DOI: 10.1515/FV-2016-0026

FOLIA VETERINARIA, 60, 3: 34—38, 2016

STUDY OF ANTIOXIDANT EFFECTS OF SELECTED TYPES OF COFFEE

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ABSTRACT

Coffee is a rich source of dietary antioxidants which protects the human body against the effects of dangerous free radicals. The aim of this study was to determine and compare the antioxidant activity, content of total phenols and flavonoids in selected types of coffee with respect to the way of their processing. The individual coffees were investigated with regard to their origin and composition. The antioxidant effects were determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay. The content of total phenols was analysed by the Folin-Ciocalteu method and the content of flavonoids in the coffee extracts was determined by a colorimetric method. The highest antioxidant activity was exhibited by the extract of unroasted ground 100 % green coffee *Arabica* (89.55 %), and the high scavenging of free radicals was achieved also by the extracts of roasted ground 100 % coffees *Arabica* and *Robusta*. The highest levels of total phenols (77.54 µg.ml^{-1}) and flavonoids (1.74 µg.ml^{-1}) were measured in the extract of unroasted ground 100 % green coffee *Arabica*. High levels of these substances were found also in extracts of roasted ground 100 % coffees *Arabica* and *Robusta*. The lowest levels of total phenols (31.24 µg.ml^{-1}) and flavonoids (0.91 µg.ml^{-1}) were detected in the extract of instant coffee *Arabica*. The processing of coffee by roasting decreased the level of the investigated antioxidant components but considerably improved the taste and aroma, the properties that make coffee one of the most popular drinks in the world.

Key words: antioxidant effect; *Coffee Arabica* (L.); free radicals

INTRODUCTION

The human body is constantly exposed to the effects of free radicals produced by various metabolic pathways. They get into the bodies through air, liquids and food. They are dangerous due to their ability: to attack cell structures, inhibit their normal functions, and thus contribute to the development of numerous diseases. Antioxidants play an important role in the battle against the undesirable effects

of free radicals. The contribution of antioxidants is extensive; they protect human bodies against premature ageing, weakening of the immune system, and other health problems caused by free radicals. The human body is capable of naturally producing its own effective antioxidants but their amount is not always sufficient and thus it is necessary to take them in the food. They are found, for example in fruits, vegetables, chocolate, red wine and cereals. Recently, increased attention has been paid to the beneficial effect of coffee on human organism. Coffee is also an excellent source of antioxidants, particularly polyphenol compounds, which participate considerably in the neutralisation of free radicals [9].

Green (raw) coffee beans are the richest source of chlorogenic acids, one of the strong antioxidants with a positive influence on health. Research has demonstrated that these acids can function as an inhibitor of glucose-6-phosphatase. Owing to chlorogenic acids, the body compensates a decrease in the level of blood glucose by the break-down of fats, and thus, green coffee is presently much sought-after for the preparation of weight reduction. However, chlorogenic acids are thermally unstable and thus undergo many changes during roasting. Roasting is, however, an inevitable way of processing of green coffee as it gives complex aroma and taste to the final product (the properties required by coffee consumers). The degree and conditions of roasting considerably affect the volatile components in coffee. Aroma of light roasted coffee differs considerably from that of dark roasted coffee [10]. Owing to its pleasant aroma, taste and stimulating effect on the body and mind, coffee is one of the most popular drinks in the world.

The aim of this study was to determine the antioxidant properties and content of flavonoids and total phenols in roasted and unroasted coffee varieties *Coffea Robusta* and *Coffea Arabica* by means of the spectrophotometric methods.

MATERIALS AND METHODS

For analysis, we purchased from a store chain, 7 coffee types (3 roasted and ground, 2 instant and one unroasted green coffee) of varieties *Coffea Robusta* and *Coffea Arabica*. The list of coffee types is presented in Table 1. For preparation of the extracts, we used 5 g quantities of ground coffee and 2 g of instant coffee. The extracts were prepared by pouring boiling water (100 °C) over coffee samples and after 10 minutes, filtering the extracts and using them for the ultimate determination. All chemicals used in the experiments were supplied by Sigma Aldrich (Germany).

Table 1. Coffee types used for the determination of antioxidant activity

Sample No.	Coffee type
1	*Arabica*: roasted ground 100 % coffee (Gold)
2	*Arabica*: roasted ground 100 % coffee (Extra special)
3	*Robusta*: roasted ground 100 % coffee
4	*Robusta*: roasted ground coffee
5	*Arabica*: Instant 100 % coffee
6	*Arabica*: Instant coffee (Crema Gold)
7	*Arabica*: unroasted ground 100 % green coffee

Spectrophotometric determination of antioxidant activity by the DPPH radical scavenging assay

The antioxidant properties were determined by the method of Heilerová et al. [6]. The principle consists in the reaction of the coffee extract with a stable 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The ability of coffee extract to scavenge free radical is directly related to the rate and extent of decolouration of the synthetic radical DPPH and at the same time as a decrease in the absorbance. The antioxidant activity of coffee extracts was calculated as a per cent of inhibition of the DPPH radical. The procedure was as follows: 3.9 ml aliquot of the stock solution of DPPH (0.0035 g per 100 ml methanol) was pipetted into a cuvette and the absorbance (A0) of this solution was measured at 515 nm. Subsequently, we prepared a reaction mixture by adding 100 µl aliquot of coffee extract. After mixing and incubation for 5 minutes, the absorbance of the reaction mixture (AA) was measured. The antioxidant activity (per cent of DPPH radical inhibition) was calculated according to the formula: % inhibition = $[(A0 - AA)/A0] \times 100$.

Spectrophotometric determination of the total phenols

The content of the total phenols in the coffee extracts was determined by the Folin-Ciocalteu method described in the study by Singleton et al. [12]. The method is

based on the oxidation-reduction reaction during which the phenol compounds are oxidised at a parallel reduction of Folin-Ciocalteu (FC) reagent and the development of a blue colour. The intensity of colouring correlates with the redox properties of phenolic compounds present in coffee extracts. The procedure was as follows: coffee extracts and the FC reagent were diluted with distilled water 1:1000 and 1 : 10, respectively. Then, 1 ml of the diluted coffee extract was pipetted into a test tube and 5 ml of diluted FC-reagent and 4 ml Na_2CO_3 (75 g.l^{-1}) were added and the content was mixed. After a 30 minute incubation at room temperature, the absorbance of the solutions were measured at a wavelength of 765 nm, against a blank which contained distilled water instead of the coffee extract. Gallic acid in concentrations of 0.038—0.3 mg.l^{-1} was used as a standard. Gallic acid absorbance ranges from 0.05 to 0.555 nm.

Determination of total flavonoids

The content of total flavonoids was determined by the colorimetric method described in the study by Kim et al. [7]. The addition of an aluminium chloride solution to flavonoids results in the formation of yellow chelate complexes. The content of flavonoids was determined spectrophotometrically by measuring the intensity of the yellow colour. The procedure was as follows: coffee extracts were diluted with distilled water 1 : 100. To 1 ml of the diluted coffee extract, we added 4 ml of distilled water and 0.3 ml $NaNO_2$ (50 g.l^{-1}). After 5 min of incubation, we added 0.3 ml AlCl3 (100 g.l^{-1}), and after an additional 6 minutes, 2 ml NaOH (1 mol.l^{-1}) and 2.4 ml distilled water, mixed the content and measured the absorbance of the samples (510 nm) against a blank which contained distilled water instead of coffee extract. The content of total flavonoids was determined using the quercetin standard (0.05—0.4 mg.l^{-1}).

Processing and presentation of the results

The results of the determinations conducted in individual coffee extracts are presented as arithmetic means (x) and standard deviations (± SD). All measurements were carried out in triplicate.

RESULTS

Of all tested coffee types, the extract of unroasted ground coffee exhibited the highest capacity (Sample 7; 89.55 %) for scavenging DPPH radicals. High antioxidant activity was determined also with extract of roasted ground 100 % *Arabica* — Sample 1 (82.5 %). The lowest percentage of free radical scavenging was observed with extract of instant coffee with high content of coffee variety *Arabica* — Sample 6 (56.16 %). Similar to antioxidant activity, the extract of unroasted ground coffee exhibited the highest content of total phenols (7754 µg.ml^{-1}) followed by extract of roasted ground 100 % *Arabica* — Sample 1 (73.64 µg. ml^{-1}). The level of total phenols was the lowest in Sample 6 (31.24 µg.ml^{-1}); the extract of instant coffee with high content of coffee variety *Arabica*. The sample 7 rated the best with regard to total flavonoids (1.74 µg.ml^{-1}). The level of total flavonoids in the extracts of the remaining types of coffee was above 1 µg.ml^{-1} with the exception of Sample 6, instant coffee with high content of variety *Arabica*, where it reached (0.91 µg.ml^{-1}). The mean levels of the values and standard deviations are presented in Table 2.

DISCUSSION

Coffee is the main source of polyphenolic compounds which are well known for their antioxidant effects. They contain chlorogenic acids with biological effects mostly related to their remarkable antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory activities. The chlorogenic acids exhibit a high capacity to scavenge reactive oxygen radicals. These polyphenols are able to inhibit inflammatory processes and propagation of tumours by means of the deactivation of pro-oxidative enzymes [5]. Some studies demonstrated that green coffee extracts have; anti-hypertension effects [8], inhibit the accumulation of fats and body weight gain [4] and modulate glucose metabolism in humans [1]. These biological effects were ascribed to the chlorogenic acids present in green coffee.

The studies involved in the determination of the antioxidant capacity of coffee of a variety of *Arabica* showed that the content of total phenols in roasted coffee is lower than in unroasted green coffee [3], which correlates with our results. Lower levels of polyphenols in roasted coffee can be ascribed to their polymerization, autoxidation or degradation during roasting [2]. The content of total phenols determined in our study correlates with the results of Cheong et al. [3]. The highest antioxidant activity was observed in the extract of green unroasted 100 % coffee

Table 2. Antioxidant capacity, total phenols and flavonoids in the types of coffee (mean ± SD)

Sample	Antioxidant capacity [%]	Total phenols [μg.ml^{-1}]	Flavonoids [μg.ml^{-1}]
1	82.5 ± 1.62	73.64 ±14.92	1.43 ± 0.18
2	78.92 ± 2.46	55.7 ± 6.11	1.05 ± 0.04
3	78.46 ± 0.66	51.37 ± 18.4	1.14 ± 0.08
4	79.93 ±1.49	59.9 ± 11.03	1.41 ± 0.05
5	71.06 ± 1.20	57.47 ± 11.24	1.02 ± 0.05
6	56.16 ± 4.09	31.24 ± 18.07	0.91 ± 0.01
7	89.55 ± 0.37	77.54 ± 15.36	1.74 ± 0.03

SD — standard deviation

Arabica. This coffee extract also showed the highest content of total phenols and flavonoids. The lowest antioxidant capacity was measured in the extract of 100 % coffee *Robusta*. Extracts of roasted ground coffees exhibited a comparable capacity to scavenge free radicals. This capacity correlated with the levels of total phenols and flavonoids. Relatively lower levels of total phenols and flavonoids were recorded in extracts of instant coffee. These samples exhibited also lower antioxidant capacity. The results of our study agree with the investigations of Ramalakshmi and Rao [12] who reported that extracts of green coffee contain higher levels of polyphenols and chlorogenic acids than extracts of instant coffee. The relatively low levels of total phenols can be explained by the degradation of these compounds during roasting and their release to water during extract preparation.

CONCLUSIONS

The best antioxidant properties and the highest levels of total phenols and flavonoids were detected in ground green coffee *Arabica* and the lowest in the extract of instant coffee with high content of variety *Arabica*. These results allowed us to conclude that the antioxidant activity of coffee decreases with roasting, but coffee still retains considerable capacity to scavenge free radicals and sufficient level of total phenols and flavonoids which also act as antioxidants.

ACKNOWLEDGEMENTS

This study was conducted with the support of National Reference Laboratory for Pesticides at UVMP in Košice.

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Selected paper from the 59th STUDENT SCIENTIFIC CONFERENCE, Section III – Food hygiene and the environment, held at the University of Veterinary Medicine and Pharmacy in Košice, SR, on April 6, 2016.