

CAFFEINE CONTENT AND ANTIOXIDANT ACTIVITY OF VARIOUS BREWS OF SPECIALTY GRADE COFFEE

Michał Miłek✉, Łukasz Młodecki, Małgorzata Dżugan

Department of Chemistry and Food Toxicology, Institute of Food Technology and Nutrition, University of Rzeszów
 Ćwiklińskiej 1A, 35-601 Rzeszów, Poland

ABSTRACT

Background. The aim of the study was to evaluate the caffeine level and antioxidant activity of brews of specialty grade coffee compared to popular coffee brands.

Materials and methods. Ten types of coffee were used, including 7 specialty Arabica, 1 Robusta and 2 popular cheap coffee brands. For caffeine determination, HPLC analysis and the spectrophotometric method were used as reference. The total polyphenol content and antioxidant capacity (DPPH and FRAP methods) were evaluated. For two selected high-quality coffees, the influence of the brewing method on the antioxidant activity and caffeine content in the brews was assayed.

Results. Regarding the caffeine content, differences between specialty coffee brews and popular products were not found, and an average level amounted to 56 and 40 mg/ml, respectively. In contrast, the antioxidant capacity of specialty coffee brews was significantly higher than for popular ones, independently of the test used. The highest scavenging ability and total phenolic content was found for S3 specialty coffee (46.15% of DPPH inhibition and 58.7 mg GAE/ml, respectively), whereas the lowest was found for popular coffee (about 35% of DPPH inhibition and about 41 mg/GAE/ml). For two selected high-quality coffees, the influence of the brewing method on the antioxidant activity and caffeine content in the brews was tested. It was found that the use of a dripper (overflow brewing method) provides the brew with the best antioxidant properties but with moderate caffeine levels.

Conclusion. It was found that ‘specialty’ quality coffees do not differ from popular brands in terms of caffeine content, but significantly outweigh them in terms of antioxidant activity. This property can be beneficial in the case of a high consumption of coffee, due to antiradical protective effects without the risk of caffeine overdose.

Keywords: coffee, Arabica, specialty grade, brewing method, total polyphenols

INTRODUCTION

Coffee is a drink eagerly consumed all over the world which has been proved to bring health benefits for humans if consumed in moderate amounts (Godoś et al., 2014). The health benefits of coffee consumption affecting the cardiovascular system and metabolism mostly depend on its antioxidant compounds. In contrast, diterpenes and caffeine may cause harmful

effects by raising lipid fraction and affecting endothelial function, respectively. Coffee is obtained from the seeds of the botanical type *Coffea (Rubiaceae)* and grains from two main species are commercially available: *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). Moreover, many botanical varieties of both Arabica and Robusta coffee are used. The Arabica

✉mmilek@ur.edu.pl, <https://orcid.org/0000-0001-9312-2506>, phone +48 17 872 1730

variety is preferred because of its stronger aroma and milder flavor; it also contains less caffeine than Robusta (Jeszka-Skowron et al., 2016; Khapre et al., 2017). The positive effects of coffee include its high antioxidant capacity, beneficial effects on the cardiovascular system, and even an anti-cancer effect (Ding et al., 2014; Gaascht et al., 2015; Messina et al., 2015; Yashin et al., 2013).

The antioxidant potential is associated with the high content of polyphenolic compounds in coffee beans, mainly phenolic acids (caffeic, chlorogenic, ferulic) and depsides, such as caffeoylquinic, dicaffeoylquinic and feruoylquinic acids. Certain flavonoids have also been identified, e.g., quercetin and kaempferol glycosides, as well as epigallocatechin (Farah and Donangelo, 2006; Górecki and Hallmann, 2020; Król et al., 2020; Pedan et al., 2020; Xu et al., 2012; Wang and Ho, 2009). Caffeine, which belongs to the alkaloids, is the best-known and the most active component of coffee. The mechanisms of its action concern the central nervous system and cause a stimulating effect on the human organism. Caffeine is also a known inhibitor of many enzymes (e.g., phosphodiesterases, acetylcholinesterase, transferases), it also interferes with adenosine and benzodiazepine receptors and neurotransmitters. Acting as an enzyme inhibitor may be beneficial (e.g., in the treatment of neurodegenerative diseases) but, on the other hand, it may lead to certain metabolic disturbances (Al-Qaisi et al., 2011; Nehlig et al., 1992; Pohanka and Dobes, 2013). The addictive effect of this alkaloid is also known (Cappelletti et al., 2015; Uddin et al., 2017). The chemical composition, and thus the biological properties, of coffee is closely related to its quality and the way the brew is prepared. Popular methods of brewing coffee include the use of a dripper, an AeroPress® device or an Italian coffee percolator (*moka pot*). Moreover, the chemical composition of the coffee brew is strongly dependent on the type and conditions of brewing, including polyphenols, diterpenes and caffeine content (Fuller and Rao, 2017; Gloess et al., 2013; Ludwig et al., 2012; Urgert et al., 1995). It has also been shown that the mineral composition of coffee depends on the brewing method, as well as the content of bioactive compounds (Janda et al., 2020). Moreover, the degree of roasting and the course of the roasting process also have a significant influence on the composition of coffee (Diviš

et al., 2019; Górecki and Hallmann, 2020; Pedan et al., 2020).

As a reaction to the decline in the quality of coffee offered on the market, the specialty coffee grade was born, which is defined as coffee grown in special and ideal climates with a distinctive taste and flavor, and with little to no defects. The term “specialty” for coffee beans means a particularly high-quality product and is currently specified by the Specialty Coffee Association (SCA) and applies to coffees which, when tested by specially trained tasters (*Q-graders*), reach at least 80 points on a 100-point scale (SCA, 2018). The specialty coffee market is growing rapidly in many countries, and the growing consumer demand for specialty coffee is attributable to consumers’ awareness regarding issues of quality, taste, health, environment, equity, and fair wages (Bacon, 2005). Quality control from the green beans to the roasting method helps bring the best coffee flavors and aromas, which are the main sensory components experienced by coffee drinkers.

In this work, several coffee brands from the specialty segment were compared in terms of antioxidant activity and caffeine content measured by two different methods. Furthermore, the influence of the brew preparation method on the health-promoting potential of selected specialty coffee samples was also estimated.

MATERIALS AND METHODS

Coffee

Ten types of coffee of different origin were used in the study, including seven specialty Arabica coffees and, for comparison, one Robusta coffee and two unknown quality coffees from popular cheap brands. The characteristics of the coffees used in the experiment are given in Table 1.

Preparation of coffee brews

All infusions were prepared in simulated commercial café conditions in cooperation with a barista, using professional equipment and with strict control of the dosed ingredients. The appropriate portion of each coffee bean was weighed for a specific coffee brewing method (described below). Coffee was ground using a WSCG-2 automatic mill (Wilfa, Oslo, Norway) immediately before preparing the infusions. To prepare

Table 1. A list of coffees used in the experiment

Sample number	Sample symbol	Botanical variety	Country of origin
1	S1	Gesha	Panama
2	S2	Red Bourbon	Burundi
3	S3	SL28, Ruiru	Kenya
4	S4	Yellow Caturra	Peru
5	S5	Caturra, Catuai	Nicaragua
6	S6	Bourbon	Rwanda
7	S7	Heirloom	Ethiopia
8	R	Robusta	Panama
9	M1	no data	no data
10	M2	no data	no data

S – Arabica specialty coffee, R – Robusta coffee, M – market coffee.

all the infusions, water was filtered using a Marella filter (Brita, Tanusstein, Germany) with an Aquaphor B25 Maxfor replaceable filter cartridge to unify the mineral content in the water. An Artisan Gooseneck electric kettle (Brewista, Cheyenne, Wyoming, USA) was used to boil the water.

Methods of brewing

For initial comparison of different coffee brands, the brews prepared using method A were used. In the second research step, the two chosen coffees of specialty grade were prepared using the A, B, and C methods of brewing.

Dripper (A). Pre-filtered water was heated to 90°C in an electric kettle. Then 12 g of coffee beans was weighed with an accuracy of 0.1 g and ground in an automatic grinder (using grinding level “Filter”, as recommended by the producer). A paper filter was placed in the server (Hario Coffee Server V60-02 set) of the dripper and hot water was poured onto it to warm up the dripper and the server, and to get rid of the paper aftertaste. This water was poured away, and then the previously ground coffee was added to the filter before the first portion of 24 ml of water was poured into the server and left for 30 seconds (the pre-infusion step).

After 30 seconds, 176 ml of water was added and all the water was allowed to flow through the ground beans. The total brewing time was 3 minutes, and the total water usage was 200 ml.

AeroPress® device (B). Pre-filtered water was heated to 88°C in an electric kettle. Then 12 g of coffee beans was weighed with an accuracy of 0.1 g and ground in an automatic grinder (using grinding level “Aeropress”, as recommended by the producer). The *AeroPress*® device was placed in the inverted position and preheated with hot water, before the previously ground coffee was poured in and the device was filled with the first portion of 35 ml of water and left for 30 seconds (pre-infusion step). After half a minute, 165 ml of water was added and mixed three times. Then a strainer with two paper filters, which were previously covered with hot water to get rid of the paper aftertaste in the brew, was added. The *AeroPress* was placed on the server and 60 seconds later the first portion of water was added and squeezed into the server to obtain a coffee brew. The total brewing time was 1 minute 40 seconds and the total water usage was 200 ml.

Percolator (C). Pre-filtered water was boiled in an electric kettle. Then 12 g of coffee beans was weighed with an accuracy of 0.1 g and ground in an automatic grinder (using grinding level “Mocca”, as recommended by the producer). Freshly ground coffee was poured into the strainer in the Italian coffee percolator (Bialetti Moka Express 3tz) without being whipped. The coffee strainer was placed in a tank under a safety valve filled with boiling water (150 ml) and the top of the coffee cup was turned off. The coffee percolator was placed on the burner to increase the pressure in the water chamber so that the water could flow through the ground coffee in the strainer and reach the upper tank through the spouts as a coffee brew. During the outflow of the brew into the upper tank of the coffee housing, the color of the outflow brew was observed. When the lighter color of the brew was observed, the heat supply to the coffee maker was cut off and the coffee machine was immersed in cold water to stop further coffee extraction. The total brewing time was 3 minutes and the total water usage was 150 ml.

Caffeine content determination

HPLC method. The HPLC method for caffeine determination was adopted from Fajara and Susanti (2017) with minor modifications. Analyses were carried out in the Laboratory of Plant Biotechnology “Aeropolis” using a Gilson chromatographic set (Gilson, Middleton, Wisconsin, USA), equipped with a binary pump (Gilson 322), a DAD detector (Gilson 172), a column thermostat (Knauer, Berlin, Germany) and an autosampler with a fraction collector (GX-271 Liquid Handler). The separation was performed using a Knauer Nucleosil II C-18 100-5 column (250 × 4.6 mm) with a precolumn (Gilson). The elution was performed isocratically with a water-methanol (95:5, v/v) mobile phase, flow rate: 1 ml·min⁻¹, and time of analysis: 7 min. The injection volume of the samples was 10 µl. The analyte was detected at a wavelength of 272 nm. For quantitative analysis of the caffeine, a standard curve was prepared using standard solution within the concentration range 0.0625–1 mg ($y = 18940x + 944.19$, $R^2 = 0.9881$). The LOQ and LOD were found to be 2 and 0.5 µg/ml respectively.

Spectrophotometric method. The content of caffeine in the prepared coffee brews was determined according to Li et al. (1990). The prepared infusions were filtered through a paper filter and 10 ml of the resulting filtrate was basified with a NaOH solution to pH 12.5–12.7. The solutions were transferred to a separatory funnel and extracted with two 10 ml portions of chloroform. After adding each portion, the solutions were shaken in a separatory funnel for one minute and allowed to stratify for 5 minutes. The organic layer with the extracted caffeine was collected into 25 ml volumetric flasks. The flasks were made up to the mark with chloroform. The absorbance of the samples was measured using a spectrophotometer (Biomate 3, Thermo Scientific, Waltham, Massachusetts, USA). The absorbance values were read for the maximum caffeine absorption band (λ_{\max} 276 nm) and at 310 nm (background). The actual caffeine absorbance was calculated from the difference in measured values. The caffeine content was calculated on the basis of the prepared standard curve for pure caffeine chloroform solutions ($y = 0.0372x + 0.1061$, $R^2 = 0.9928$).

Total phenolic content assay

Total phenolic content was determined using a Folin-Ciocalteu reagent, according to Singleton and Rossi (1965) with minor modifications. Briefly, 0.2 ml of each coffee brew was added to 1 ml of 10% Folin-Ciocalteu reagent followed by 0.8 ml of 7.5% (w/v) Na₂CO₃ solution. After mixing, the samples were kept in the dark for 120 min and then the absorbance was read at 760 nm against blank (Biomate 3 spectrophotometer, Thermo Scientific, Waltham, Massachusetts, USA). Results are expressed as mg of gallic acid equivalents per milliliter of brew (mg GAE·ml⁻¹) based on a calibration curve ($y = 0.0555x$, $R^2 = 0.9976$).

Antioxidant assays

DPPH. DPPH radical inhibition was measured according to the assay described by Blois (1958) with minor modifications. Briefly, 0.2 ml of each coffee brew was mixed with 1.8 ml of DPPH radical methanolic solution (0.1 mmol·l⁻¹) and kept in the dark for 60 min. After incubation, the absorbance of the samples was measured at 517 nm against blank. The reduction of DPPH radical was calculated using the following equation:

$$\text{DPPH percentage reduction} = (A_0 - A_s) / A_0 \times 100$$

where:

A_0 – the absorbance of control,

A_s – the absorbance of the samples with extract.

The results were also expressed as µmol of Trolox equivalents per milliliter of brew (µmol TE·ml⁻¹) based on a calibration curve ($y = 1.4124x$, $R^2 = 0.9793$).

FRAP. The FRAP assay (Ferric Reducing Ability of Plasma) was carried out as previously described by Benzie and Strain (1996) with some minor modifications introduced by Bertonec et al. (2007). The FRAP reagent contained 2.5 ml of a 10 mmol·l⁻¹ TPTZ solution in 40 mmol·l⁻¹ HCl, 2.5 ml of 20 mmol·l⁻¹ FeCl₃ and 25 ml of 0.3 mol·l⁻¹ acetate buffer (pH 3.6). 1.8 ml of the prepared reagent was added to 0.2 ml of coffee brew and the absorbance of the reaction mixture was measured spectrophotometrically (Biomate 3 spectrophotometer, Thermo Scientific, Waltham, Massachusetts, USA) at 593 nm after incubation at 37°C for 10 min against blank. The results were expressed as µmol of Trolox equivalents per milliliter of brew

($\mu\text{mol TE}\cdot\text{ml}^{-1}$) based on a calibration curve ($y = 0.026x$, $R^2 = 0.9980$).

Statistical analysis

Calculations of the average values (3 independent infusions, 2 repetitions of analysis) and calibration curves were carried out in MS Excel 5.0. Pearson's correlation coefficient was used to analyze the correlation of the obtained results. Statistically significant differences were determined by a one-way ANOVA variance analysis with the Tukey test (Statistica 13, StatSoft).

RESULTS AND DISCUSSION

Caffeine content in specialty coffee

The caffeine content in brews prepared with method A for the individual analyzed coffee grades determined by the HPLC method is presented in Figure 1. A great variability was observed between particular products offered as high-quality coffee. Among the coffee samples analyzed, the highest caffeine content was recorded in the Robusta variety (0.66 mg/ml of brew or 10.97 mg per gram of coffee), which is consistent with

all data indicating this species as richer in caffeine (Dias and Benassi, 2015; Jeszka-Skowron et al., 2016; Khapre et al., 2017). When comparing specialty coffees with those commonly available on the market, in most cases, a higher caffeine content was determined in specialty coffees. Only coffees labeled as S2 and S4 contained less caffeine (0.113 and 0.235 mg/ml, which corresponds to 1.89 and 3.92 mg per gram of coffee, respectively) than popular market coffees (average content: 0.276 mg/ml, or 4.61 mg per gram of coffee). Both market coffees were very similar in terms of caffeine content. The values of the caffeine level obtained in the study are of the same order of magnitude as the data reported in the literature by various authors (dePaula and Farah, 2019; Dias and Benassi, 2015; Hećimović et al., 2011). Often, the caffeine content is given per portion of the infusion (e.g., a cup), which makes it difficult to compare the data. McCusker et al. (2003) investigated the caffeine content in specialty coffees – converted to the metric system, they obtained values ranging from 0.3 to 0.55 mg/ml of brew. This strictly corresponds to the alkaloid content in the samples we tested. The geographic origin of coffee, the conditions of its brewing and processing, and the

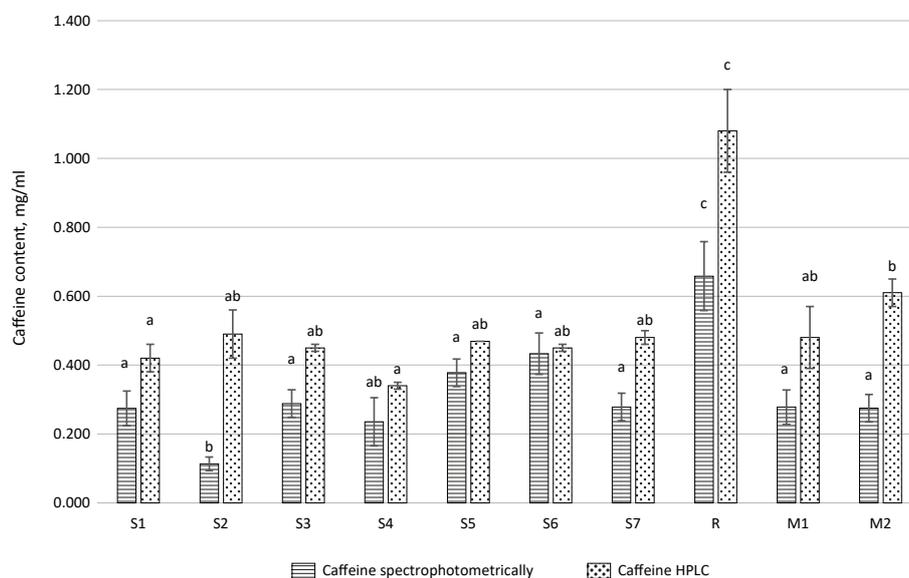


Fig. 1. Caffeine content in coffee brews determined spectrophotometrically and by the HPLC method. Means sharing the same superscript letter (within data concerning one analytical method) are not significantly different (Tukey's honest significant difference test, $p < 0.05$)

method of preparing the infusion may also have an impact on the caffeine content (Hečimović et al., 2011; McCusker et al., 2003).

There is currently no set acceptable daily intake (ADI) value for caffeine, but the dose of ≤ 2.5 mg/kg body weight/day has been used in risk assessments (Pollard et al., 2015). The European Food Safety Authority (EFSA) gives single doses up to 200 mg as a safe limit for daily caffeine intake for adults, which corresponds to 3 cups of 100 ml manual dripped coffee or 1 cup of 100 ml soluble coffee + 1 cup 100 ml cold brewing coffee or 2 cups of 100 ml aero press coffee + 1 cup of 100 ml infusion coffee bag (dePaula and Farah, 2019; EFSA, 2015).

There were significant ($p < 0.05$) differences between the caffeine results obtained by the two methods used. Due to this, fresh brews were used in the HPLC assay, whereas in the spectrophotometric examinations the sample was prepared by extraction of caffeine using chloroform. The results obtained were lower in comparison to the HPLC results as a consequence of incomplete analyte recovery. A similar tendency was observed by Hečimović et al. (2011) in the case of using chloroform extraction. Moreover, a great variability between the two methods was observed (by

3–75%), which indicates the problem with gaining full effectiveness of the extraction. The chromatographic method of caffeine quantitation is used as a routine method in this type of assay and it is characterized by better accuracy and repeatability of results. As the spectrophotometric method is cheaper and easily accessible, we compared it with HPLC. However, one strong disadvantage of the spectrophotometric method is the use of volatile chloroform as a solvent.

Total phenolic content and antioxidant capacity

The Folin-Ciocalteu method was used to determine the content of polyphenols in the coffee infusions, whereas the antioxidant capacity of the tested brews was examined by two methods based on the different mechanisms' methods (DPPH and FRAP). The results are presented in Table 2.

Among the tested coffee infusions, the variation in total polyphenol content and antioxidant capacity was low. However, the S3 sample was the best in terms of this and was only significantly different ($p < 0.05$). It is worth noting that both the polyphenol content and antioxidant capacity were lower in the case of market coffees compared to those of specialty quality. The average content of these compounds calculated for all

Table 2. Total phenolic content and antioxidant capacity data for tested coffee brews

Sample symbol	Total phenolic content mg GAE/ml	FRAP $\mu\text{mol TE/ml}$	DPPH	
			% of radical inhibition	$\mu\text{mol TE/ml}$
S1	50.56 \pm 3.92 ^a	26.51 \pm 2.15 ^{bc}	40.02 \pm 0.99 ^{abc}	11.74 \pm 1.49 ^{abc}
S2	43.82 \pm 1.32 ^{abc}	24.35 \pm 1.37 ^c	33.84 \pm 2.64 ^c	10.12 \pm 1.93 ^c
S3	58.70 \pm 4.64 ^d	33.08 \pm 4.31 ^a	46.15 \pm 2.06 ^a	13.35 \pm 1.77 ^a
S4	47.21 \pm 0.92 ^{abc}	29.68 \pm 4.11 ^{abc}	37.86 \pm 3.38 ^{abc}	11.18 \pm 2.12 ^{abc}
S5	48.32 \pm 4.74 ^{ac}	24.66 \pm 0.94 ^{abc}	36.41 \pm 1.16 ^{abc}	10.80 \pm 1.54 ^{abc}
S6	44.76 \pm 1.53 ^{abc}	24.88 \pm 0.02 ^{abc}	37.51 \pm 1.24 ^{abc}	11.08 \pm 1.56 ^{abc}
S7	49.05 \pm 5.25 ^a	29.86 \pm 4.03 ^{abc}	38.51 \pm 0.99 ^{abc}	11.35 \pm 1.49 ^{abc}
R	47.35 \pm 4.79 ^{abc}	25.52 \pm 1.33 ^{abc}	36.87 \pm 2.15 ^{abc}	10.92 \pm 1.80 ^{abc}
M1	41.12 \pm 0.46 ^b	22.86 \pm 0.52 ^c	34.77 \pm 2.97 ^{ab}	10.37 \pm 2.01 ^{ab}
M2	41.84 \pm 2.19 ^{bc}	23.78 \pm 0.78 ^{bc}	35.06 \pm 4.54 ^{bc}	10.44 \pm 2.43 ^{bc}

Data presented as mean value \pm standard deviation ($n = 3$). The means marked with different letter superscripts in a column differ significantly ($p < 0.05$).

Table 3. Correlation matrix for caffeine quantitation methods and antioxidant capacity

	Caffeine UV	Caffeine HPLC	TPC	FRAP	DPPH
Caffeine UV	1				
Caffeine HPLC	0.744	1			
TPC	0.066	-0.165	1		
FRAP	-0.121	-0.285	0.852	1	
DPPH	0.053	-0.245	0.941	0.856	1

tested specialty coffees is 40.69 mg GAE/ml, which is a value close to that obtained by Hallmann et al. (2010), who reported the average polyphenol content in conventional coffees of 39.83 mg/ml. In addition, in other studies these values were at a similar level and were within 20–50 mg GAE/g (Abdeltaif et al., 2018; Chłopicka et al., 2015; Hečimović et al., 2011). Similarly, in the case of antioxidant potential (analyzed with the FRAP test and DPPH radical quenching), the S3 sample exhibited the highest activity, whereas significantly lower results were found in market coffees. A strong positive correlation was found between the polyphenol content determined by the Folin-Ciocalteu method and the total antioxidant capacity determined by the FRAP method ($r = 0.852$) as well as by the DPPH method ($r = 0.941$). Both methods of determining the antioxidant potential were also strongly correlated ($r = 0.855$). No significant correlation was observed between caffeine content and antioxidant activity (Table 3).

Coffee is a known source of antioxidants, which has been confirmed by numerous studies (Liang and Kitts, 2014; Yashin et al., 2013). Research conducted by Díaz-Rubio and Saura-Calixto (2007) compared the total antioxidant capacity in coffee brewed using the filter method, and an average activity of 1565.2 $\mu\text{mol TE}/100\text{ ml}$ of brews was determined. In our tests, the average content of Trolox was 2651.8 $\mu\text{mol TE}/100\text{ ml}$ when calculated for the same unit. In our tests, the ability to inhibit the DPPH radical is 33–46%, which is a relatively low result. This may be a consequence of the sample dilution used in the assay. According to other sources, coffee infusions quench this synthetic radical with an efficiency of over 60% (Hudáková et al., 2016; Pokorná et al., 2015). The results converted to Trolox equivalents are more reliable and suitable

for comparison. The results expressed in this way are even higher than some data available in the literature (da Cruz et al., 2018; Stalmach et al., 2006). No significant differences were found for the Robusta variety of coffee we examined.

It has been shown that certain anti-radical properties are exhibited by bioactive compounds present in coffee, such as caffeine, nicotinic acid or trigonelline, but to a lesser extent than phenolic acids (Daglia et al., 2004). Phenolic acid derivatives are the main antioxidant compounds in coffee and their content and profile depend on many factors: coffee type, cultivation and processing conditions. Moreover, in the roasting process, new compounds (e.g., polymeric melanoidins) with potential antioxidant activity may be formed (Yashin et al., 2013).

The effects of the brewing method

It has been shown that the method used to brew the coffee affects its properties, including phenol content and antioxidant potential (Díaz-Rubio and Saura-Calixto, 2007; Yashin et al., 2013). Therefore, the two specialty products were selected, S4 (as the lowest caffeine source) and S5 (as the richest one), for evaluation of the influence of the brewing method on the brew composition. The results of the caffeine content in various brews determined by HPLC, total polyphenol content, and antioxidant capacity are summarized in Table 4.

Having analyzed the content of polyphenols and the antioxidant potential of two selected coffee species prepared by three different methods, it can be noticed that the results are at a similar level with a slight advantage for the brew prepared in a dripper. Among the three brewing methods used, the content of polyphenolic compounds and antioxidant capacity was the

Table 4. Caffeine content, total phenolic content and antioxidant capacity of coffee brews prepared using different methods

Sample symbol	Brewing method	Caffeine content mg/ml	Total phenolic content mg GAE/ml	Antioxidant capacity		
				FRAP $\mu\text{mol TE/ml}$	DPPH	
				$\mu\text{mol TE/ml}$	% of radical inhibition	$\mu\text{mol TE/ml}$
S4	Dripper (A)	0.46 \pm 0.01 ^a	53.08 \pm 1.78 ^a	29.31 \pm 0.15 ^{ac}	41.66 \pm 1.65 ^a	12.17 \pm 1.67 ^a
	AeroPress® (B)	0.46 \pm 0.04 ^a	45.41 \pm 2.55 ^b	25.82 \pm 1.17 ^b	34.07 \pm 3.13 ^b	10.18 \pm 2.06 ^b
	Percolator (C)	0.98 \pm 0.04 ^b	50.72 \pm 3.82 ^a	28.40 \pm 0.70 ^c	43.93 \pm 1.90 ^a	12.77 \pm 1.73 ^a
S5	Dripper (A)	0.70 \pm 0.14 ^c	51.82 \pm 1.63 ^a	30.92 \pm 3.35 ^a	38.62 \pm 1.16 ^c	11.38 \pm 1.54 ^c
	AeroPress® (B)	0.75 \pm 0.04 ^c	46.09 \pm 0.97 ^b	25.89 \pm 0.98 ^b	40.37 \pm 4.13 ^{ac}	11.84 \pm 2.32 ^{ac}
	Percolator (C)	0.98 \pm 0.04 ^b	44.77 \pm 1.99 ^c	23.54 \pm 0.09 ^b	37.28 \pm 0.58 ^c	8.40 \pm 1.39 ^c

Data presented as mean value \pm standard deviation ($n = 3$). The means marked with different letter superscripts in a column differ significantly ($p < 0.05$).

lowest for method B. It is worth emphasizing that the infusion obtained by method A contained significantly less caffeine than method C. This makes method A more beneficial in preserving the health-promoting properties of coffee while reducing the caffeine content. Dependencies of the studied parameters on the brewing method used, including “alternative” methods, were also observed by Lin et al. (2009). In their case, American style coffee (espresso diluted with hot water) and espresso were characterized by having the highest anti-radical activity as well as the highest caffeine content. The use of a dripper resulted in a significant reduction in the content of caffeine but also the content of polyphenols (Lin et al., 2009). Similarly, the comparable antioxidant activity of coffee prepared in an AeroPress® device and in a dripper was observed by Janda et al. (2020). It has been shown that the method of brewing has a significant impact on the caffeine content. Espresso coffee has the highest content and, among those tested by us, coffee made with a percolator. The infusions with the lowest caffeine content include those obtained by using a dripper or infusion bags (de Paula and Farah, 2019).

CONCLUSION

Great variability was observed in caffeine content between brews of specialty grade Arabica coffee. However, the content was significantly lower than in the

Robusta variety. In contrast, in terms of total polyphenol content and antioxidant capacity, these samples did not differ significantly, excluding one sample originating from Kenya, which was more abundant in antioxidants. Among the three tested methods of brewing, the dripper produced a brew with high antioxidant activity without simultaneously enhancing the caffeine level. Additionally, the benefits of using the HPLC method for caffeine determinations were confirmed as it is faster and allows the loss of analyte during sample preparation to be avoided in spectrophotometric determinations.

REFERENCES

- Abdeltaif, S. A., Sirelkhatim, K. A., Hassan, A. B. (2018). Estimation of phenolic and flavonoid compounds and antioxidant activity of spent coffee and black tea (Processing) waste for potential recovery and reuse in Sudan. *Recycling*, 3(2), 27. <https://doi.org/10.3390/recycling3020027>
- Al Qaisi, Z. H. J., Abbas, S. A. R., Abdullah, A. H. (2011). Effect of caffeine on some transferase enzymes activities. *Int. J. Chem.*, 3(2), 140–148. <https://doi.org/10.5539/ijc.v3n2p140>
- Bacon, C. (2005). Confronting the coffee crisis: Can fair trade, organic, and specialty coffees reduce small-scale farmer vulnerability in Northern Nicaragua? *World Dev.*, 33(3), 497–511. <https://doi.org/10.1016/j.worlddev.2004.10.002>

- Benzie, I. F. F., Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of “antioxidant power”: The FRAP Assay, *Anal. Biochem.*, 239, 70–76.
- Bertoncelj, J., Doberšek, U., Jamnik, M., Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chem.*, 105(2), 822–828. <https://doi.org/10.1016/J.FOODCHEM.2007.01.060>
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199–1200. <https://doi.org/10.1038/1811199a0>
- Cappelletti, S., Piacentino, D., Sani, G., Aromatario, M. (2015). Caffeine: Cognitive and physical performance enhancer or psychoactive drug? *Curr. Neuropharmacol.*, 13, 71–88. <https://doi.org/10.2174/1570159X13666141210215655>
- Chłopicka, J., Niedziela, A., Bartoń, H. (2015). Aktywność antyoksydacyjna i całkowita zawartość polifenoli w naparach kawy w zależności od rodzaju kawy i sposobu jej przygotowania [Antioxidant activity and total polyphenols in coffee infusion prepared under different conditions impact of brewing method and time]. *Bromatol. Chem. Toksykol.*, 48, 278–292 [in Polish, with English abstract].
- Daglia, M., Racchi, M., Papetti, A., Lanni, C., Govoni, S., Gazzani, G. (2004). *In vitro* and *ex vivo* antihydroxyl radical activity of green and roasted coffee. *J. Agric. Food Chem.*, 52, 1700–1704. <https://doi.org/10.1021/jf030298n>
- da Cruz, R. G., Vieira, T. M. F. S., de Lira, S. P. (2018). Potential antioxidant of Brazilian coffee from the region of Cerrado. *Food Sci. Technol.*, 38(3), 447–453. <https://doi.org/10.1590/1678-457X.08017>
- dePaula, J., Farah, A. (2019). Caffeine consumption through coffee: Content in the beverage, metabolism, health benefits and risks. *Beverages*, 5(2), 37. <https://doi.org/10.3390/beverages5020037>
- Dias, R., Benassi, M. (2015). Discrimination between Arabica and Robusta coffees using hydrosoluble compounds: Is the efficiency of the parameters dependent on the roast degree? *Beverages*, 1(3), 127–139. <https://doi.org/10.3390/beverages1030127>
- Díaz-Rubio, M. E., Saura-Calixto, F. (2007). Dietary fiber in brewed coffee. *J. Agric. Food Chem.*, 55, 1999–2003. <https://doi.org/10.1021/jf062839p>
- Ding, M., Bhupathiraju, S. N., Chen, M., Van Dam, R. M., Hu, F. B. (2014). Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: A systematic review and a dose-response meta-analysis. *Diabetes Care*, 37(2), 569–586. <https://doi.org/10.2337/dc13-1203>
- Diviš, P., Pořízka, J., Křikala, J. (2019). The effect of coffee beans roasting on its chemical composition. *Potrav. Slov. J. Food Sci.*, 13(1), 344–350. <https://doi.org/10.5219/1062>
- EFSA (2015). European Food Safety Authority (EFSA Panel on Dietetic Products, Nutrition and Allergies). Scientific Opinion on the Safety of Caffeine; European Food Safety Authority: Parma, Italy, 2015.
- Fajara, B. E. P., Susanti, H. (2017). HPLC determination of caffeine in coffee beverage. *IOP Conf. Ser. Mater. Sci. Eng.*, 259(1), 012011. <https://doi.org/10.1088/1757-899X/259/1/012011>
- Farah, A., Donangelo, C. M. (2006). Phenolic compounds in coffee. *Braz. J. Plant Physiol.*, 18(1), 23–36. <https://doi.org/10.1590/S1677-04202006000100003>
- Fuller, M., Rao, N. Z. (2017). The effect of time, roasting temperature, and grind size on caffeine and chlorogenic acid concentrations in cold brew coffee. *Sci. Rep.*, 7(1), 1–9. <https://doi.org/10.1038/s41598-017-18247-4>
- Gaascht, F., Dicato, M., Diederich, M. (2015). Coffee provides a natural multitarget pharmacopeia against the hallmarks of cancer. *Genes Nutr.*, 10(6), 1–17. <https://doi.org/10.1007/s12263-015-0501-3>
- Gloess, A. N., Schönbächler, B., Klopprogge, B., D’Ambrosio, L., Chatelain, K., Bongartz, A., ..., Yeretzyan, C. (2013). Comparison of nine common coffee extraction methods: Instrumental and sensory analysis. *Eur. Food Res. Technol.*, 236(4), 607–627. <https://doi.org/10.1007/s00217-013-1917-x>
- Godoš, J., Pluchinotta, F. R., Marventano, S., Buscemi, S., Li Volti, G., Galvano, F., Grosso, G. (2014). Coffee components and cardiovascular risk: beneficial and detrimental effects. *Int. J. Food Sci.*, 65, 925–936. <https://doi.org/10.3109/09637486.2014.940287>
- Górecki, M., Hallmann, E. (2020). The antioxidant content of coffee and its *in vitro* activity as an effect of its production method and roasting and brewing time. *Antioxidants*, 9(4), 308. <https://doi.org/10.3390/antiox9040308>
- Hallmann, E., Ožga, M., Rembiałkowska, E. (2010). The content of bioactive compounds in selected kind of coffee from organic and conventional production. *J. Res. Appl. Agric. Eng.*, 55(3), 99–104.
- Hečimović, I., Belščak-Cvitanović, A., Horžić, D., Komes, D. (2011). Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. *Food Chem.*, 129, 991–1000. <https://doi.org/10.1016/j.foodchem.2011.05.059>
- Hudáková, J., Marcincáková, D., Legáth, J. (2016). Study of antioxidant effects of selected types of coffee. *Folia Vet.*, 60(3), 34–38. <https://doi.org/10.1515/fv-2016-0026>

- Janda, K., Jakubczyk, K., Baranowska-Bosiacka, I., Kapczuk, P., Kochman, J., Rębacz-Maron, E., Gutowska, I. (2020). Mineral composition and antioxidant potential of coffee beverages depending on the brewing method. *Foods*, 9(2). <https://doi.org/10.3390/foods9020121>
- Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K., De Peña, M. P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *Eur. Food Res. Technol.*, 242(8), 1403–1409. <https://doi.org/10.1007/s00217-016-2643-y>
- Khapre, Y., Kyamuhangire, W., Kihara Njoroge, E., Kathurima, C. W. (2017). Analysis of the diversity of some arabica and robusta coffee from kenya and uganda by sensory and biochemical components and their correlation to taste. *IOSR J. Environ. Sci. Toxicol. Food Technol.*, 11(10). 39–43. <https://doi.org/10.9790/2402-1110023943>
- Król, K., Gantner, M., Tatarak, A., Hallmann, E. (2020). The content of polyphenols in coffee beans as roasting, origin and storage effect. *Eur. Food Res. Technol.*, 246, 33–39. <https://doi.org/10.1007/s00217-019-03388-9>
- Li, S., Berger, J., Hartland, S. (1990). UV spectrophotometric determination of theobromine and caffeine in cocoa beans. *Anal. Chim. Acta*, 232(C), 409–412. [https://doi.org/10.1016/S0003-2670\(00\)81263-5](https://doi.org/10.1016/S0003-2670(00)81263-5)
- Liang, N., Kitts, D. D. (2014). Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules*, 19(11), 19180–19208. <https://doi.org/10.3390/molecules191119180>
- Lin, S. Y., Wang, L. H., Lin, Y. H., Han, Y. H., Wang, Y. S., Yeh, C. S. (2009). Antioxidative activity and caffeine content of coffee from different preparation methods, Taiwan. *J. Agric. Chem. Food Sci.*, 47(5), 268–275.
- Ludwig, I. A., Sanchez, L., Caemmerer, B., Kroh, L. W., De Peña, M. P., Cid, C. (2012). Extraction of coffee antioxidants: Impact of brewing time and method. *Food Res. Int.*, 48(1), 57–64. <https://doi.org/10.1016/j.foodres.2012.02.023>
- McCusker, R. R., Goldberger, B. A., Cone, E. J. (2003). Caffeine content in specialty coffee. *J. Anal. Toxicol.*, 27, 520–522.
- Messina, G., Zannella, C., Monda, V., Dato, A., Liccardo, D., De Blasio, S., ..., Monda, M. (2015). The beneficial effects of coffee in human nutrition. *Biol. Med.*, 7(4), <https://doi.org/10.4172/0974-8369.1000240>
- Nehlig, A., Daval, J. L., Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Rev.*, 17(2), 139–170. [https://doi.org/10.1016/0165-0173\(92\)90012-B](https://doi.org/10.1016/0165-0173(92)90012-B)
- Pedan, V., Stamm, E., Do, T., Holinger, M., Reich, E. (2020). HPTLC fingerprint profile analysis of coffee polyphenols during different roast trials. *J. Food Compos. Anal.*, 94, 103610. <https://doi.org/10.1016/j.jfca.2020.103610>
- Pohanka, M., Dobes, P. (2013). Caffeine inhibits acetylcholinesterase, but not butyrylcholinesterase. *Int. J. Mol. Sci.*, 14, 9873–9882. <https://doi.org/10.3390/ijms14059873>
- Pokorná, J., Venskutonis, P. R., Kraujalyte, V., Kraujalis, P., Dvůrák, P., Tremlová, B., ..., Oštádalová, M. (2015). Comparison of different methods of antioxidant activity evaluation of green and roast *C. arabica* and *C. robusta* coffee beans. *Acta Aliment.*, 44(3), 454–460. <https://doi.org/10.1556/066.2015.44.0017>
- SCA (2018). Coffee standards. Santa Ana, USA: Specialty Coffee Association. <https://sca.coffee/research/coffee-standards>
- Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.*, 16, 144–158.
- Stalmach, A., Mullen, W., Nagai, C., Crozier, A. (2006). Online HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. *Braz. J. Plant Physiol.*, 18(1), 253–262. <https://doi.org/10.1590/S1677-04202006000100018>
- Uddin, M. S., Sufian, M. A., Hossain, M. F., Kabir, M. T., Islam, M. T., Rahman, M. M., Rafe, M. R. (2017). Neuropsychological effects of caffeine: Is caffeine addictive? *J. Psychol. Psychoter.*, 7(2), 1000295. <https://doi.org/10.4172/2161-0487.1000295>
- Urgert, R., van der Weg, G., Kosmeijer-Schuil, T. G., van de Boverikamp, P., Hovenier, R., Katan, M. B. (1995). Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews. *J. Agric. Food Chem.*, 43(8), 2167–2172. <https://doi.org/10.1021/jf00056a039>
- Wang, Y., Ho, C. T. (2009). Polyphenols chemistry of tea and coffee: A century of progress. *J. Agric. Food Chem.*, 57(18), 8109–8114. <https://doi.org/10.1021/jf804025c>
- Xu, J. G., Hu, Q. P., Liu, Y. (2012). Antioxidant and DNA-protective activities of chlorogenic acid isomers. *J. Agric. Food Chem.*, 60(46), 11625–11630. <https://doi.org/10.1021/jf303771s>
- Yashin, A., Yashin, Y., Wang, J. Y., Nemzer, B. (2013). Antioxidant and antiradical activity of coffee. *Antioxidants*, 2(4), 230–245. <https://doi.org/10.3390/antiox2040230>