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THE CONTENT OF POLYPHENOLS AND CAFFEINE IN SPENT COFFEE GROUNDS OBTAINED FROM VARIOUS HOME BREWING METHODS

S u m m a r y

Background. Grounds generated during coffee brewing as waste produced in the world in millions of tons per year seem to be an interesting source of bioactive substances for further use. The aim of the study was to evaluate the content of bioactive components in coffee grounds obtained from coffee brewing using popular methods. Coffee grounds obtained as a result of brewing Arabica coffee using five methods: pouring, drip, crucible, coffee maker and espresso machine were tested. The content of phenolic acids and total phenols, as well as antioxidant activity (by means of DPPH$^{\bullet}$ and FRAP methods) and caffeine (for the first time by means of high-performance thin layer chromatography (HPTLC) technique) were assessed.

Results and conclusion. A coffee brewing method significantly affects the residue of bioactive compounds in grounds. The results obtained allow to conclude that the methods in which the contact of a ground grain with water is short, and a grain is coarsely ground, leave the most polyphenols and caffeine in coffee grounds. Such techniques were those using a drip and espresso machine (total phenolic content up to 12.29 and 14.88 mg gallic acid equivalents/g, respectively) and pouring in the case of caffeine (21 mg/g). In turn, coffee grounds obtained from brewing coffee in a crucible, in which most of the determined substances were extracted into the infusion, had the least bioactive compounds. Out of all the extraction systems being evaluated, aqueous ethanol (50 %) was indicated as the most effective one. The use of spent coffee grounds as a source of bioactive substances may be a valuable way to valorize large amounts of gastronomic waste.

Keywords: antioxidant activity, brewing methods, caffeine, coffee grounds, HPTLC

Introduction

The multitude of bioactive substances contained in coffee beans determines the multidirectional effect of coffee drinks, both on well-being and concentration, as well as on the body's efficiency and health. Coffee beans have a stimulating effect, and due

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to polyphenolic compounds contained in them, they have a health-promoting effect on the body. Caffeine contained in them has the effect of stimulating the rate of metabolism by increasing fat oxidation [2]. The benefits of consuming even a small amount of coffee determine its popularity and a widely developed culture of drinking coffee beverages.

The preparation of coffee, whether in cafés, households or in industrial processing plants, entails the need to manage or dispose of waste from the production of beverages, concentrates or other products that require coffee beans to be produced. It is currently estimated that 6 million tons of coffee grounds are produced annually worldwide [11], of which about 50 % comes from the industrial production of instant coffee and coffee shops, and the rest from the home preparation of beverages [17]. Therefore, two main sources of coffee grounds can be distinguished: the industry and gastronomy, as well as households, which differ in a technology used and, consequently, in the characteristics of generated waste. Due to the high content of organic bioactive ingredients in spent coffee grounds, they can be used as food additives after prior cleaning and appropriate preparation, e.g. by the extraction of appropriate compounds.

According to Balzano et al. [4], the fractionation of coffee grounds could contribute to increasing their usefulness in the food industry. For example, an isolated oil fraction could be used for biotech ethanol production, whereas carbohydrates and crude fiber would be an excellent substrate for fermentation by colon microbiota, producing anti-inflammatory short-chain fatty acids [15]. Campos-Vega et al. [8] indicate the potential cholesterol-lowering effect of unsaturated acids isolated from coffee grounds, which could help prevent heart disease. In turn, Diaz de Otalora et al. [9] investigated the indirect nutritional value of used coffee grounds as a concentrate additive to the feed of dairy sheep, which resulted in increased milk yield and concentration of macronutrients such as protein and fat in milk. Coffee grounds extracts rich in antioxidants and antimicrobial compounds can be used as natural preservatives to extend the shelf life of certain products. Gemechu [12] cites an example of inhibition of lipid peroxidation in minced pork after adding an ethanol extract of coffee grounds. In a study by Monente et al. [19], the authors proved the antimicrobial effect of ethanol extracts of used coffee grounds, mainly by inhibiting the growth of gram-positive bacteria. In his article, Gemechu [12] draws attention to the possibility of using dietary fiber purified and isolated from coffee grounds, which could be an addition to slimming preparations. Another study indicated the extraction of dietary fiber as an opportunity to recover other nutrients and non-nutrients, such as proteins and compounds with antioxidant activity [8].

All the properties of spent coffee grounds tested so far can be the basis for using this raw residual material as a potential source of bioactive compounds, thus becoming an interesting and promising food ingredient. The aim of the study was to determine

the content of bioactive compounds remaining in coffee grounds after brewing coffee using various methods available at home.

Material and methods

Chemicals and reagents

All chemical reagents, unless stated otherwise, were purchased from Chempur (Piekary Śląskie, Poland).

Preparation of coffee grounds

Ten grams of freshly ground (MK-06M electric grinder, MPM, Milanówek, Poland) coffee Barista Espresso coffee (100 % Arabica; Tchibo) was used to obtain the grounds, using five various brewing methods.

Coffee maker: Brewing coffee using a coffee maker was done as follows: the bottom water tank of the coffee maker was filled with cold water so as not to overflow the level of the safety valve (200 cm^3). Then, two teaspoons of freshly ground coffee (approx. 10 g) were poured into the coffee container, the parts of the coffee maker were screwed together and placed on the burner on medium fire.

Drip: the ground coffee was placed in the filter; a small amount of water was first poured so as to cover the ground coffee and the whole thing was left for 30 seconds. Coffee was then poured in circular motions, taking care not to pour water over the walls of the filter. Water was added twice successively so as not to leave the ground coffee beans without contact with water. The entire coffee brewing process took about three minutes.

Crucible: A copper crucible with a long handle was used for the experiment, into which 10 g of freshly, finely ground coffee was poured, 200 cm^3 of cold water was poured over and unrefined cane sugar was added in the amount of 10 g. The whole thing was thoroughly mixed and placed on a burner over low heat. The mixture prepared in this manner was heated until the solution rose and foamed, and the crucible was removed from the heat immediately after the solution began to bubble in a characteristic way.

Espresso machine: The Saeco Minuto HD8761 automatic espresso machine (Saeco, Gaggio Montano, Italy) was used, which is designed for the preparation of coffee-espresso. The machine is equipped with a ceramic coffee grinder with adjustable grinding degree, which in turn will allow the use of whole grains in the cooking process. Features: power 1850 W, pressure 15 bar, volume water tank 1.8 dm^3, coffee container 250 g, self-cleaning system. Using this method, 35 cm^3 of infusion was obtained from 10 g of coffee.

Pouring hot water: Water in a kettle was boiled, then left for 3 min. to cool down to approx. 94 °C. In the meantime, about 10 g of medium-ground coffee beans were

poured into the glass, and then 200 cm^3 of water at the right temperature was poured over it. Coffee was brewed for two - three minutes, then poured through a sieve to remove the grounds. The spent grounds obtained from all coffee brewing methods were dried in a laboratory dryer (40 ^oC; SLN 35 Simple, POL-EKO, Wodzisław Śląski, Poland) for five hours, to water content not exceeding 3 % (assessed by a weight method) and extracted for further determinations.

Coffee ground extraction

Three types of coffee grounds extracts were prepared: aqueous, aqueous- ethanol (50 %) and ethanolic (95 % vol). In each case, 10 ml of solvent was poured over 2 g of dry grounds, which were then extracted in an ultrasonic bath (700 W; Sonic-10, Polsonic, Warszawa, Poland) for 20 minutes. The extracts were filtered through paper and used for further determinations.

Antioxidant capacity and polyphenols content

Total phenolic content (TPC) was determined according to Singleton and Rossi [23] with modification, enabling measurement using a microplate reader. Briefly, 20 µl of 40-times diluted extract were pipetted into microplate wells, then 100 µl of 10 % Folin-Ciocalteu reagent and 80 µl of 7.5 % Na_2CO_3 solution were added. The plate was incubated for 1 hour in the dark, then the absorbance was measured at 760 nm using EPOCH2 microplate reader (Biotek, Winooski, VT, USA). The results were expressed in mg of gallic acid equivalents (GAE) per gram of coffee grounds, using a calibration curve ($y = 0.3364 \cdot x$, $r^2 = 0.9914$).

The determination of phenolic acids by the Arnov method was based on the methodology described by Kulichova et al. [14]. Briefly, 30 µl of 10-times diluted sample were pipetted into a spectrophotometric plate, then 150 µl of distilled water, 30 µl of 0.5 M HCl, 30 µl of Arnov reagent, 30 µl of 1 M NaOH and 30 µl of distilled water was added. The absorbance of the samples was measured immediately in a microplate reader (EPOCH 2, Biotek) at a wavelength of 490 nm. The total phenolic acid content was calculated using the calibration curve ($y = 0.0446 \cdot x$, $R^2 = 0.9873$) and expressed in mg of caffeic acid per 1 g of coffee grounds.

The determination of the antioxidant activity of coffee grounds using the FRAP method was carried out according to Bertoncelj et al. [5], modified as follows: 20 µl of 40-times diluted sample was introduced on a microplate, 180 µl of FRAP reagent was added and incubated for 10 min. at 37 °C. The sample plate was then placed in a microplate reader (EPOCH 2, Biotek) to measure the absorbance of the test samples at a wavelength of 593 nm. The absorbance value of the samples was calculated using the calibration curve ($y = 0.1523 \cdot x$, $r^2 = 0.9995$). The results obtained were expressed in µmol of Trolox per 1 g of the sample of the tested coffee grounds.

The determination of the antiradical activity by the DPPH$^{\bullet}$ method was performed according to the Blois [6] method, modified for use in multi-well plates. Briefly, 20 µl of the tested extracts (40-times diluted) were introduced on a microplate, 180 µl of DPPH$^{\bullet}$ reagent (Sigma Aldrich, Saint Louis, MO, USA) was added and left for 30 min. in the dark. The plate was then placed in a microplate reader (EPOCH 2, Biotek) and the absorbance was measured at 517 nm. The antioxidant activity of the sample was calculated using the calibration curve ($y = 15.554 \cdot x$, $r^2 = 0.9975$). Results were expressed in µmol of Trolox per 1 g of the sample of the tested coffee grounds.

HPTLC determination of caffeine content

The high-performance thin layer chromatography (HPTLC) analysis was prepared as follows: samples of the extracts were applied with a microsyringe (2 µl) on a 20 x 10 cm HPTLC plate (ALUGRAM ® Xtra SIL G/UV254, Merck, Darmstadt, Germany). The samples were applied in the form of strips using an automatic applicator (Linomat 5, Camag, Muttenz, Switzerland). An aqueous solution of caffeine (99%, Sigma Aldrich, Saint Louis, MO, USA) was used for calibration, put on the plate in parallel with the test extracts. Calibration range: 0.25 ÷ 2 µg/ml. Limit of detection (LOD): 0.125 µg/ml and limit of quantitation (LOQ): 0.25 µg/ml have been established. The plate was developed using an ADC-2 automatic chromatographic chamber (Camag). The developing phase was a mixture of chloroform, ethyl acetate and formic acid (5:4:1). The solvent front migration distance was 70 mm. The Camag TLC Visualizer was used to analyze the compounds separated by HPTLC, images were taken in UV light at wavelength 254 nm. Images were analyzed using Camag's VisionCats software. The caffeine content of the extracts was calculated from the intensity of the bands (converted to peaks), according to the equation of the standard curve ($y = 1.558 \cdot 10^{-5} \cdot x$; $r^2 = 0.9999$) using the VisionCats software. The result of the quantitative analysis was expressed in µg/g of coffee grounds.

Statistical analysis

All samples were extracted in duplicate and analyzed in three technical replicates. The results were covered by a statistical analysis in the Statistica 13 software. The mean and standard deviation for each sample were calculated, as well as the correlation between the results expressed by Pearson's r coefficient in Microsoft Excel 2019. The ANOVA was used to assess the significance of differences between the means. The Brown-Forsythe test was applied to check the homogeneity of variance and normal distribution of data was tested using Shapiro-Wilk test. As a post-hoc test, Tukey's test was applied ($p = 0.05$).

Results and discussion

The total polyphenol content and phenolic acids in extract of the tested spent coffee grounds are presented in Table 1. In most cases, the highest content of polyphenols was found in water-ethanol extracts, with the exception of poured coffee, where the aqueous extract was the richest in these substances (11.15 mg GAE/g d.w.) than water-ethanol extract (8.16 mg GAE/g d.w.). It was found that with water mainly hydrophilic acids were extracted, whereas the use of ethanol favors the separation of hydrophobic compounds, hence the optimal extraction phase is water-ethanol (Fig. 1). However, this observation shows the possibility to select the adequate extractants which allow to separate expected polyphenols fraction. Taking into account the method by which the largest amount of polyphenols can be left over in coffee grounds is the drip coffee brewing method, where the content of these compounds in the sample was 14.88 mg GAE/g d.w. Other methods in which the amount of polyphenols is at a good recovery level are brewed coffee grounds in the espresso machine (12.29 mg GAE/g d.w.), infused coffee (11.15 mg GAE/g d.w.) and coffee from the coffee maker (10.03 mg GAE/g d.w.). The smallest content of the tested compounds was determined in a sample of coffee grounds from a crucible, only 6.52 mg GAE/g d.w. Differences in the total content of phenolic compounds in spent coffee grounds were previously shown not only depending on the brewing method but also on geographical origin. The reported values ranged from 25.13 mg GAE/g d.m. for light Mexican brewed Mocha coffee to 46.23 mg GAE/g d.w. for dark coffee from Nicaragua brewed using drip [13]. Lower values, similar to those obtained in our study, are reported by Musatto et al. [20], who obtained from 6 to 18.2 mg GAE/g coffee grounds using different concentrations of methanol and a different ratio of solvent to raw material. The results obtained for polyphenols residues in ground coffee, taking into account different types of sample extraction (3.39 to 14.88 mg GAE/g d.w.), are in line with other authors' findings, who analyzed the composition of bioactive compounds in these waste type. Abbasi-Parizad et al. [1] indicate the content of phenolic compounds in the amount of 10.05 ± 0.44 mg GAE/g of dry mass of coffee grounds. Mussatto et al. [20] found the level in the range of 6 - 17.9 mg GAE/g, while Lopez-Linares et al. [16] of about 0.48 mg GAE/g. The content of phenolic acids in the article by Abbasi-Parizad et al. was 1.098 ± 0.038 mg/g [1], while in our experiment the amount of these compounds ranged from 0.84 to 3.34 mg CAE/g d.w., with the highest concentration of acids recorded in most cases in ethanolic extracts, and the lowest in aqueous ones. The influence of the type of solvent and extraction conditions of coffee beans (Arabica and Robusta) on the content of bioactive substances in brews was also previously studied. It was found that the use of ethanol resulted in thebhigher recovery of caffeine and lower chlorogenic acids and Maillard reaction products, which were better extracted with water [7]. Since among the polyphenols present in coffee, non-phenolic acids constitute a small percentage [24], based

on the determination of phenolic acids, it can be suspected that the data on TPC in water and water-ethanol extracts could be overestimated. It is known that the Folin-Ciocalteu reagent is not specific enough for phenolic compounds, it may be reduced by other substances (e.g. unsaturated fatty acids, reducing carbohydrates, amino acids). It is postulated that it can be used to better express the antioxidant activity of samples, however, it is still a popular method for determining the content of polyphenols in plant extracts and food samples [10].

Table 1. Total polyphenolic compound and phenolic acid content, as well as antioxidant capacity of coffee grounds extracts

Tabela 1. Całkowita zawartość związków polifenolowych i kwasów fenolowych oraz aktywność antyoksydacyjna ekstraktów fusów kawowych

Brewing method/extraction solvent Metoda parzenia/rozpuszczalnik ekstrakcyjny		Total phenolic content Całkowita zawartość związków fenolowych [mg GAE/g d.w.]	Total phenolic acids content Całkowita zawartość kwasów fenolowych [mg CAE/g d.w.]	Antioxidant capacity Aktywność przeciwutleniająca [µmol TE/g d.w.]	
				FRAP	DPPH$^{\bullet}$
Drip / Drip	ethanol / etanol	3.92±0.39^{aA}	3.32±0.04^{aA}	19.70±1.57^{aA}	9.36±0.44^{aA}
	water / woda	10.44±0.03^{aB}	2.02±0.04^{aB}	51.25±1.48^{aB}	25.76±1.29^{aB}
	ethanol / etanol 50 %	14.88±0.01^{aC}	2.60±0.08^{aC}	68.88±2.82^{aC}	38.03±1.16^{aC}
Crucible Tygielek	ethanol / etanol	3.54±0.45^{aA}	3.22±0.25^{aA}	15.50±1.12^{bA}	8.89±1.32^{abA}
	water / woda	5.66±0.01^{bB}	1.12±0.02^{bB}	25.94±1.12^{bB}	12.57±0.54^{bA}
	ethanol / etanol 50 %	6.52±0.31^{bB}	1.32±0.02^{bB}	30.53±0.00^{bC}	15.31±0.64^{bB}
Coffee maker Kawiarka	ethanol / etanol	3.39±0.15^{aA}	3.34±0.33^{aA}	13.53±0.85^{aA}	9.84±3.14^{abA}
	water / woda	5.84±0.07^{bcB}	1.23±0.01^{cB}	26.99±1.18^{bB}	13.05±0.00^{bA}
	ethanol / etanol 50 %	10.03±0.04^{cC}	1.75±0.27^{cB}	46.42±0.00^{cC}	23.05±0.34^{cB}
Pouring Zalewanie wrzątkiem	ethanol / etanol	3.70±0.61^{aA}	2.90±0.05^{bA}	18.94±2.99^{cA}	8.32±0.27^{bA}
	water / woda	11.5±0.06^{aB}	2.11±0.15^{bcA}	56.99±1.25^{cB}	26.37±0.21^{aB}
	ethanol / etanol 50 %	8.16±0.04^{dC}	0.84±0.25^{dB}	39.72±0.66^{dC}	18.29±0.10^{bC}
Espresso machine Ekspres do kawy	ethanol / etanol	3.58±0.16^{aA}	2.83±0.02^{bA}	17.66±1.77^{dA}	6.02±0.27^{cA}
	water / woda	6.52±0.07^{cB}	1.27±0.31^{dB}	31.85±0.33^{dB}	14.60±1.28^{bB}
	ethanol / etanol 50 %	12.29±0.07^{eC}	2.20±0.01^{eA}	56.01±2.10^{eC}	29.04±0.44^{dC}
Mean Średnia	ethanol / etanol	3.63±0.38	3.12±0.27	17.07±2.81	8.48±1.90
	water / woda	7.93±2.46	1.55±0.46	38.60±13.44	18.47±6.50
	ethanol / etanol 50 %	10.38±3.07	1.74±0.66	48.31±13.76	24.74±8.41

Objaśnienia /Explanatory notes:

^{a,b,c,d,e} - means marked with the same lowercase superscripts differ among samples brewed with different methods within one extraction solvent used, ^{A,B,C} - means marked with the same uppercase superscripts differ among samples brewed with different extraction solvents within one method used

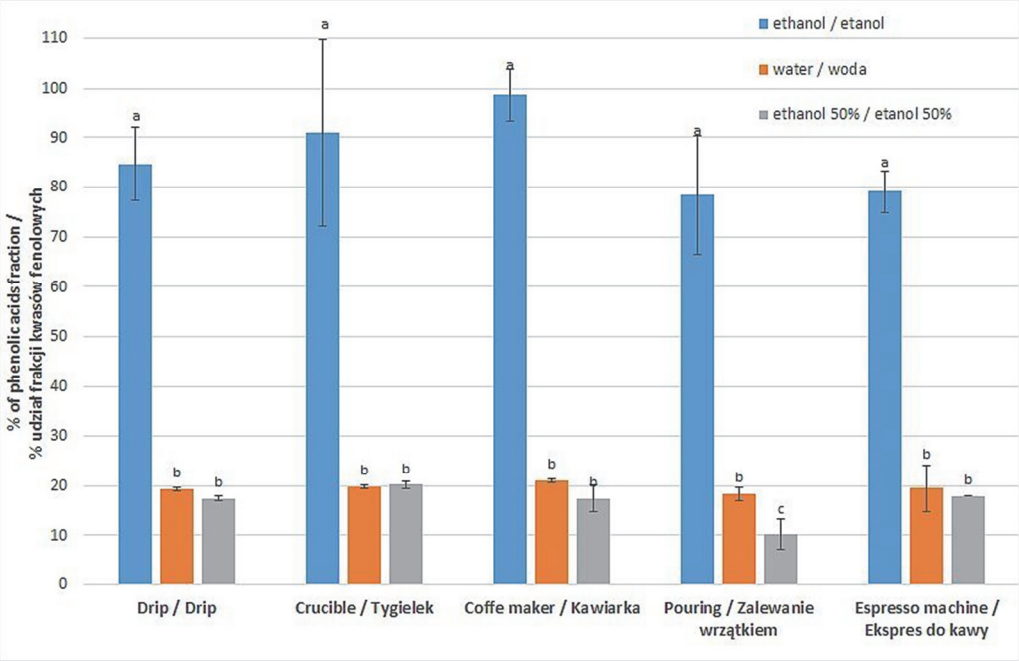

Fig. 1. Percentage share of the phenolic acids fraction in the total phenolic content, a,b,c - means marked with the same superscripts differ among samples brewed with different methods within one extraction solvent used

Rys. 1. Procentowy udział frakcji kwasów fenolowych w całkowitej zawartości związków fenolowych, a,b,c – średnie oznaczone tymi samymi literami różnią się istotnie w ramach jednego zastosowanego rozpuszczalnika ekstrakcyjnego

The antioxidant capacity evaluated by two methods based on different mechanisms allows to conclude that the vast majority of samples showed the greatest antioxidant activity in the case of water-ethanol extraction (Table 1). Only the poured coffee ground sample differs from the others, the sample extracted with distilled water gave a higher result in both methods used, namely FRAP and DPPH$^{\bullet}$. The results obtained by both methods show a very high degree of correlation (r = 0.982). The highest antioxidant activity was shown by a sample of extracts from coffee grounds brewed using the drip method, then in the espresso machine, while the poorest properties were shown by the infusion obtained using a crucible. The measured antioxidant activity of water-ethanol extracts was created mainly by polyphenolic compounds which confirmed calculated Pearson's correlation coefficient values (0.985 and 0.991 for TPC-DPPH and TPC-FRAP, respectively). The high antioxidant activity of extracts from coffee grounds brewed using drip and espresso machine indicates a less effective extraction of these compounds into the solution during coffee brewing, which led to a less valuable

infusion in terms of antioxidant properties compared to coffee brewed using other methods. Another explanation may be the effect of pressure treatment during coffee brewing on the structure of coffee grounds, which makes it easier to leach antioxidant components from beans [7, 25]. Moreover, coffee made in a crucible, brewed in a coffee maker or poured into a glass is in contact with hot water for a long time, and a coffee grain itself is finely ground, which facilitates the extraction of compounds for the brew and their lower content in coffee grounds. It was observed visually that the coffee grounds obtained by brewing coffee in a coffee maker and a crucible were more fine-grained compared to those obtained by other methods. It is well known that the method of brewing coffee has a significant impact on the antioxidant properties of the brew [18, 21], therefore it is reflected in the opposite way in waste after brewing. The effect of the temperature of water used for brewing on the antioxidant capacity of the infusions was observed (hot brewing gave higher values) [21]. It might be expected that the content of antioxidants in the coffee infusion should be inversely correlated with their content in the coffee grounds obtained from the brewing process, however, the described studies focused on assessing coffee grounds only, disregarding the quality of the infusion.

The quantitative HPTLC method was used for the first time to determine the content of caffeine residues in coffee grounds. Based on the initial assessment, water-ethanolic extracts were selected for quantitative analysis. Caffeine visible under UV 254 nm light in the form of a dark band on a green background was noted in all tested samples (R_f = 0.35; Figure 2), which confirms that caffeine recovery during coffee brewing is below 100%.

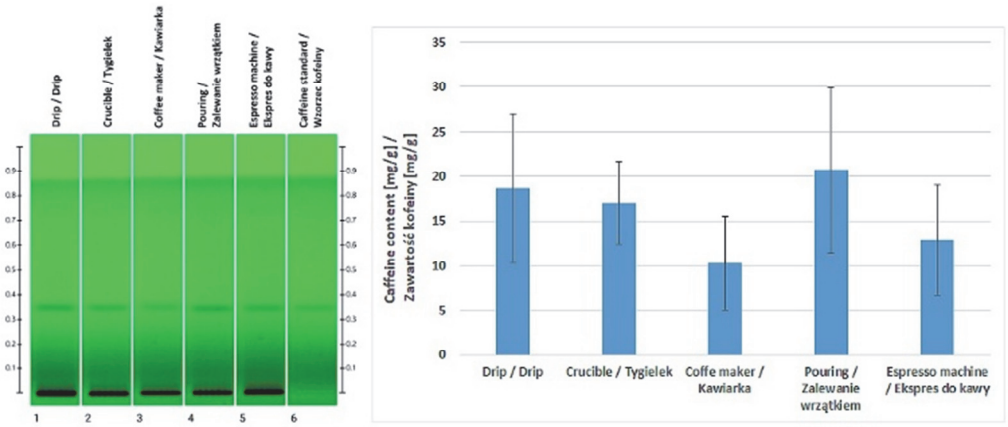

Fig. 2. Caffeine content in coffee grounds assessed by HPTLC method
Rys. 2. Zawartość kofeiny w fusach oceniona metodą HPTLC

Although results per gram of coffee grounds ranged from 10.26 to 20.71 mg (1000 to 2000 mg/100 g) of caffeine, the observed differences were not significant ($P>0.05$). However, the lowest residual level was observed for coffee maker and espresso machine samples which suggest these coffee brews were the strongest in terms of caffeine level, whereas pouring and drip method brews contain less of that stimulating ingredient. Previous research showed that the use of a coffee maker produces a higher caffeine content than drip or AeroPress device, which is in line with observations on coffee grounds [18].

Depending on the geographical origin of the coffee, the caffeine content in coffee grounds after brewing in the espresso machine ranged between 194.1 and 391.9 mg/100 g [3], which is lower than the value obtained by us. Also a lower content (0.35% of caffeine) was determined in coffee grounds in the study by Prihadi and Maimulyanti [22]. However, this data was obtained using the HPLC method and different extraction conditions were applied. What is more, the caffeine content in coffee grounds is affected not only by the method of brewing coffee and extracting grounds, but also by the quality of coffee itself and its variety. However, in other study [18], no significant differences between specialty coffee brews and popular products regarding the caffeine content were found (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, regardless of the test used. For two selected high-quality coffees, the impact 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 a moderate caffeine level, compared to coffee maker and AeroPress device [18].

Conclusion

1. It was shown that depending on the coffee brewing method, the quality of spent coffee grounds varies in terms of polyphenols and caffeine content. The popular home methods of brewing coffee in a drip and espresso machine, provides coffee grounds with the highest content of antioxidant compounds.
2. A high residue level of caffeine in the tested waste with the use of a simple and relatively cheap HPTLC method was quantified for the first time.
3. As above mentioned, brewing methods are popular in gastronomy, where waste is produced on a large scale, the results obtained indicate that their use for the recovery of bioactive ingredients is justified. However, due to technological differences in the production of coffee grounds, such waste generated in the industrial processing of coffee on a large scale should also be verified.

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ZAWARTOŚĆ POLIFENOLI I KOFEINY W FUSACH UZYSKANYCH PRZY UŻYCIU RÓŻNYCH DOMOWYCH METOD PARZENIA KAWY

S t r e s z c z e n i e

Wprowadzenie. Fusy powstające podczas parzenia kawy jako odpad wytwarzany na świecie w milionach ton rocznie wydają się być ciekawym źródłem substancji bioaktywnych do dalszego wykorzystania. Celem pracy była ocena zawartości składników bioaktywnych w fusach kawowych uzyskanych w wyniku parzenia kawy wybranymi, popularnymi metodami. Badano fusy uzyskane w wyniku parzenia kawy Arabica pięcioma metodami: prostego zalewania, dripa, tygielka, kawiarki i ekspresu. Oceniono zawartość kwasów fenolowych i fenoli ogółem oraz aktywność przeciwutleniającą (przy użyciu metod DPPH$^{\bullet}$ i FRAP) a także zawartość kofeiny (po raz pierwszy przy użyciu techniki wysokosprawnej chromatografii cienkowarstwowej HPTLC).

Wyniki i wnioski. Użyta metoda parzenia kawy istotnie wpłynęła na pozostałość związków bioaktywnych w fusach kawowych. Uzyskane wyniki pozwalają stwierdzić, że metody, w których kontakt zmielonego ziarna z wodą jest krótki, a ziarno rozdrobnione grubo, pozostawiły w fusach najwięcej związków fenolowych i kofeiny. Takimi technikami są metoda z użyciem dripa i ekspresu do kawy (całkowita zawartość fenoli odpowiednio do 12,29 i 14,88 mg równoważników kwasu galusowego/g) oraz proste zalewanie wrzątkiem w przypadku kofeiny (21 mg/g). Z kolei najuboższe w związki bioaktywne były fusy kawowe uzyskane po parzeniu kawy w tyglu, w których większość oznaczonych substancji została wyekstrahowana do naparu kawowego. Jako najskuteczniejszy z ocenianych systemów ekstrakcji wskazano mieszaninę etanolu i wody (50 %). Wykorzystanie zużytych fusów jako źródła substancji bioak-

tywnych, w tym przeciwutleniaczy z grupy polifenoli oraz kofeiny, może stanowić wartościowy sposób waloryzacji powstających w dużej ilości odpadów gastronomicznych.

Słowa kluczowe: aktywność przeciwutleniająca, metody parzenia, kofeina, fusy kawowe, HPTLC