

# Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation

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**Abstract** Chlorogenic acids and caffeine are important for flavor formation as well as the health effect of green coffee brews and its extracts. The content of these compounds was determined by HPLC–DAD analysis in twelve samples of coffee from Robusta and Arabica types of different geographical origin including steamed and decaffeinated coffees. Generally, Robusta coffee extracts contain twice as much caffeine as Arabica, and its content varies from 3.41 % per dry mass in Arabica type from Laos or Rwanda to 8.16 % in Robusta coffee from Indonesia. The highest concentration of 5-*O*-caffeoylquinic acid (5-CQA) was obtained for both coffees from Uganda. Decaffeination process does not affect the concentration of this main chlorogenic acid, but steaming of the coffee beans with hot water produced a significant decrease in the level of 5-CQA. Antioxidant activity of coffee extracts was measured by CUPRAC and F–C assays, which really measure the reducing power of the sample components. Extracts of green coffee beans from Vietnam possessed the highest antioxidant activity in both assays.

**Keywords** Green coffee · Chlorogenic acids · Caffeine · Antioxidant activity · HPLC–DAD

## Introduction

Coffee is one of the most popular drinks nowadays all over the world. It contains more than 700 compounds which are responsible for its aromatic and unique flavor. Genus *Coffea arabica* and *Coffea canephora* var. *robusta* are the most important species of *Coffea*, and they constitute 60–40 % of world production. Arabica usually comes from South America (mainly from Brazil) and upland and mountain areas of East Africa while Robusta (mainly from Vietnam) from lowland of Central and West Africa and South Asia [1].

Coffee as a functional food with antioxidant properties reduces the incidence of cancer, diabetes and liver disease, protects against Parkinson's disease and reduces mortality risk [2, 3]. Green coffee bean extract shows a hypotensive effect in rats [4] and reduces visceral fat and body weight [5, 6]. These properties are connected with bioactive compounds, not only chlorogenic acids and their derivatives, but also caffeine, theophylline and theobromine, cafestol, kahweol, tocopherols and trigonelline [7–12].

Green coffee beans contain higher level of 5-*O*-caffeoylquinic acid (5-CQA), even twofold higher than in roasted coffee depending on the time of roasting [12]. Caffeine in coffee reduces oxidative stress and protects antioxidant system: in hypoxia-induced pulmonary epithelial cells; it is an inhibitor of hydrogen peroxide-induced lipid peroxidation products in human skin fibroblasts, and it reduces tissue lipid peroxidation and ROS [13, 14].

Antioxidant activity of coffee beans depends on characteristics of phenolic compounds, especially chlorogenic acids which possess in vitro and in vivo antioxidant capacity [15], and these phenolics are highly bioavailable in vitro

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[16]. Antioxidant activity of green coffee extracts of Arabica is positively correlated with calcium level [17].

It is well known, especially by consumers and experts, that Arabica coffee has better quality than Robusta. Therefore, exists methods for improving the quality of Robusta coffee. Steaming of Robusta coffee is used to create the specific acidic taste and flavor unique for Arabica coffees. This process also removes specific aromas: “musty” and “earthy” found in Robusta. Steaming coffee beans, especially Robusta coffee beans, also eliminates stomach-unfriendly substances such as chlorogenic acids, free diterpenes: cafestol, kahweol, dehydrokahweol and dehydrocafestol. These compounds can be reduced depending on the steaming parameters [18]. Monsooned coffee is a special wind treatment of coffee seeds to have good body, low acidity, and pleasant aroma and flavor in the cup [18]. Decaffeination process is performed prior to the roasting and is usually attributed to Arabica coffee [19, 20]. Organic solvent extraction is the most common and cheapest method of decaffeination, and coffee industry uses as solvents: dichloromethane or ethyl acetate, associated with the use of water or vapor prior to and after extraction. Decaffeination process, especially with water solvent, causes loss of not only flavor components of coffee, but also may lose the chlorogenic acids (CQA) and their related compounds [19].

The aim of the study was to compare the content of chlorogenic acids and caffeine in green coffee Arabica and Robusta extracts due to their origin and preparation of beans (decaffeinated and steamed coffee beans). Folin–Ciocalteu (F–C) assay and cupric ion reducing antioxidant capacity (CUPRAC) method were used to evaluate the antioxidant properties of coffee brews. Possible correlations between the content of particular compounds and antioxidant activity are also in the scope of this study.

## Materials and methods

### Chemicals

Gallic acid, chlorogenic acid, caffeine and F–C reagent were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). MS-grade acetonitrile and methanol were from POCH (Gliwice, Poland), and MS-grade formic acid was from Sigma–Aldrich. Ultrapure water from a Milli-Q system (Millipore, Bedford, MA, USA) with a conductivity of 18  $\Omega\text{m}$  was used in all experiments.

### Material and extraction process

Twelve green coffee beans of different origin *Coffea arabica*: Brazil (TG), Rwanda (Ordinary), China, Laos and

*Coffea robusta*: Vietnam (Gr2), Vietnam (Gr2) decaffeinated by dichloromethane and Vietnam (Gr2) steamed (3 bar pressure for 30 min), India (Cherry), Indonesia, Laos (FAQ), Uganda (Sc) and Uganda (Bugishu), were obtained from a producer (Strauss Café, Poland). The geographical origin of the samples and their types were confirmed by the supplier. The moisture content of coffee beans was above 12 %. 0.5 g of milled beans was extracted by 20 mL of distilled water (94 °C) for 10 min. Then, the solution was cooled to room temperature, centrifuged (5 min, 4500 rpm) and decanted. The extracts were lyophilized (Lyophilizator Alpha 1-2 LD plus; Martin Christ, Germany). Before analysis, extracts were dissolved in 1 mL of Millipore water and filtered through 0.2- $\mu\text{m}$  polytetrafluoroethylene syringe filter from Agilent Technologies (Santa Clara, CA, USA).

### Chromatographic analysis of chlorogenic acids and caffeine

Caffeine was determined using chromatographic system equipped with analytical HPLC unit model 1100 (Agilent Technologies, CA, USA) equipped with a binary pump and an automated sample injector and DAD lamp (Agilent Technologies, CA, USA). Fifty microliters of samples was injected into a C18 column (100 mm  $\times$  2.1 mm I.D.; 2.6  $\mu\text{m}$ ) from Phenomenex (Torrance, CA, USA) maintained at 25 °C. The mobile phase employed in the analysis consisted of 8 mM formic acid in water (pH 2.8) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL  $\text{min}^{-1}$ . Elution was initiated at 10 % A and maintained for 5 min; the percentage of solvent A was increased to 20 % in 10 min and maintained for 10 min, then increased to 50 % in 20 min and maintained for 3 min, and finally increased to 80 % for 15 min. Detection was accomplished with a diode array detector (DAD), and chromatograms were recorded at 325 nm for chlorogenic acids and 276 nm for caffeine. Identification of 5-CQA was performed by comparing the retention time and the photodiode array spectra with those of its reference standard compound. 3-CQA and 4-CQA were identified by the isomerization of 5-CQA standard. Quantitation of 5-caffeoylquinic acid (5-CQA) was performed by comparing the peak areas with those of the standards. Quantitation of the other chlorogenic acids was performed using the area of 5-CQA standard combined with their respective molar extinction coefficients as reported earlier [21, 22].

### Cupric ion reducing antioxidant capacity

A 1 mL aliquot of  $\text{CuCl}_2$  solution (0.01 mol  $\text{L}^{-1}$ ) was mixed with 1 mL of neocuproine alcoholic solution ( $7.5 \times 10^{-3}$  mol  $\text{L}^{-1}$ ) and 1 mL of acetate buffer (1 mol  $\text{L}^{-1}$ , pH 7), followed by mixing with 0.5 mL of coffee

**Table 1** pH, antioxidant activity and caffeine concentration in green coffee extracts

| Coffee          | pH   | CUPRAC (mM Trolox kg <sup>-1</sup> ) | F–C assay (g GAE kg <sup>-1</sup> ) | Caffeine (g kg <sup>-1</sup> ) |
|-----------------|------|--------------------------------------|-------------------------------------|--------------------------------|
| <i>Robusta</i>  |      |                                      |                                     |                                |
| Vietnam         | 4.47 | 7.49 <sup>e</sup> ± 0.04             | 482 <sup>f</sup> ± 4                | 74.3 <sup>c</sup> ± 3.3        |
| Vietnam decaf.  | 4.63 | 6.20 <sup>d</sup> ± 0.09             | 498 <sup>e</sup> ± 1                | 3.9 <sup>a</sup> ± 1.2         |
| Vietnam steamed | 4.29 | 5.85 <sup>c</sup> ± 0.03             | 340 <sup>b</sup> ± 9                | 70.2 <sup>b</sup> ± 1.6        |
| India Cherry    | 4.78 | 6.47 <sup>d</sup> ± 0.06             | 365 <sup>b</sup> ± 12               | 74.4 <sup>c</sup> ± 0.4        |
| Indonesia       | 4.86 | 6.88 <sup>de</sup> ± 0.16            | 385 <sup>c</sup> ± 1                | 81.7 <sup>d</sup> ± 1.8        |
| Laos            | 4.90 | 6.46 <sup>d</sup> ± 0.24             | 409 <sup>d</sup> ± 25               | 70.4 <sup>b</sup> ± 1.8        |
| Uganda          | 4.84 | 6.72 <sup>d</sup> ± 0.11             | 407 <sup>d</sup> ± 21               | 68.6 <sup>b</sup> ± 0.7        |
| Uganda Bugishu  | 5.03 | 5.56 <sup>b</sup> ± 0.01             | 307 <sup>b</sup> ± 24               | 70.4 <sup>b</sup> ± 1.8        |
| <i>Arabica</i>  |      |                                      |                                     |                                |
| Brazil          | 4.92 | 5.11 <sup>a</sup> ± 0.17             | 191 <sup>a</sup> ± 17               | 36.2 <sup>b</sup> ± 2.2        |
| Laos            | 4.60 | 4.66 <sup>a</sup> ± 0.01             | 220 <sup>a</sup> ± 1                | 38.5 <sup>b</sup> ± 1.8        |
| China           | 4.73 | 4.64 <sup>a</sup> ± 0.28             | 196 <sup>a</sup> ± 1                | 34.1 <sup>b</sup> ± 1.7        |
| Rwanda          | 4.92 | 4.51 <sup>a</sup> ± 0.18             | 198 <sup>a</sup> ± 1                | 34.1 <sup>b</sup> ± 2.5        |

Results presented as dry mass of lyophilized brews of coffee ± SD

Mean values with different letters are significantly different in Tukey's test ( $p \leq 0.05$ )

infusion and 0.6 mL of water [23, 24]. The tube containing the sample and reagents was incubated in a water bath at 50 °C for 20 min, after which it was cooled under running water. The absorbance against a reagent blank was measured at 450 nm. A calibration curve was constructed with Trolox, and the antioxidant activity was expressed as Trolox equivalent (TE) in mM kg<sup>-1</sup> of dry mass extract. Data are presented as the average of three independent measurements.

All spectrophotometric determinations were performed on a Perkin Elmer model Lambda 20 UV–VIS spectrophotometer with cuvettes of 1 cm length. Spectra were recorded in the range from 220 to 800 nm with 0.2-nm resolution. Data were processed with WinLab software.

#### Folin–Ciocalteu assay

Aliquots (1 mL) of coffee infusion were introduced into test tubes followed by 0.1 mL of F–C reagent and 0.9 mL of water [25]. The tubes were allowed to stand for 5 min. At the end of this period, 1 mL of Na<sub>2</sub>CO<sub>3</sub> solution (70 g L<sup>-1</sup>) and 0.4 mL of water were added and a further period of 10 min was allowed for stabilization of the blue color formed. The absorbance against a reagent blank was measured at 765 nm. Data were expressed as gallic acid equivalent (GAE) in mg kg<sup>-1</sup> of dry mass extract. All determinations were carried out in triplicate.

#### Statistical analysis

Results are expressed as mean ± standard deviation (at least three replicates). Analysis of variance and significant

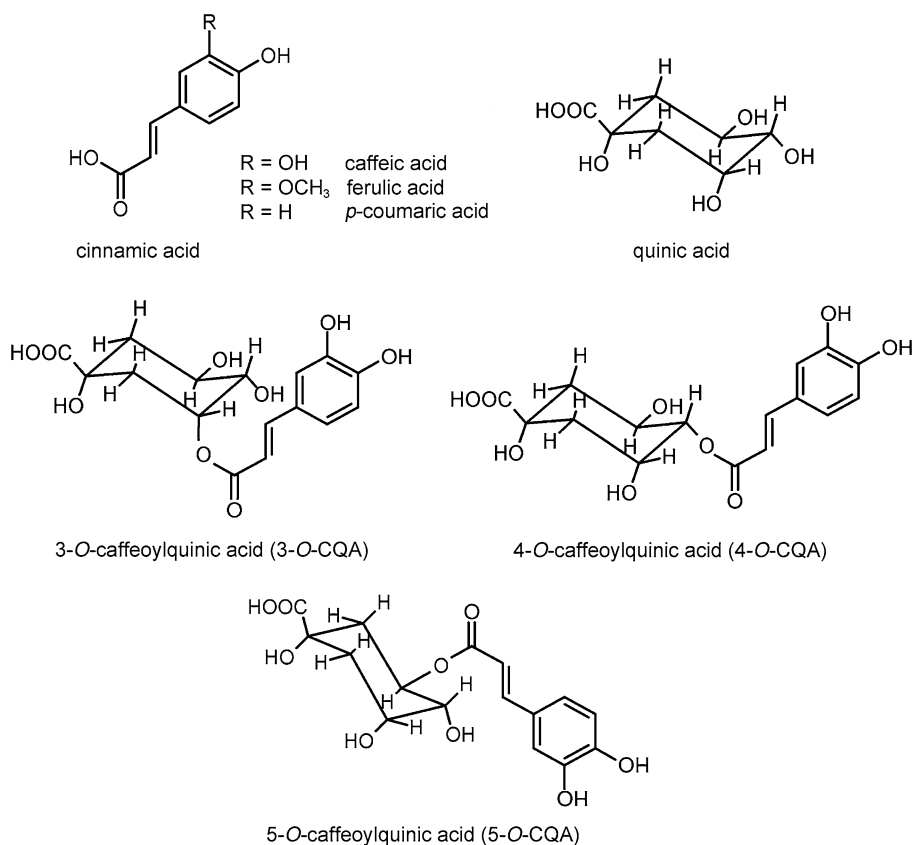
differences among means and correlation analysis were performed with one-way ANOVA. The significance level was based on a confidence level of 95.0 %. Principal component analysis of results was also presented. The experimental data were analyzed using Statistica 10.0 program (StatSoft Inc., Tulsa, OK, USA).

## Results and discussion

### Content of caffeine

The levels of caffeine in studied green coffee brews are given in Table 1. Its content varies from 34.1 g kg<sup>-1</sup> dry mass of extract in Arabica coffees from Laos or Rwanda to 81.6 g kg<sup>-1</sup> d.m. in Robusta coffee from Indonesia. Generally, Robusta coffee extracts contain twice as much caffeine as Arabica. The caffeine content in green bean extracts of *Coffea arabica* was very similar 34.1–38.5 g kg<sup>-1</sup>, while in *Coffea robusta* was in the range between 3.9 (for Vietnam decaf); 68.6 (Uganda Sc) to 81.6 g kg<sup>-1</sup> dry mass. Steaming of green coffee beans has not significantly changed the content of caffeine (decrease of only 0.4 %). The results of caffeine are comparable with the results received earlier [26]. The caffeine content was lower than results obtained by Dziki et al. [16], but they used methanol in ASE extraction of green Arabica coffees [16].

“Decaffeinated” coffee does not mean 100 % caffeine-free. In fact, a decaf coffee only needs to be 97 % caffeine-free according to the USDA, while Brazilian legislation allows at most 0.1 % of residual caffeine in decaffeinated coffee [27].

**Fig. 1** Major chlorogenic acids present in coffee**Table 2** 3-CQA, 4-CQA and 5-CQA content in green coffee extracts in  $\text{g kg}^{-1}$ 

| Coffee          | 3-CQA                     | 4-CQA                     | 5-CQA                    | Total              |
|-----------------|---------------------------|---------------------------|--------------------------|--------------------|
| <i>Robusta</i>  |                           |                           |                          |                    |
| Vietnam         | 21.77 <sup>a</sup> ± 1.12 | 27.33 <sup>a</sup> ± 1.01 | 109.9 <sup>b</sup> ± 4.5 | 159.0 <sup>b</sup> |
| Vietnam decaf.  | 57.18 <sup>c</sup> ± 2.01 | 59.77 <sup>c</sup> ± 1.80 | 104.4 <sup>b</sup> ± 3.2 | 221.4 <sup>c</sup> |
| Vietnam steamed | 35.45 <sup>b</sup> ± 2.54 | 39.24 <sup>b</sup> ± 1.74 | 56.0 <sup>a</sup> ± 2.4  | 130.7 <sup>a</sup> |
| India Cherry    | 18.91 ± 1.04              | 24.05 ± 0.89              | 137.5 ± 5.1              | 180.5              |
| Indonesia       | 27.25 ± 1.91              | 32.10 ± 0.96              | 126.2 ± 3.1              | 185.6              |
| Laos            | 16.47 ± 1.56              | 22.15 ± 1.32              | 144.3 ± 4.5              | 182.9              |
| Uganda          | 19.31 ± 1.67              | 24.74 ± 1.02              | 136.76 ± 6.5             | 180.8              |
| Uganda Bugishu  | 15.03 ± 1.88              | 20.87 ± 1.41              | 135.32 ± 7.1             | 171.2              |
| <i>Arabica</i>  |                           |                           |                          |                    |
| Brazil          | 14.88 ± 2.44              | 19.70 ± 1.98              | 117.14 ± 6.3             | 151.7              |
| Laos            | 15.83 ± 1.13              | 19.11 ± 1.35              | 97.17 ± 4.5              | 132.1              |
| China           | 16.28 ± 1.39              | 20.55 ± 1.46              | 115.50 ± 4.3             | 152.3              |
| Rwanda          | 16.74 ± 1.57              | 21.39 ± 1.61              | 122.00 ± 3.4             | 160.1              |

Results presented as dry mass of lyophilized brews of coffee ± SD  
 Mean values in columns with different letters are significantly different in Tukey's test ( $p \leq 0.05$ )

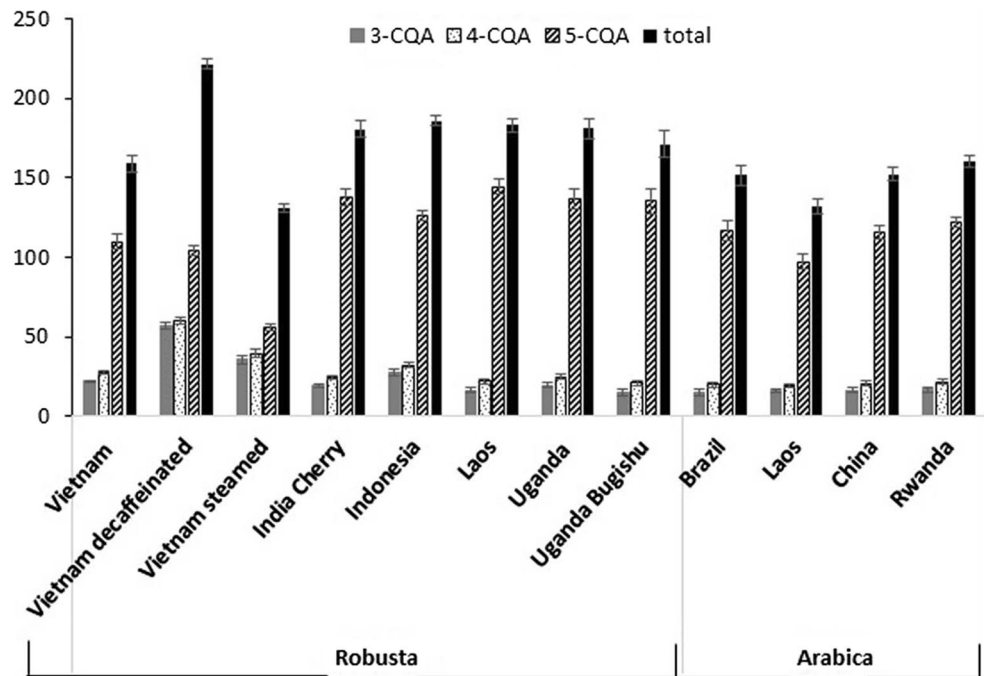
### Concentration of chlorogenic acids

Green coffee beans possess chlorogenic acids, and their derivatives and their contents are 3.5–7.5 % (d.m.) for Arabica and 7.0–4.0 % (d.m.) for Robusta coffees [28]. 3-, 4- and 5-CQA are dominated chlorogenic acids in green coffee beans and extracts [12, 16].

Three major chlorogenic acids present in green coffee samples were determined by chromatographic analysis (Fig. 1). The highest concentration of 5-CQA among Robusta coffee type was obtained for both coffees from Uganda (135–137  $\text{g kg}^{-1}$  of dry mass). The concentration of 5-CQA in the studied Arabica coffee brews was in the range of 97–122  $\text{g kg}^{-1}$  dry mass of dry extract. These results are 3–4-fold higher than in methanol–water extracts (ASE, 100 °C, extraction time 2 min) [16] but similar to results of beans obtained earlier [29]. On the other hand, when isopropanol and water in ratio of 60:40 were employed, the chlorogenic acid (5-CQA) content was two-fold higher [30].

Decaffeination process used on Vietnam Robusta coffee beans does not affect the concentration of 5-CQA, but increases (almost twice) the total sum of chlorogenic acids

**Fig. 2** Content of 3-*O*-CQA, 4-*O*-CQA and 5-*O*-CQA in green coffee extracts (in g kg<sup>-1</sup> dry mass)



in this coffee brew due to the increase in other chlorogenic acids (Table 2).

Moreira et al. [20] determined the chlorogenic acids contents in ground and instant, light and dark roasted, regular and decaffeinated Brazilian Arabica commercial coffee samples, and observed usually lower CQA contents in all decaffeinated samples, in comparison with non-decaffeinated ones. Farah et al. [19] observed loss of 10 % in chlorogenic acids contents of decaffeinated and roasted Arabica coffee samples, in comparison with non-decaffeinated samples roasted in the same conditions. Decaffeination process caused the increase 3-CQA and 4-CQA probably due to lixiviation process. There is a state that these acids are adjacent to the cell walls of coffee beans and seems to be associated with caffeine [19].

In contrast to decaffeination process, steaming of these coffee beans with hot water produced a significant decrease in the level of the main chlorogenic acid (5-CQA), while the concentration of 4-CQA and 3-CQA was slightly increased (Fig. 2).

Degradation of chlorogenic acids during steaming of green beans has been reported as a consequence of increased water uptake [31]. The degradation could also explain that high temperature affects 5-CQA in coffee beans [21].

It is well known that phenolics content in green coffee beans depends on growth conditions of plant, such as location, light, drainage, temperature and weather and depends on the process used on beans [19].

#### Antioxidant activity of green coffee extracts

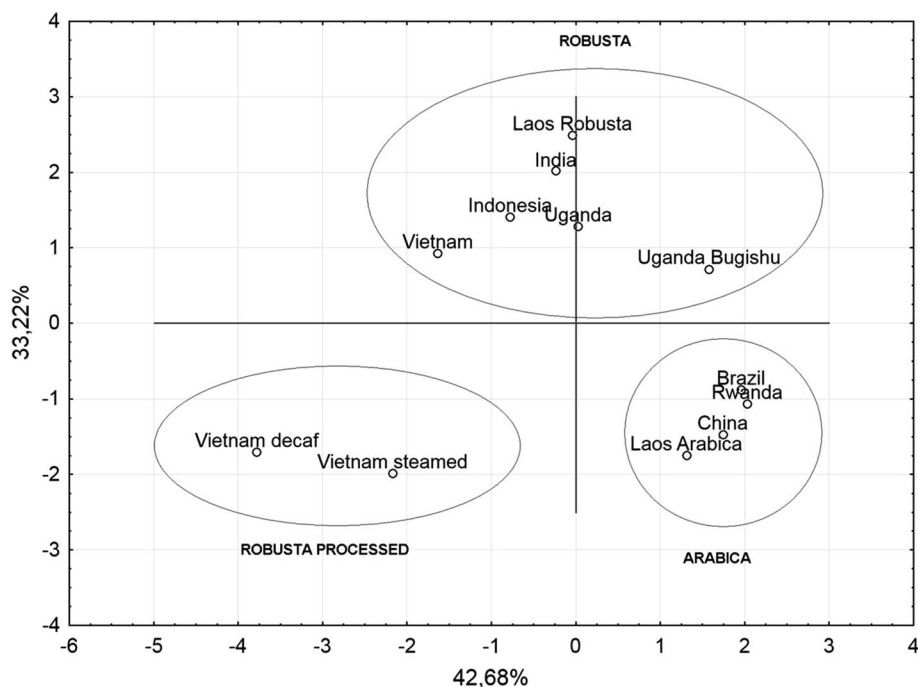
Antioxidant activity of coffee brews was measured by CUPRAC and F-C assays based on a single-electron transfer reaction. In both assays, the antioxidant activity equals to the reducing capacity of a sample. One of the most important advantages of the CUPRAC assay is that simple sugars and citric acid are not oxidized and pH of reaction is close to the physiological pH [32].

Robusta coffee extracts showed significantly higher antioxidant activity in Folin–Ciocalteu assay than Arabica extracts (Table 1). The mean values (expressed as gallic acid equivalent in g kg<sup>-1</sup> of d.m. extract) were 399.7 and 201.8 for Robusta and Arabica coffees, respectively. Similar relationship was obtained for CUPRAC assay. It was found that Robusta coffees contained more reducing substances than Arabica (Table 1). Similar results have been reported [16, 17, 33] only for Arabica water extracts; however, in the last two papers methanol, ethyl acetate, ethyl ether and dichloromethane were used for the extraction of the reducing substances. Other authors determined higher level of these substances; however, they used other than hot water solvents, such as hexane, methanol and water–isopropanol mixture [30, 34].

Extracts of green coffee beans from Vietnam possessed the highest antioxidant activity in both assays. There was no significant influence of decaffeination process of these coffee beans on antioxidant activity in F-C assay, but the decrease in CUPRAC assay (17 %) was observed. Steaming of coffee beans with hot water decreased the antioxidant



**Fig. 3** Principal component analysis of Arabica and Robusta green coffee extracts



activity in F–C assay: (31 %) and CUPRAC assay: (22 %) in comparison with unprocessed beans.

The coffees from Uganda (Uganda Sc and Uganda Bugishu) showed the lowest antioxidant activities between Robusta coffees, although Uganda Bugishu coffee is considered as the best Uganda coffee [35].

pH of Vietnam Robusta coffee was different from extracts obtained from processed beans. After steaming of beans by hot water, pH of the extract decreased, but in the case of decaffeination pH increased (Table 1). Any correlation was not found between pH and antioxidant activity of studied coffee extracts.

The positive correlation between F–C and CUPRAC assays (0.807) with the use of Pearson's linear correlation coefficient was found. Although both used methods are based on the redox properties of the sample components, but they differ in terms of reduction potentials, kinetics and experimental conditions. The positive correlation between the sum of three major chlorogenic acids and F–C assay (0.667) was also obtained. Lower correlation may be due to the fact that F–C reagent is not specific only for phenolic compounds [36].

Principal component analysis has been applied to evaluate differences between Arabica and Robusta coffees as well as preparation process of coffee beans (Fig. 3). PCA performed on the complete data set of Arabica and Robusta samples confirmed that coffee samples from Robusta formed a separate cluster in the PC1 versus PC2 plot (75.90 % of total system variability). Thus, it can explain that Robusta coffee brews contain the highest content of

chlorogenic acids, caffeine and antioxidant activity. Simultaneously, preparation process of coffee beans influenced on the differences in PCA, and this was confirmed in separate cluster.

## Conclusions

Not only species of *Coffea* or geographical origin but also different preparation processes influence the content of major compounds in the extract of green coffee as well as its antioxidant activity determined by F–C and CUPRAC assays. Decaffeination process and steaming used on Vietnam Robusta coffee beans influence 3-, 4- and 5-CQA content. Decaffeination process increased the level of sum of 3-, 4- and 5-CQA. Steaming coffee possesses better quality for coffee experts, but the sum of 3-, 4- and 5-CQA was at the lowest level, even lower than in Arabica coffees.

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## Compliance with ethical standards

**Conflict of interest** None.

**Compliance with ethics requirements** This article does not contain any studies with human or animal subjects.

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