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Original research paper

INVESTIGATION OF THE ANTIOXIDANT PROPERTIES OF EXTRACTS OF SPENT COFFEE GROUNDS

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Abstract: Coffee is one of the most consumed beverages worldwide. During coffee processing, a significant amount of by-products is generated, with coffee grounds being the primary by-product resulting from both beverage preparation and instant coffee production. The yield of bioactive compounds extracted from coffee grounds depends on the type of coffee and its growing and processing conditions. The antioxidant activity of coffee grounds is mainly attributed to their high phenolic content.
The main goal of this study was to investigate the antioxidant activity of dried coffee grounds remaining in the machine after espresso preparation, focusing on determining the total phenolic content, flavonoids and antioxidant activity through FRAP, DPPH and ABTS tests. Four solvents were used for the extraction and isolation of antioxidant components: 70% ethanol, distilled water and mixtures of 70% ethanol: water in different ratios (70% water and 30% ethanol, and 30% water and 70% ethanol).
Based on the research results, it was concluded that the solvents of ethanol and its mixtures with water present a better solution for maximizing the contents of phenols and flavonoids, as well as for achieving the highest antioxidant activity in spent coffee grounds extracts. The results of this research also suggest that coffee waste could be used as a significant source of bioactive compounds, provided that appropriate extraction solvents are used. This study highlights the potential of spent coffee grounds as a sustainable source of antioxidants, contributing to the reduction of food waste. By valorizing coffee waste, this research is in line with sustainability goals and offers a valuable approach to recycling food by-products.

Key words: *espresso coffee waste, by-products, antioxidant activity, bioactive compounds, waste valorization*

INTRODUCTION

Coffee is the second largest commodity in the world (Daviron & Ponte, 2005). Coffee drink has been consumed for more than a thousand years and it is also one of the most popular beverages in the world today (Panusa, Zuorro, Lavecchia, Marrosu & Petrucci, 2013). Coffee consumption is increasing worldwide and its sales are increasing in developing economies, especially in India and Pakistan (Butt & Sultan, 2011). In the Balkan region, including Serbia,

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traditional black coffee is probably consumed more frequently than other types of coffee, while the results of the study presented in the same paper (Ranić et al., 2015) showed that espresso and filter coffee are consumed by 20.3 and 5.3% of subjects, respectively.

According to the International Coffee Organization, the annual production of coffee increased from 140 to 152 million 60 kg bags since 2010 (Janissen & Huynh, 2018). Millions of cups of coffee are consumed every day in the world, both in restaurants and cafeterias and in households, and in this way, created spent coffee grounds are measured in tons, additionally, these residues are considered waste or occasionally used as fertilizer (Bravo et al., 2012). With this increase in production, reducing coffee by-products becomes a demanding challenge, taking into account that if the annual production of coffee is about 6 million tons, the amount of by-products is not negligible, i.e. the use of basic by-products of coffee processing is of great importance, both from an ecological and an economic point of view (Janissen & Huynh, 2018). Although the coffee by-products such as coffee husk, parchment, pulp, mucilage and silverskin have traditionally been used for low-value applications, such as animal feed or composting for soil fertilization, coffee waste may end up in landfills where it typically decomposes spontaneously, producing unpleasant odors and acidic leachates that can damage the neighboring soil through lixiviation (Rebollo-Hernanz et al., 2023). Transforming the waste products of the coffee processing industry into animal feed and fertilizer, or using biotechnology to convert them into biofuels, enzymes and aroma compounds are promising approaches to sustainably address this problem (Lee, Cho, Maskey, Nguyen & Bae, 2023).

The use of some of the secondary products may be limited due to their composition (Janissen & Huynh, 2018). Coffee is a composite mixture of more than a thousand different phytochemicals including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds and also possesses multifunctional properties as a food supplement (Saeed et al., 2019). The sources of coffee's antioxidant activity are polyphenols, caffeine, trigonelline and melanoids, i.e. naturally occurring components and those that are produced during coffee roasting (Bae, Park, Im & Song, 2014). The yield of bioactive compounds extracted from coffee grounds depends on various factors such as the type of coffee, and growing and processing conditions. According to this, the main idea and goal of this study was to investigate the antioxidant activity of dried spent espresso coffee grounds by analyzing the total phenolic content, flavonoids and antioxidant activity (FRAP, DPPH and ABTS test). Cuba espresso coffee was investigated, while four solvents were used for the extraction and isolation of antioxidant components, all to find the most suitable combination for the possible extraction of antioxidant components from the coffee waste sample.

MATERIALS AND METHODS

Chemicals

The following reagents were purchased from Sigma–Aldrich Chem (Steinheim, Ger-many): Folin-Ciocalteu reagent, (±)-catechin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS (2,2′-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), TPZT (2,4,6-tris (2-pyridil)-s triazine). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma– Aldrich Chem (Steinheim, Germany). All other chemicals and reagents used in the experimental work were analytical purity.

Sample

The sample used in this research was residue of Cuba coffee. The coffee residue was procured from of a local coffee shop, and it is actually a coffee cake that remains in the machine after preparing an espresso drink.

Sample preparation

Drying

Sample of residues of Cuba coffee was dried in a dryer at 105 °C to a constant mass.

Extraction

Extracts were prepared from the previously dried sample of Cuba coffee grounds using classical extraction methods. For the extraction, 25 g of Cuba espresso coffee grounds samples and 250 ml of solvent were measured. Pure water (H_2O) and 70% ethanol solution were used as the extraction solvents in the following ratios: 100:0 (1C in Table 1); 0:100 (2C in Table 1); 70:30 (3C in Table 1) and 30:70 (4C in Table 1). Each extraction was done in two steps, so at the end of the extraction approximately 500 ml extract was collected.

25 g of coffee grounds were measured, transferred to Erlenmeyer of 500 ml and poured with 250 ml of selected solvent. After this step, the Erlenmeyer with the mixture was placed on a magnetic stirrer and connected to the return cooler with constant mixing. The extraction temperature was 80 °C, while the extraction time was 1 h. After the first extraction, the mixture was filtered through the filter paper. The supernatant was separated in a flask of 500 ml, and the remaining precipitate was re-extracted with 250 ml of the same solvent under the same conditions. After the second extraction, the filtration was repeated, the resulting supernatant was pooled with the previous one, and the residual precipitate was thrown away.

Pairing

The obtained supernatants from the previous step were evaporated on a vacuum evaporator to a volume of approximately 15 ml at a temperature between 40-60 °C, for 1-3 hours, depending on the sample.

Vacuum drying

After pairing, the paired samples were transferred to crucibles (previously weighed) and then dried in a vacuum dryer to a constant mass at a temperature of 30 °C and a pressure of 20 mbar.

After the drying step, the content of total phenolic and flavonoid content and antioxidant activity using three tests (FRAP, DPPH and ABTS) was observed.

Total phenolic content (TPC)

The extracts for the further analysis of phenolic compounds were prepared according to the method of González-Gómez et al. (2010) with some modifications. Dried coffee extracts were first ground in a blender IKA A11 basic and then each sample was poured over with 50 ml of the extraction solvent (95% methanol). After the extraction (24 h) extracts were first filtered and then stored in the refrigerator before the analysis. These extracts were then used to determine total phenolic content, total flavonoid content and antioxidant activity (FRAP, DPPH, and ABTS test).

The content of total phenols in all dried coffee extract samples was determined spectrophotometrically, by the Folin-Ciocalteau method (Singleton & Rossi, 1965), using gallic acid as a standard. The content of total phenolics was expressed as gallic acid equivalent (mg GAE/100 g).

Total flavonoid content (TFC)

The content of total flavonoids in all dried coffee extract was determined spectrophotometrically, using the colorimetric method with aluminium chloride (Harborne, 1998). The content of total flavonoids was expressed as catechin equivalent (mg CE/100 g DW).

Antioxidant activity (FRAP, DPPH and ABTS test)

The slightly modified method presented by Benzie and Strain (1996) was used to measure the sample's ability to reduce Fe^{3+}in FRAP assay, while a modified method originally presented by Brand-Williams, Cuvelier and Berset (1995) was used to measure the sample's ability to scavenge 2,2-diphenyl-1 picrylhydrazyl free radicals (DPPH·) and, finally, the modified method originally described by Re et al. (1999) was used to measure the ABTS free radical scavenging ability of samples. FRAP The results were finally expressed as mg Fe^{2+}/g (for FRAP assay); and as mg Trolox/g (for DPPH and ABTS assay).

RESULTS AND DISCUSSION

The investigation included spent coffee ground extracts. For the most appropriate quality indicators of extracts total phenolic content (TPC), total flavonoid content (TFC) and three tests of antioxidant activity (FRAP, DPPH and ABTS) were conducted. Experimental data are presented in Tables 1 and 2.

Total phenolic content

The extraction process presents the most important stage in the isolation of polyphenols, and the most commonly used extraction solvents of polyphenolic components from plant materials are methanol, ethanol, acetone and ethyl acetate. However, with pure highly polar phenolic acids such as benzoic and cinnamic acids, extraction cannot be performed, and for these reasons, their aqueous solutions are mainly used. Because of the low toxicity, high yields and economy, an aqueous ethanol solution is particularly suitable for the extraction process (Milutinović et al., 2013). The highest total phenolic content in the Cuba samples (Table 1) was obtained with 70% ethanol as a solution (169.84 mg GAE/g) in sample 2C, followed by samples 4C and 3C where the phenol content decreased as the ethanol content

decreased whereas the usage of water for extraction proved to be the least effective (sample 1C) for isolation of polyphenolic components from Cuba espresso coffee grounds (122.52 mg GAE/g).

Even water possesses numerous advantages compared to organic solvents, but aqueous extracts, in addition to polyphenols, usually contain a larger amount of other components, especially carbohydrates that have a large share in the composition of coffee grounds. Additionally, the pH value of water as a solvent could significantly affect the extraction of certain polyphenol components (Escribano-Balin and Santos-Buelga, 2003). In the paper by Milutinović et al. (2013), the highest total phenolic content was obtained using an ethanol solution, then a methanol solution, while water also proved to be the least effective for the extraction of polyphenolic compounds. In the research by Coelho, Robalo, Boyaddzhieva and Stateva (2021) microwave-assisted extraction of phenolic compounds from spent coffee grounds was investigated and it was concluded that the optimized extraction conditions resulted in a high yield of total polyphenols (117.7 mg GAE/g) and strong antioxidant activity (143.8 μmol TE/g), measured by the DPPH assay, so the use of microwave-assisted extraction, along with green solvents, proved to be an effective method for recovering phenolic compounds with strong antioxidant properties from coffee waste, demonstrating its potential for sustainable extraction in various applications.

Total flavonoid content

Regarding the content of total flavonoid content in the Cuba espresso coffee grounds extract, it increases in the following order, depending on the solvent used: water < water: 70% ethanol (70:30) < water:70% ethanol (0:100) < water: 70% ethanol (30:70). The highest total flavonoid content was obtained with a combination of ethanol and water in ratio 70:30 (284.33 mg CE/g), then using ethanol as a solvent (257.78 mg CE/g), followed by a combination of ethanol and water in the ratio 30:70 (196.19 mg CE/g) while water proved to be the least effective for extracting flavonoids from espresso coffee grounds (177.44 mg CE/g). In their research, Panusa et al. (2013) determined the content of total polyphenols and flavonoids in coffee grounds, a mixture of Arabica and Robusta coffee grounds, as well as in coffee from espresso machines. Extraction was performed with pure water as well as a combination of ethanol and water in a ratio of 60:40. It was noted that the solvent had a significant effect on the extraction efficiency. On average, the con-tent of polyphenols and flavonoids increased by about 1.5 and 2.2 times, respectively, when water was replaced by aqueous ethanol.

Antioxidant activity

FRAP test

Unlike the DPPH radical inhibition method, which is suitable for antioxidants soluble in organic solvents (ethanol, methanol), the FRAP

Table 1.
Content of total phenolic (TPC) and flavonoid (TFC) compounds in different coffee grounds extracts

Sample*	Solution	TPC (mg GAE/g)	TFC (mg CE/g)
1C	H_2O:70% ethanol (100:0)	122.52	174.44
2C	H_2O:70% ethanol (0:100)	169.84	257.78
3C	H_2O:70% ethanol (70:30)	138.47	196.19
4C	H_2O:70% ethanol (30:70)	154.40	284.33

*C-Cuba

Table 2.
Experimental data of antioxidant activity (FRAP, DPPH and ABTS test).

Sample*	Solution	FRAP (mg Fe^{2+}/g)	DPPH (mg Trolox/g)	ABTS (mg Trolox/g)
1C	H_2O:70% ethanol (100:0)	81.33	146.65	388.87
2C	H_2O:70% ethanol (0:100)	150.81	261.71	419.55
3C	H_2O:70% ethanol (70:30)	96.81	171.95	401.92
4C	H_2O:70% ethanol (30:70)	108.08	214.11	402.02

*C-Cuba

method determines the antioxidant properties of water-soluble antioxidants. The results of measuring the antioxidant activity using the FRAP method in Cuba coffee grounds extract depending on the type of solvent are shown in Table 2. It could be seen that the sample extracted with 70% ethanol (150.81 mg Fe^{2+}/g) possessed the highest antioxidant activity, followed by the sample extracted with water and 70% ethanol in the ratio 30:70 (108.08 mg Fe^{2+}/g), as expected. It could be seen that lower FRAP values were obtained in the samples extracted with lower ethanol in the extract solution. Milutinović et al. (2013) reported the following FRAP values in their experiment, using different extraction solvents: 2.57±0.11 mmol Fe^{2+}/l for the ethanol extract, 2.10±0.10 mmol Fe2+/l for the methanol extract and 0. 98±0.02 mmol Fe^{2+}/l for the aqueous extract. Also, Milutinović et al. (2013) stated that there was a direct linear dependence between the content of total polyphenols and the antioxidant activity expressed through the FRAP value, that is, a higher content of polyphenols in the extract gives higher FRAP values.

DPPH test

In complex systems like natural extracts containing various polyphenolic components, it is essential to conduct a comparative study of antioxidant activity using at least two standardized tests. This approach considers the different mechanisms of action of antioxidants found in foods (Costa et al., 2018). Regarding to this, the antioxidant activity of coffee grounds extracts was determined by DPPH, ABTS and FRAP tests.

From the experimentally obtained data (Table 2), it could be seen that the sample of the extract of Cuba coffee grounds extracted with a 70% ethanol solution (261.71 mg Trolox/g sample), as well as the sample of the waste coffee extract where a combination of water:70% ethanol in the ratio of 30:70 was used as a solvent (214.11 mg Trolox/g sample) had a higher antioxidant activity compared to the remaining two samples of Cuba coffee grounds extract where water and a combination of water:70% ethanol in the ratio of 70:30 were used as a solution.

The lowest value of antioxidant activity according to the DPPH method was found in the coffee grounds extract sample where the solvent was water (146.65 mg Trolox/g sample). Samples of coffee grounds extract where 70% ethanol solution and a combination of water:70% ethanol (30:70) were used as solvents are characterized by a high content of flavonoids, so high antioxidant activity for these samples according to the DPPH method is expected. These results confirm the fact that flavonoids are largely responsible for the antioxidant activity of plants.

In the research by Milutinović et al. (2013), it was concluded that the ethanol extract possesses the highest antioxidant activity at a concentration of 100 μg/ml. Yen, Wang, Chang and Duh (2005) found that at a concentration of 0.2 mg/ml of the aqueous extract of coffee grounds removed 95.4%, and tocopherol 95.3% of total DPPH radicals, and also no notable statistical difference was found between the inhibitory effect of these two antioxidants. Based on this it was concluded that the aqueous extract effectively reduced the content of free radicals (Milutinović et al., 2013).

Generally, based on the results shown in Table 2 and the total phenolic contents, it could be concluded that as the content of total polyphenols in the extracts decreases, the ability of DPPH radicals neutralization decreases.

ABTS test

From the measured data (Table 2) it could be noticed that the sample of the extract of Cuba coffee grounds extracted with 70% ethanol solution (419.55 mg Trolox/g sample) exerted a higher antioxidant activity than the remaining three samples. A negligible difference in antioxidant activity could be observed between the samples where the used solvent was a combination of water:70% ethanol in a ratio of 70:30 (401.92 mg Trolox/g sample), and a 30:70 ratio of water:70% ethanol (402.02 mg Trolox/g sample). The lowest value of antioxidant activity according to the ABTS method was found in the sample of coffee grounds extract with water a solvent 388.87 mg Trolox/g sample).

CONCLUSIONS

In this research, the contents of phenolic and flavonoid compounds, as well as the antioxidant activity of Cuba coffee ground extracts were analyzed using different solvents during the extraction process.

The highest phenol content in the samples from Cuba coffee grounds was recorded in the sam-

ple extracted with 70% ethanol (169.84 mg GAE/g). Regarding the total flavonoid content, the highest value in Cuba coffee ground extracts (284.33 mg CE/g) was obtained by extraction with a mixture of ethanol and water (70:30).

The study's results and conclusions confirmed that the choice of solvent significantly impacts the extraction efficiency of bioactive compounds from coffee ground samples. Ethanol and its mixtures with water were found to be the most effective solvents for maximizing the content of phenols and flavonoids, as well as achieving the highest antioxidant activity. These results suggest that coffee waste could be used as a significant source of bioactive compounds, provided appropriate extraction solvents are used.

Bioactive substances isolated from coffee grounds have potential use in the pharmaceutical, cosmetic and food industries. Also, it is suggested that the high antioxidant capacity of ethanol coffee grounds extract could be used to slow down oxidative changes in various food products since the food industry tends to use natural components and replace synthetic ones.

The results suggest that antioxidants can be isolated from coffee grounds, which could be utilized in food production to partially or entirely replace synthetic antioxidants.

However, it should be noted that the composition of spent coffee grounds may vary depending on factors such as coffee type and roasting method, which may affect the consistency of the yield of bio-active compounds. Future research plans would certainly focus on investigating the impact of these variables and optimizing extraction methods for different types of coffee.

AUTHOR CONTRIBUTIONS

Conceptualization, A.M., S.S. and B.P.; Methodology, A.T.H., Z.Š., B.C. and M.J.; Investigation, A.M., S S., Z.Š. and B.C.; formal analysis, A.M. and B.P.; validation, A.T.H., Z.Š. and M.J.; writing-original draft preparation, A.M., S.S., B.C. and B.P.; Writing-review and editing, A.T.H., Z.Š. and M.J.; Supervision, M.J.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ISPITIVANJE ANTIOKSIDATIVNIH SVOJSTAVA EKSTRAKATA TALOGA KAFE

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Sažetak: Kafa je jedan od najpopularnijih napitaka širom sveta. Tokom prerade kafe stvara se značajna količina nusproizvoda, pri čemu je talog kafe glavni nusproizvod koji nastaje i prilikom pripreme napitka i u proizvodnji instant kafe. Prinos bioaktivnih jedinjenja iz taloga kafe zavisi od vrste kafe, uslova uzgoja i obrade. Antioksidativna aktivnost taloga kafe se uglavnom pripisuje visokom sadržaju fenola.
Glavni cilj ove studije bio je ispitivanje antioksidativne aktivnosti osušenog taloga kafe koji ostaje u aparatu nakon pripreme espresa, sa fokusom na određivanje ukupnog sadržaja fenola, flavonoida i antioksidativne aktivnosti (FRAP, DPPH i ABTS testovi). Za ekstrakciju i izolaciju antioksidativnih komponenata korišćena su četiri rastvarača: 70% etanol, destilovana voda i mešavine 70% etanola i vode u različitim odnosima (70% voda i 30% etanol, kao i 30% voda i 70% etanol).
Na osnovu rezultata istraživanja, zaključeno je da etanol i njegove mešavine sa vodom predstavljaju bolja rešenja za ekstrahovanje većeg sadržaja fenola i flavonoida, kao i za postizanje najviše antioksidativne aktivnosti u ekstraktima taloga iskorišćene kafe. Rezultati ovog istraživanja takođe ukazuju na to da bi talog kafe mogao biti značajan izvor bioaktivnih jedinjenja, pod uslovom da se koriste odgovarajući rastvarači za ekstrakciju.

Ključne reči: *talog espresso kafe, nusproizvodi, antioksidativna aktivnost, bioaktivna jedinjenja, iskorišćenje otpada*

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