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Characterization of polyphenol content and antioxidant capacity of spent coffee grounds

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In this study spent coffee grounds were investigated as a source of natural antioxidants. The polyphenolic content and antioxidant capacity of the extracts from fresh roasted coffee was determined and compared to those of spent coffee grounds (after a common espresso preparation). An optimized water-ethanol solvent was used. The extracts were analyzed for the total polyphenol content by Folin-Chiocalteau phenol reagent, by UV spectrophotometry for the content of chlorogenic acid. The antioxidant activity was investigated using the DPPH assay. It was found that the coffee remains still contain significant amount of polyphenols and show high antioxidant capacity. In our case study, spent coffee grounds (SCG) contain 17 mg polyphenols/g SCG, 15.8 mg chlorogenic acid/g SCG, and its antioxidant capacity was 86 mg DPPH/g SCG. The remaining polyphenolic content, chlorogenic acid and antioxidant capacity of spent coffee grounds represent 28%, 31% and 32.5%, respectively, as compared to untreated fresh coffee. In conclusion, spent coffee is far from being exhausted by a simple hot water extraction, and it can be considered as a rich, valuable and widely available source of useful natural bioactive substances with antioxidant activity.

Keywords: Spent coffee, Extraction, Polyphenols, Antioxidant capacity

INTRODUCTION

Coffee has been for decades the most commercialized food product and most widely consumed beverage in the world, taking second place after the water. 85% of human population drinks coffee. The world production in 2016 was about 9 million tons, the biggest producer being Brazil with 3 million tons. The other major producers are Vietnam, Colombia, Indonesia, and Ethiopia. Europe, especially northern European Scandinavian countries take first place in consumption led by Finland - 12 kg per year per capita. The consumption in Balkan countries is less, although significant (Greece – 5.5 kg, Bulgaria – 3.5 kg, Romania – 2.5 kg) [1].

Coffee production and consumption create a huge amount of wastes. There are research studies aimed at valorization of this waste. It has been investigated for biodiesel production [2,3], as sorbent [4,5], as source of sugars [6]. Spent coffee grounds are often used at small scale, mainly for composting and fertilizing [7,8] as they are known to slowly release nitrogen in the soil. The major amount is going to waste.

Normal biological functions naturally produce highly reactive molecules called free radicals or reactive oxygen species. They have toxic and damaging effects in the body, which are counteracted by antioxidants produced by the body or taken with foods. When there is insufficient antioxidant capacity to balance the effect of free radicals, the result is oxidative stress. Scientists now believe that many of the disease processes in the body involve oxidative stress as a common pathway [9,10].

Coffee contains more than 500 different compounds. Besides its refreshing effect due to caffeine, it contains many healthy constituents. Among them, coffee phenolics with the main representative chlorogenic acid (CGA) have attracted great attention by the scientific and medical communities due to their strong antioxidant properties that have positive influence against the oxidative stress [11,12].

A common practice in making a beverage is by shortly contacting milled roasted coffee with hot water. It seems doubtful that this simple treatment with hot water can fully extract the useful substances. So, it might be expected that coffee grounds still contain non-extracted heathy compounds.

The aim of this work was to study the remains after coffee extraction with hot pressurized water (espresso preparation) in order to establish whether they still contain antioxidant substances (chlorogenic acid and other polyphenols) in a quantity that may deserve additional treatment in view of obtaining enriched extracts with antioxidant activity from largely available wastes.

EXPERIMENTAL

Materials

Roasted and milled coffee (called fresh coffee hereafter) and spent coffee grounds after expresso

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preparation were collected from a local coffee shop. Spent coffee grounds were dried and kept refrigerated at dark along with the fresh coffee. Sieve analysis was made and the following fractions were found: the major quantity (about 80%) is of size 0.2-0.5 mm, about 20% is of size 0.5 mm or bigger, negligible quantity (0.6%) is less than 0.2 mm.

Chemicals

The following chemicals were used for the extracts preparation and for analyses: 96% ethanol of food quality, methanol 99.9% (from Lab Scan), Folin-Ciocalteu reagent (2N solution), DPPH, gallic acid, chlorogenic acid, all from Sigma, anhydrous Na_2C0_3 (from Valerus).

Extraction procedure

CGA and the majority of other polyphenols in the coffee are well soluble in methanol, ethanol and their mixtures with water [13]. Although studies using methanol as solvent exist [14], in this study ethanol-water mixtures were used for the reason of lower toxicity of the solvent.

Extraction was made with non-fractionated solid material and a corresponding solvent. As two-hour and one-hour extraction results were equal, one hour of process duration was applied. In order to eliminate solubility limitations, a big solvent-to-solute ratio (hydromodule) of 20 was chosen.

5 g of milled fresh coffee or dry spent coffee grounds were mixed in a flask with 100 ml of solvent with different ethanol content (from 0 to 96%). The suspension was vortexed for one hour at 70ºC in a thermostated water bath shaker Gyrotory G76, New Brunswick Scientific Co. Then the solid-liquid system was filtered, a sample of the liquid phase (liquid extract) was taken for analyses, another liquid sample was dried in order to determine the yield of extraction. All experiments were made in double, and good reproducibility was observed.

Analyses

Analysis for chlorogenic acid. This analysis was made according to the method described in [15,16] using UV-Vis spectrophotometer UV-1600PC, VWR international. The UV spectrum, 260-400 nm, was registered from ethanol extract after suitable dilution with ethanol against the reference cuvette with solvent. The concentration of chlorogenic acid was determined from the absorption maximum at 324-328 nm using a calibration curve prepared with pure chlorogenic acid.

Analysis of total phenolic content. The total phenolic content was determined spectrophotometrically with the Folin-Ciocalteu reagent [17,18]. 0.02 mL of the extract was mixed with 0.1 mL of 2N Folin-Ciocalteu reagent and 0.3 mL of Na_2CO_3 (20 % w/v), all diluted to 2 mL with distilled water. The resulting mixture was incubated at room temperature for 2 hours for color development. The absorbance of the samples was measured at 765 nm using double beam UV/VIS spectrophotometer UV-1600PC, VWR international. Calibration curve with gallic acid was made, and the total phenolic content was expressed as gallic acid equivalents. The reference cuvette contained all reagents except the sample extract.

Antioxidant capacity (AOC). AOC was determined by the DPPH method, which is largely used because of its simplicity and reproducible results [19,20]. This method is based on the reaction of antioxidant substances with methanol solution of DPPH, resulting in neutralization of free radicals emitted by DPPH. The latter absorbs at 517 nm, but upon reduction by an antioxidant the absorption decreases, and the color changes from deep violet to yellow. The absorption was measured spectrophotometrically. The analytical protocol was as follows:

The blank sample was adjusted by measuring a mixture of 1 mL solvent and 4 mL methanol solution of DPPH against methanol (A_0). The analyzed sample was obtained by mixing 1 mL of plant extract with 4 mL of 0.004 % (0.1 mM) solution of DPPH in methanol. After 60 min incubation in dark, the light absorbance of the sample was measured (A_e) against methanol at 517 nm. The scavenging concentration (SC) of the sample was calculated by the expression:

$$SC\ [\%] = (1 - A_e/A_0) \times 100 \qquad (1)$$

The antioxidant capacity was expressed as SC50 value, which represents the concentration of a sample that inhibits 50% of the free radicals added to the system. SC50 value can be determined from the chart that expresses SC as a function of the extract concentration C_e.

The graphical relationship $SC = f(C_e)$ for an extract was obtained by measuring the absorption of a series of samples containing different amounts of this extract added to the solvent [mL/L]. Appropriate dilution of the samples is necessary in order to fall in the linear part of the graph in IC interval 0 to above 50 %. The extract concentration reducing 50% of free radicals can be calculated from the linear equation by setting SC=50, or determined from the chart as the abscissa of the intersection point of the horizontal line from the

50% SC ordinate and the data line. A smaller value of *C* corresponds to higher AOC, i.e. a smaller quantity of this extract is needed for neutralization of 50 % of the free radicals. SC50 concentration can be transformed and expressed as mg DPPH neutralized by 1 g of raw material (rm) [mg DPPH/g rm] or by 1 g of dry extract (de) [mg DPPH/g de]. In this case the representation is more logic, because higher values correspond to higher AOC.

Extraction yield. After extraction, 10 mL samples of the liquid extract were dried at 80°C until constant weight was reached (henceforth referred to as dry extract - de). Laboratory analytical balance Sartorius with 0.1 mg accuracy was used.

RESULTS AND DISCUSSION

Optimizing the solvent composition

For determination of the solvent composition, at which maximum quantity of target bioactive components is recovered, the extractions were carried out with different concentrations of ethanol in the solvent (0, 24, 40, 48, 60, 72, 96%).

The results for spent coffee grounds are shown in Fig. 1 as mg extracted substance (TPC or CGA) per gram of raw material (mg/g_{rm}).

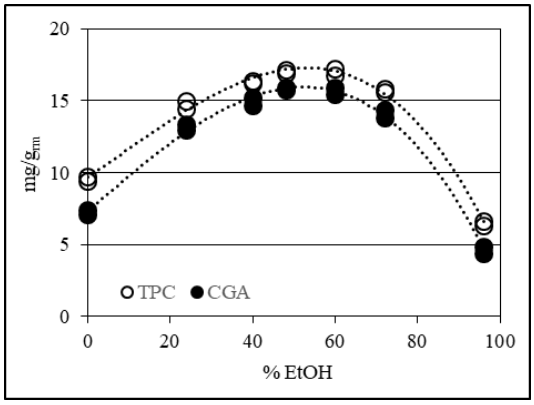

Fig. 1. Effect of ethanol concentration on the quantity of extracted substances from the raw material

It is seen that ethanol concentration is most efficient and equally suitable between 48 and 60%. From economic point of view it is advisable to choose the less concentrated solvent containing 48% ethanol. Also, water is a better solvent than concentrated ethanol (96%).

The quantitative result for the highest content of the studied bioactive compounds in the spent coffee, as taken from Fig. 1, is 17 mg TPC and 15.8 mg CGA per gram of solid matter. Accounting for the fact that chlorogenic acid is phenolic compound, it might be concluded that CGA is the main phenolic representative in the waste (93%), while the other phenolics altogether represent 7% of TPC.

The picture becomes slightly different when considering the obtained dry extract (see Fig. 2). The extract obtained with 60% ethanol seems to be more concentrated. However, one-way analysis of variance (ANOVA) with significance level 0.05 (made by Microsoft software) has shown that concentrations of TPC between 24 and 72% ethanol are statistically equal, the same being valid for 48 and 60% ethanol extracts of CGA. So, the solvent concentration selected initially (48%) still appears to be appropriate for the optimal extraction of the target substances. Again, water is a better extractant than concentrated ethanol. It is worth mentioning that after extraction the content of TPC in the raw material (17 mg/g_{rm}) has raised to 265 mg/g_{de} in the extract, i.e. the extract is 16 times more concentrated. The same is valid for CGA (concentration increased from 15.8 to 245 mg/g).

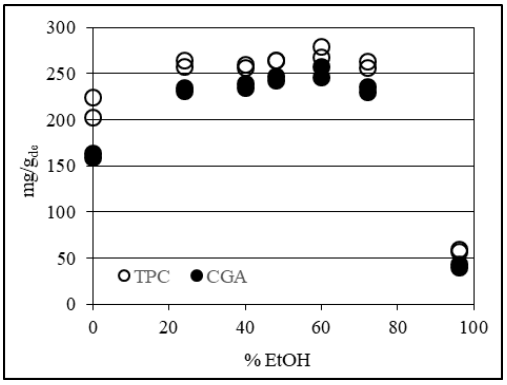

Fig. 2. Influence of solvent composition on the concentration of total polyphenols (TPC) and chlorogenic acid (CGA) in the dry extract

Fig. 3 depicts the total quantity of solid matter (de) extracted from the raw material. The tendency is similar to that shown in Figs. 1 and 2, namely maximum yield is obtained with a solvent containing 40-60% ethanol. Confronting the maximum values (obtained with 48% ethanol) for total extract (65 mg/g_{rm}) to these for TPC (17 mg/g_{rm}), it follows that our target antioxidant compounds represent about 26% of the total dry matter extracted from spent coffee grounds.

Concerning a study using methanol as solvent [14], some different results are obtained there. Pure methanol is reported to be a better solvent than water, and the optimal solvent concentration is 60% methanol.

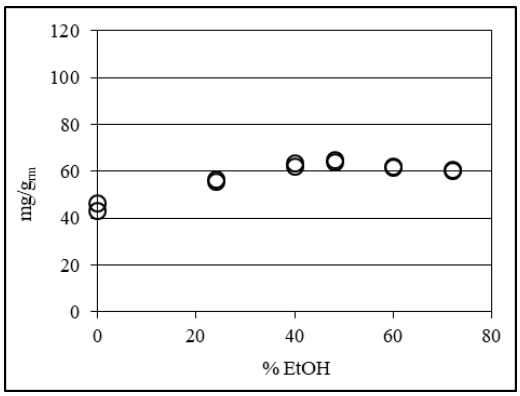

Fig. 3. Effect of ethanol concentration on the yield of extracted matter

Although not far from these data, our result regarding the solvent concentration states that less concentrated and less expensive 48% ethanol is slightly better than a more concentrated solvent. However, we have obtained more concentrated phenolic extracts with water than with ethanol. Additionally, as compared to methanol, we use a non-toxic solvent still extracting similar quantity of phenolic compounds as in [14].

The antioxidant capacity of extracts from spent coffee grounds obtained at different solvent composition is shown in Fig. 4. Clear tendency of higher AOC corresponding to higher phenolic concentration is seen (cf. Fig. 1 and Fig. 4). The solvent containing 48% ethanol produces extracts with the highest antioxidant capacity.

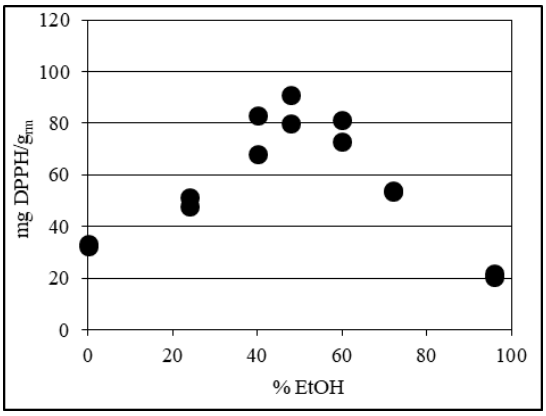

Fig. 4. Effect of ethanol concentration on the AOC of raw material

Table 1. Comparison of results for fresh and spent coffee

	1	2	3	4	5
	Fresh coffee	Spent coffee	Espresso drink (2-1)	Spent/Fresh (2:1) %	Spent/Espresso (2:3) %
TPC [mg/g_{rm}]	61± 0.68	17± 0.08	44		
CGA [mg/g_{rm}]	51± 0.68	15.8± 0.05	35.2		
AOC [mg_{DPPH}/g_{rm}]	265± 9.36	86± 5.45	-		

Regarding Table 1, it is seen that about 1/3 of the bioactive substances in fresh coffee have not been extracted during the espresso preparation and remain in the spent coffee grounds (see column 4, lines 1 and 2). The result for antioxidant capacity closely corresponds to the phenolic content, i.e. spent coffee possesses 1/3 of fresh coffee AOC (column 4, line 3) and contains around 40% of the phenolics found in espresso drink (column 5). So, fresh coffee is far from being exhausted by coffee preparation, and its remains contain significant amount of useful antioxidants.

CONCLUSION

This study presents comparative results for the content of polyphenols in roasted and milled coffee beans and in spent coffee grounds obtained after espresso preparation. The extraction is made with non-toxic water-ethanol mixtures. Optimal solvent concentration of 48% ethanol is found to extract maximum polyphenols. The results attest that significant quantity of useful healthy antioxidant substances remain in the spent coffee grounds after short treatment of milled coffee with hot pressurized water (espresso preparation). Nearly 30% of initial content of polyphenolic compounds remain non-extracted. Concerning the antioxidant activity of spent coffee, it represents about 1/3 of the AOC of fresh coffee. The dry extract obtained from spent coffee contains about 25% of polyphenols.

Based on the quantitative results of this study, it might be concluded that spent coffee grounds can be considered as a rich potential source of useful natural antioxidant compounds, which can be obtained from largely available wastes.

Comparison of results for fresh and spent coffee

Table 1 reports the results for the content of target components and antioxidant capacity in fresh coffee along with spent coffee. All results are obtained at identical extraction conditions using the optimized solvent concentration.

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ОПРЕДЕЛЯНЕ НА СЪДЪРЖАНИЕТО НА ПОЛИФЕНОЛИ И АНТИОКСИДАНТНИЯ КАПАЦИТЕТ НА УТАЙКА ОТ КАФЕ

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(Резюме)

В настоящата работа е изследвана утайка от кафе като източник на природни антиоксиданти. Съдържанието на полифеноли и антиоксидантният капацитет на екстракти от прясно изпечено кафе са определени и сравнени с тези на утайка от кафе (след приготвяне на обикновено еспресо). Използван е оптимизиран разтворител вода-етанол. Екстрактите са анализирани за тотално съдържание на полифеноли с фенолния реагент на Folin-Chiocalteau, а за съдържание на хлорогенна киселина – с УВ спектрофотометрия. Антиоксидантната активност е изследвана с помощта на DPPH метода. Установено е, че остатъците от кафе съдържат значителни количества полифеноли и проявяват висок антиоксидантен капацитет. В конкретния случай утайката от кафе (УК) съдържа 17 mg полифеноли/g УК, 15.8 mg хлорогенна киселина acid/g УК и антиоксидантният й капацитет е 86 mg DPPH/g УК. Съдържанието на полифеноли и хлорогенна киселина, както и антиоксидантният капацитет на утайката от кафе са съответно 28%, 31% и 32.5% от тези на необработено прясно кафе. В заключение следва, че полезните вещества в кафето далеч не са изразходвани чрез обикновена екстракция с гореща вода и утайката от кафе представлява богат, ценен и широко разпространен източник на полезни природни биоактивни вещества с антиоксидантен капацитет.