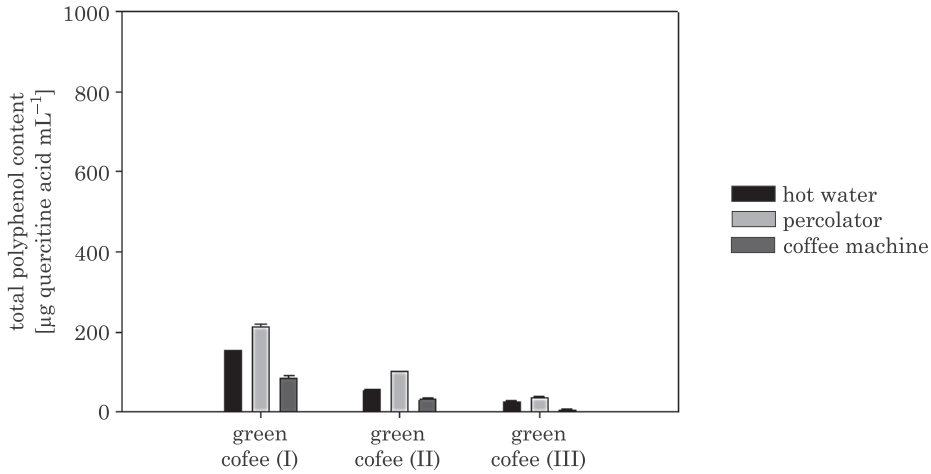


Fig. 6. Total flavonoid content in green coffee beverages depending on three tested brewing methods: I – first brew; II – second brew; III – third brew

over the coffee beans, 212.80±6.39 µg quercetin $ml^{-1}$ for the percolator and 86.00±7.05 µg quercetin $ml^{-1}$ for the coffee machine (first brew); 55.50±2.54 µg quercetin $ml^{-1}$, 102.60±0.85 µg quercetin $ml^{-1}$ and 32.70±4.32 µg quercetin $ml^{-1}$ (second brew); 26.00±2.65 µg quercetin $ml^{-1}$, 36.6±4.51 µg quercetin $ml^{-1}$ and 5.6±2.83 µg quercetin $ml^{-1}$ (third brew). Significant differences were found in first, second and third brew between different beverages. Quercetin is a flavonol, the most wide spread sub-class of flavonoids. As with total antioxidant activity and polyphenol content, flavonoid content was highest in green coffee brewed using a percolator; lower levels were observed in the coffee prepared by pouring hot water over coffee beans, and finally the coffee machine. On average, flavonoids comprised 44% of total polyphenol content, for both roasted and green beans (HEČIMOVIĆ et al. 2011).

## Caffeine content

Caffeine is the most important active ingredient responsible for the stimulatory effect of coffee, and its levels may vary depending on the type of bean and length of roasting process. The results according to brewing process are shown in Figure 7 for the roasted coffee and Figure 8 for the green coffee.

For the beverages from roasted coffee beans, the average content of caffeine [mg $ml^{-1}$] were 0.59±0.05 mg caffeine $ml^{-1}$ from pouring hot water over the beans, 0.53±0.03 mg caffeine $ml^{-1}$ for percolator coffee and 0.32±0.02 mg caffeine $ml^{-1}$ for coffee machine coffee. For the beverages

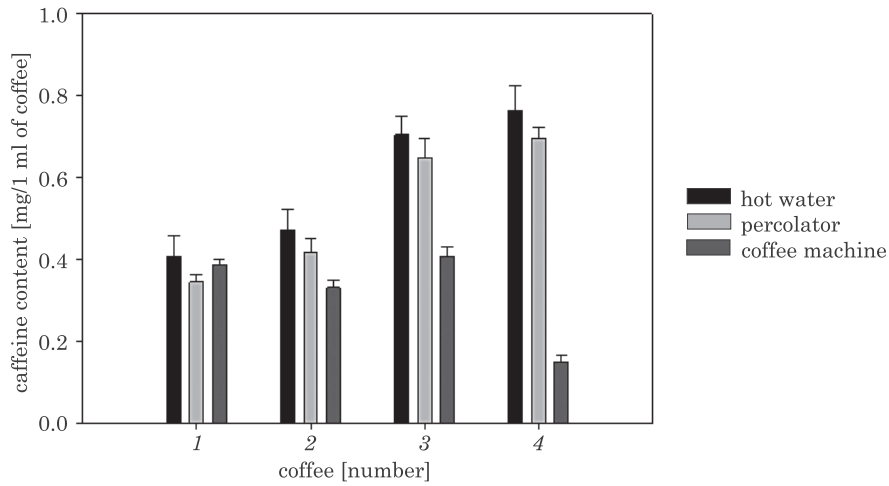


Fig. 7. Caffeine content of four roasted coffee beverages depending on three different brewing methods

from green coffee

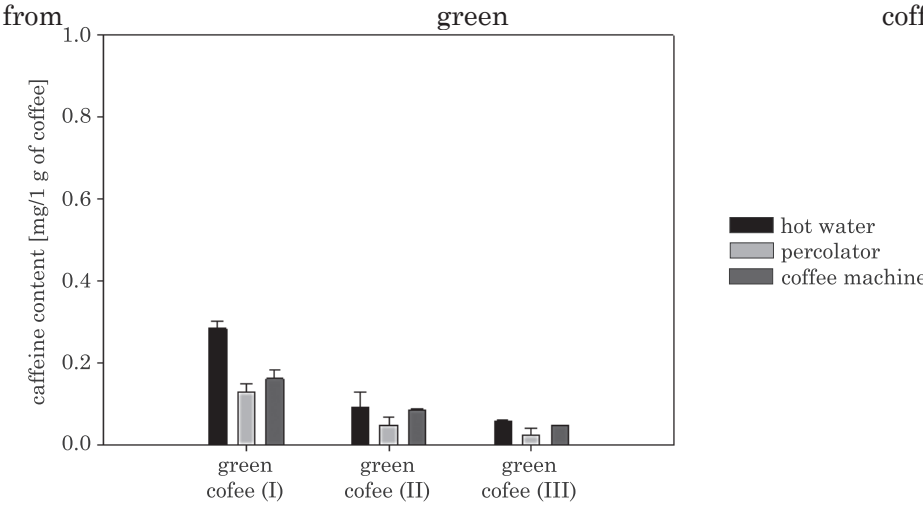


Fig. 8. Caffeine content of green coffee beverages depending on three different brewing methods: I – first brew; II – second brew; III – third brew

beans, the respective values were 0.28±0.02, 0.13±0.02 mg caffeine $ml^{-1}$ and 0.16±0.02 mg caffeine $ml^{-1}$ after the first brew, 0.09±0.03, 0.05±0.02 mg caffeine $ml^{-1}$ and 0.09±0.0004 mg caffeine $ml^{-1}$ after the second brew, and 0.06±0.005, 0.02±0.1 mg caffeine $ml^{-1}$ and 0.05±0.0008 mg caffeine $ml^{-1}$ after the third brew. A slightly higher level of caffeine was found in roasted and unroasted coffee beverages prepared by pouring hot water over the beans, than by using a coffee percolator or coffee machine. The caffeine content of green coffee beans varies between 0.9% and 1.3% dry

matter for Arabica and 1.5% and 2.5% for Robusta coffees. Typical caffeine levels in a cup of coffee vary between 50 and 100 mg (LUDWIG et al. 2014). Caffeine content differs based on brewing method and were ceive similar results (BELL et al. 1996).

The beneficial influence of coffee consumption has been highlighted in previous studies. Coffee intake appears to be associated with a lower risk of type 2 diabetes mellitus (van DAM and HU 2005,van DAM 2006), some cancers (BØHN et al. 2014, NKONDJOCK 2009) and Parkinson's disease (HERNÁN et al. 2002).

## Conclusions

Antioxidant activity of infusions was high for roasted and green coffee (first brew) but independent on the condition of brewing. No significant differences in total polyphenol and total flavonoids content between roasted coffee were measured while using simple infusion, coffee machine and percolator. However, significant differences in total polyphenol and total flavonoids content were found for green coffee infusions prepared in percolator. Statistically significant differences in caffeine content have been noticed for roasted and unroasted coffee beans in the following infusions: hot water, percolator and coffee machine for roasted coffee and hot water, coffee machine and percolator for unroasted coffee. Our findings indicate also that infusions from green coffee beans have lower antioxidant activity, as well as lower phenolic acid, flavonoid and caffeine content. Consecutive brews, performed according to the manufacturer's instructions, result in significantly lower levels of bioactive components.

To summarize, conducted experiments did not unequivocally answer the question, if the type of brewing method affects significantly the antioxidant activity in coffee beverages.

Simple infusion allows to obtain the highest content of biologically active substance in coffee. The results depend on type of selected coffee and, among other things, the method of its storage, the degree of grinding.

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