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Evaluation of regular and decaffeinated (un)roasted coffee beans based on antioxidant capacity and total phenolic content

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In this study, the effect of coffee beans processing (decaffeination and roasting) on their antioxidant capacity and total phenolic content has been investigated. In a total of 64 regular as well as decaffeinated (un)roasted coffee samples, these characteristic parameters were determined spectrophotometrically using the DPPH free-radical method and the Folin-Ciocalteu assay. While the total phenolic content remained unchanged regardless of the processing method used, the antioxidant capacity of the samples varied depending on their treatment being affected mainly by the decaffeination procedure chosen.

Keywords: Coffee beans; Antioxidant capacity; DPPH; Total phenolic content, Folin-Ciocalteu reagent.

Introduction

Coffea arabica (Arabica) and *Coffea canephora* (Robusta) are the most consumed and therefore the most economically important varieties of coffee beans in the world [1,2]. Since coffee beans contain more than 2000 compounds, their samples represent a very complex matrix. The coffee brew is a very popular drink worldwide, mainly thanks to its unique organoleptic properties. However, many consumers also appreciate its antioxidant and biological properties, because of which it is classified as the so-called functional food [3].

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Caffeine together with caffeoylquinic acid isomers, such as chlorogenic acid (5-*O*-caffeoylquinic acid), cryptochlorogenic acid (4-*O*-caffeoylquinic acid), and neochlorogenic acid (3-*O*-caffeoylquinic acid), represent the most significant and well-known antioxidants occurring in coffee. These constituents bring potential health benefits and also strongly affect the flavour of the resulting coffee brew (bitterness, acidity, and/or astringency) [4,5]. However, the composition of the resulting beverage, and thus its organoleptic and physicochemical properties, are affected by a number of factors, including the variety and pedoclimatic conditions for growing the coffee tree, the manner of processing the coffee beans, and/or the method of preparing the coffee brew [2,6–8].

Roasting is a very complex process throughout which, as a result of the Maillard and Strecker reactions (followed by several other procedures and reactions), the chemical composition of the coffee is entirely altered. Many biologically active substances degrade due to high roasting temperatures, but new ones are formed [9–13].

As caffeine is still a contentious component of our diet, the popularity of caffeine-free coffee products is going to the fore. The most common methods of decaffeinating green coffee beans (before the roasting process) include extraction with organic [14–16] or so-called natural deep eutectic solvents [16–18], the Swiss water process [16,19], or supercritical fluid extraction [16,20]. Unfortunately, all these techniques have a negative impact on the biologically active substances present in coffee [17,20].

For this reason, the aim of this study was to evaluate the effect of roasting and decaffeination processes of coffee beans upon the antioxidant capacity and total phenolic content in the resulting coffee brew.

Experimental

Chemicals and reagents

Standards of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, and 2M Folin-Ciocalteu reagent (all ≥ 98 %) were purchased from Merck (Darmstadt, Germany). Dichloromethane, methanol, and sodium carbonate (all analytical grade) were purchased from LachNer (Neratovice, Czech Republic). Water of high purity was prepared using Milli-Q purification system (Merck Millipore, Germany).

Standards and samples

Calibration solutions of Trolox and gallic acid were prepared in methanol and water, respectively. The concentration range of Trolox was 0.01–0.1 μmol/30 μL giving the calibration equation parameters: $y = 632\ (9.1)\ x + 10.1\ (0.54)$ and $R^2 = 0.9912$, while the concentration range of gallic acid was 5–30 μg/100 μL resulting in the calibration equation parameters: $y = 31.2\ (0.25)\ x + 0.04\ (0.01)$ and $R^2 = 0.9984$.

The coffee bean samples were either purchased on Czech markets or obtained from local coffee roasteries (BotaCoffee, Brno; Pepe Coffee, Hradec Králové; roastery in Kroměříž; Coffeespot, Babice, Gaetano Caffe, Pardubice; and Frolíkova káva, Borohrádek). The list of the analysed samples is given in Table 1, gathering the corresponding information on their commercial brand/local roastery, roasting degree (light, light-medium, medium, medium-dark, and dark), coffee beans variety (Arabica, Robusta, or their mixture), and processing (roasting and decaffeination) used. Samples Nos. 1–38 and samples Nos. 39–55 represent roasted and unroasted regular coffee beans, respectively. Samples Nos. 56–64 are decaffeinated unroasted (Nos. 56 and 57) or roasted (Nos. 58–64) coffee beans. The method of decaffeination — namely, the supercritical fluid extraction with CO_2 — was known for only two samples (Nos. 57 and 62). In order to assess the effect of roasting and decaffeination on antioxidant capacity, selected regular and decaffeinated samples were purchased in the roasted, as well as unroasted forms. These were samples "BRAZIL": No. 36 (regular roasted) and No. 48 (regular unroasted); and "COLOMBIA": No. 33 (regular roasted), No. 51 (regular unroasted), No. 56 (decaffeinated unroasted), and No. 64 (decaffeinated roasted).

Sample pre-treatment

Unlike roasted coffee beans, the unroasted samples had to be oven-dried for 12 hours at 40 °C before grinding (the moisture loss was involved in the further calculation). Afterwards, approximately 7 g (un)roasted coffee beans were ground on the day of analysis using a hand grinder (Tescoma, Zlín, Czech Republic) and extracted with 50 mL boiling water for 5 min. Prior spectrophotometric measurements, the sample was filtered through a folded filter paper and diluted 20 times with distilled water.

Table 1 Analysed coffee bean samples with details of their variety and processing*

Sample No.	Commercial name/BRAND or ROASTERY	Beans variety	Beans processing
1	Espresso Strong Gaetano Daneli/GAETANO ROASTERY	A	Medium RB
2	Espresso Extra Mild/DANIEL´S COFFEE	A	Medium RB
3	Peru Andina Raritat/TCHIBO	A	Medium RB
4	Top Spot Espresso/COFFEESPOT ROASTERY	A	Medium RB
5	Columbie Excelso La Claudina/COFFEESPOT ROASTERY	A	Medium RB
6	Papua New Guinea/LIZARD COFFEE	A	Dark RB
7	Café Delicado/MARILA	A	Medium RB
8	Christmas Blend/STARBUCKS	A	Dark RB
9	Caffe Crema/MÖVENPICK	A	Dark RB
10	El Autentico/MÖVENPICK	M	Dark RB
11	Gran Espresso/LAVAZZA	M	Dark RB
12	Privat Kaffe African Blue/TCHIBO	A	Light RB
13	Classico/ILLY	A	Medium RB
14	Velluto/MANUEL CAFFE	30 % A	Dark RB
15	Aroma Piú/MANUEL CAFFE	40 % A	Dark RB
16	Aroma Bar/MANUEL CAFFE	80 % A	Dark RB
17	Bio Organic/BELLAROM	A	Medium-dark RB
18	Mocha Italia/COSTA COFFEE	M	Medium RB
19	Brasil Santos/BOTACOFFEE ROASTERY	A	Light-medium RB
20	Crema/BELLAROM	A	Medium-dark RB
21	Limeta/OXALIS	NS	Medium RB
22	Christmass mixture 2017/COFFEESPOT ROASTERY	A	Light RB
23	Mexico Shg Ep Finca Las Chicharras/KROMĚŘÍŽ ROASTERY	A	Medium RB
24	Espresso Caffé Grand/KROMĚŘÍŽ ROASTERY	50 % A	Medium-dark RB
25	Vietnam Gr. 1 Robusta Wet Polished Scr.16/KROMĚŘÍŽ ROASTERY	R	Medium-dark RB
26	Indonesia Green/KROMĚŘÍŽ ROASTERY	A	Medium-dark RB
27	Caffe Diemme/MISCELA ORO	A	Medium-dark RB
28	Brazila Fazenda Lagoa/KROMĚŘÍŽ ROASTERY	A	Medium-dark RB
29	Guetamala Grande/TCHIBO	A	Light RB
30	Espresso/MÖVENPICK	80 % A	Dark RB
31	Brazil Santos Diamond/KROMĚŘÍŽ ROASTERY	A	Medium-dark RB
32	Peru/PEPECOFFEE ROASTERY	A	Medium RB
33	Colombia Supremo/PEPECOFFEE ROASTERY	A	Light-medium RB
34	Guatemala/PEPECOFFEE ROASTERY	A	Light-medium RB
35	Costa Rica/PEPECOFFEE ROASTERY	A	Medium RB
36	Brazil/PEPECOFFEE ROASTERY	A	Medium RB

Table 1 Analysed coffee bean samples with details of their variety and processing* (continued)

Sample No.	Commercial name/BRAND or ROASTERY	Beans variety	Beans processing
37	Honduras/PEPECOFFEE ROASTERY	A	Light-medium RB
38	India Cherry/PEPECOFFEE ROASTERY	R	Dark RB
39	Honduras/FROLÍKOVA KÁVA ROASTERY	A	RB (Roasting degree NS)
40	Brazilie Cerrado/FROLÍKOVA KÁVA ROASTERY	A	RB (Roasting degree NS)
41	Brazil Santos/BOTACOFFEE ROASTERY	A	UB
42	Peru/BOTACOFFEE ROASTERY	A	UB
43	Vietnam Gr.1/KROMĚŘÍŽ ROASTERY	R	UB
44	Colombia Supremo/BOTACOFFEE ROASTERY	A	UB
45	Guatemala/BOTACOFFEE ROASTERY	A	UB
46	Honduras/BOTACOFFEE ROASTERY	A	UB
47	Ethiopia Boji Kochere/BOTACOFFEE ROASTERY	A	UB
48	Brazil/PEPECOFFEE ROASTERY	A	UB
49	Costa Rica/PEPECOFFEE ROASTERY	A	UB
50	Guatemala/PEPECOFFEE ROASTERY	A	UB
51	Colombia/PEPECOFFEE ROASTERY	A	UB
52	Peru /PEPECOFFEE ROASTERY	A	UB
53	India/PEPECOFFEE ROASTERY	R	UB
54	Arabika/FROLÍKOVA KÁVA ROASTERY	A	UB
55	Honduras/PEPECOFFEE ROASTERY	A	UB
56	Colombia/PEPECOFFEE ROASTERY	A	UB
57	Brasil Decaf Fazenda Da Lagoa/BOTACOFFEE ROASTERY	A	UB
58	Decaffeinated Espresso Coffee Pod/COSTA COFFEE	M	Dark DeUB
59	Decaroma/MANUEL CAFFE	40 % A	Dark DeUB
60	Grani Deca/ILLY	A	Dark DeRB
61	Privat Kaffe Colombia Fino/TCHIBO	A	Light DeRB
62	Caffe Decaffeinato/LAVAZZA	60 % A	Medium DeRB
63	Columbia Supremo Decaf/KROMĚŘÍŽ ROASTERY	A	Dark DeRB
64	Colombia/PEPECOFFEE ROASTERY	A	Medium DeRB

*The pure coffee beans variety is given without percentage (= 100 %). When the content of Arabica is < 100 %, the rest is Robusta. As the information on the proportion of each variety in the given sample has not been provided, it is listed under a term "mixture".

Abbreviations used: A, Arabica; DeRB, decaffeinated roasted beans; DeUB, decaffeinated unroasted beans; M, mixture; NS, not specified; R, Robusta; RB, regular roasted beans; UB, regular unroasted beans.

Instrumentation and analysis

The antioxidant capacity assay (DPPH method) along with the total phenolic content (TPC) were determined spectrophotometrically using a UV/VIS-2450 spectrophotometer (Shimadzu) with a 1 cm quartz cuvette (Fisher Scientific, Pardubice, Czech Republic).

Antioxidant capacity assay: The antioxidant capacity of the sample extract was measured by the method presented by Rivero-Peréz et al. [21] with slight modification. First, the DPPH reagent was dissolved in methanol to obtain the stock solution with an approximate concentration of 0.1 mmol/L. This stock solution was further diluted with methanol until obtaining a working solution with an absorbance of *ca*. 0.8. Subsequently, 3 mL of this working solution was mixed with 30 μL of a 20-times diluted sample extract or Trolox calibration solution or water (for a blank test). After 30 min of incubation in the dark, the absorbance intensity at a wavelength of 515 nm was measured. The percentage decrease in absorbance was converted to an equivalent amount of Trolox per gram of sample (Trolox equivalent antioxidant capacity; TEAC) using the calibration curve.

Total phenolic content assay: TPC was determined using the method described by Šilarová et al. [22]. Briefly, 100 μL of the 20times diluted extract or gallic acid calibration solution or water (for a blank test) were added to the Folin-Ciocalteu reagent, and the increase in absorbance intensity at 750 nm was recorded and converted to gallic acid equivalents (GAE) per gram of coffee beans using the corresponding calibration curve.

Method validation and data processing

The calibration data were measured at nine concentration levels, each one in five replicates (n = 5) and interpolated by linear least squares regression (QC Expert 2.9, Trilobyte, Staré Hradiště, Czech Republic). Influential points were identified using graphical diagnostics (Pregibon, Williams, and L-R graphs) and potential outliers eliminated. The linearity of the calibration curves was verified by residual plots and the significance of the intercept of regression straight-lines examined using Student's t-test.

All experiments were repeated five times (n = 5), the final results being calculated and presented as confidence intervals $\bar{x} \pm s \cdot t_{1-\alpha}$, where $\bar{x}$ is the arithmetic mean, s is the standard deviation, and $t_{1-\alpha}$ the critical value of Student's t-distribution for five replicates (2.776) at a significance level α of 0.05 (95% probability).

Results and discussions

Coffee is a complex mixture of compounds with antioxidant properties, content and representation of which are influenced by several factors, such as the variety and geographical origin of coffee beans, the strength and method of beans roasting (natural and torrefacto), and the brewing method used [23]. Although, many publications have already dealt with the determination of antioxidant capacity in various coffee samples [2,24,25], their results are not consistent; apparently, due to the use of various methods based on different mechanisms. Among the well-established assessments of antioxidant capacity, the ABTS and/or DPPH methods are usually employed. Since both methods had yielded similar results in many studies [2,5], only the DPPH method was selected in this research, primarily for its simplicity. In addition to the antioxidant capacity assessment, the total phenolic content of coffee extracts was determined by the traditional Folin-Ciocalteu method.

Before determining the antioxidant capacity and TPC, it was always necessary to optimise the appropriate volume of coffee brew added to the reaction mixture, as well as the reaction time required. The optimal reaction conditions together with the resulting calibration data are given in the Experimental part. The obtained mean TEAC and GAE values for all analysed samples along with the corresponding standard deviations are graphically shown in Figure 1.

While the TEAC values varied significantly depending on the coffee sample analysed (130–336 μmol/g), the TPC content was very similar, with GAE values around 40 mg/g. Interestingly, the roasted coffee samples provided similar antioxidant capacity and TPC to the unroasted samples. Although 5-caffeoyl-quinic acid (also known as chlorogenic acid), representing the main phenolic compound in coffee, is degraded during roasting [2], TPC and antioxidant capacity remain both similar, indicating that other compounds with antioxidant properties (e.g. melanoidins) are formed. The effect of roasting on antioxidant capacity has been the subject of many studies and it is worth noting that their conclusions differ. In our study, the roasting did not affect the antioxidant capacity, which is consistent with the results published by Contreras-Calderón et al. [23] and Vignoli et al. [2]. However, other authors [26–31] have found that light and medium roasted coffee beans exhibit a higher antioxidant capacity than that found for the dark roasted ones. On the other hand, in the study by Schouten et al. [29], the antioxidant capacity and TPC of roasted coffee beans were higher compared to unroasted ones; nevertheless in this case, only a limited number of coffee samples were subjected to analysis.

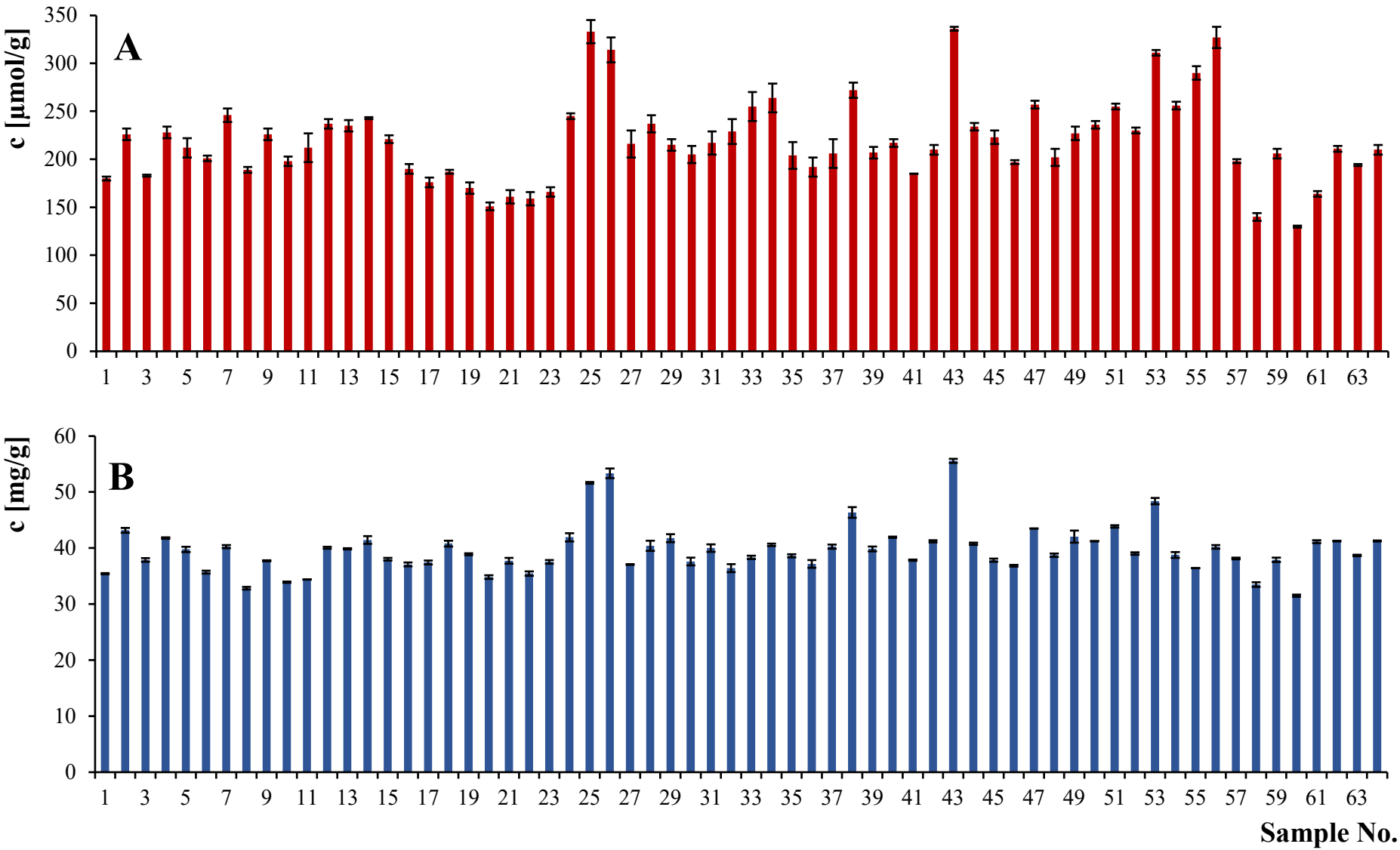

Fig. 1 Antioxidant capacity (A) and total phenolic content (B) of individual coffee brews

The increase in antioxidant capacity of roasted coffees is mainly attributed to the release of low molecular weight phenols and/or the formation of products associated with the Maillard reactions [31]. Furthermore, the caffeoylquinic acid isomers, primarily represented by the isomers of cryptochlorogenic, neochlorogenic, and chlorogenic acids, can be easily altered into their respective derivatives at a high roasting temperature [25]. Thus, the antioxidant capacity is strongly dependent on the processes of degradation and re-formation of compounds with antioxidant properties, which can also be affected by the variety of coffee beans. These ongoing processes may likely be the reason for the frequent inconsistency of hitherto published results.

In general, the highest TPC values simultaneously with the highest antioxidant capacity results were observed in Robusta coffee samples (especially samples No. 25, 38, 43, and 53). However, both parameters were also very high in Arabica coffee sample No. 26. In the previous study by Klikarová et al. [32], this sample was also subjected to chromatographic analysis to determine its caffeine content. Unnaturally high caffeine amount (23.4 mg/g), which is more typical of the Robusta variety, was found in this sample. In the studies by Jeszka-Skowron et al. [4,5] and Vignoli et al. [2], the Robusta variety showed a significantly higher antioxidant capacity than Arabica variety. For this reason, it is suspected that the sample No. 26 could be intentionally mislabelled in order to increase its market price. On the contrary, the lowest antioxidant capacity was recorded for the dark roasted decaffeinated coffee samples Nos. 58 and 60. As indicated above, the degree of roasting did not affect the presence of antioxidants in this study, thus playing a marginal role only. The opposite is true for the decaffeination process, as all caffeine-free coffee samples yielded lower TEAC values, clearly demonstrating that the caffeine removal has a great impact on antioxidant capacity. The positive effect of caffeine on antioxidant capacity, confirming our conclusions, has been presented by Vignoli et al. [2].

In all 64 samples, the most important isomers of caffeoylquinic acid were analysed chromatographically and the results are presented in a study by Klikarová et al. [32]. The correlation between the results obtained spectrophotometrically in this study and those analysed chromatographically [32] (and for our purposes reported as the sum of chlorogenic acid isomers and the sum of all determined compounds) is clearly illustrated in Figure 2. From the respective diagrams it is evident that the highest correlation was achieved for both spectrophotometric methods (DPPH and TPC) with $r = 0.7528$. Furthermore, the chromatographic results correlate better with the spectrophotometric data when caffeine is included in the sum of all compounds, confirming the significance of the antioxidant properties of caffeine. The relatively low correlation between spectrophotometric and chromatographic results can be explained by the fact that many compounds with antioxidant properties have not been monitored by HPLC. Although spectrophotometric techniques cannot reveal the exact composition of the samples, they can be useful for easy and rapid screening of their quality.

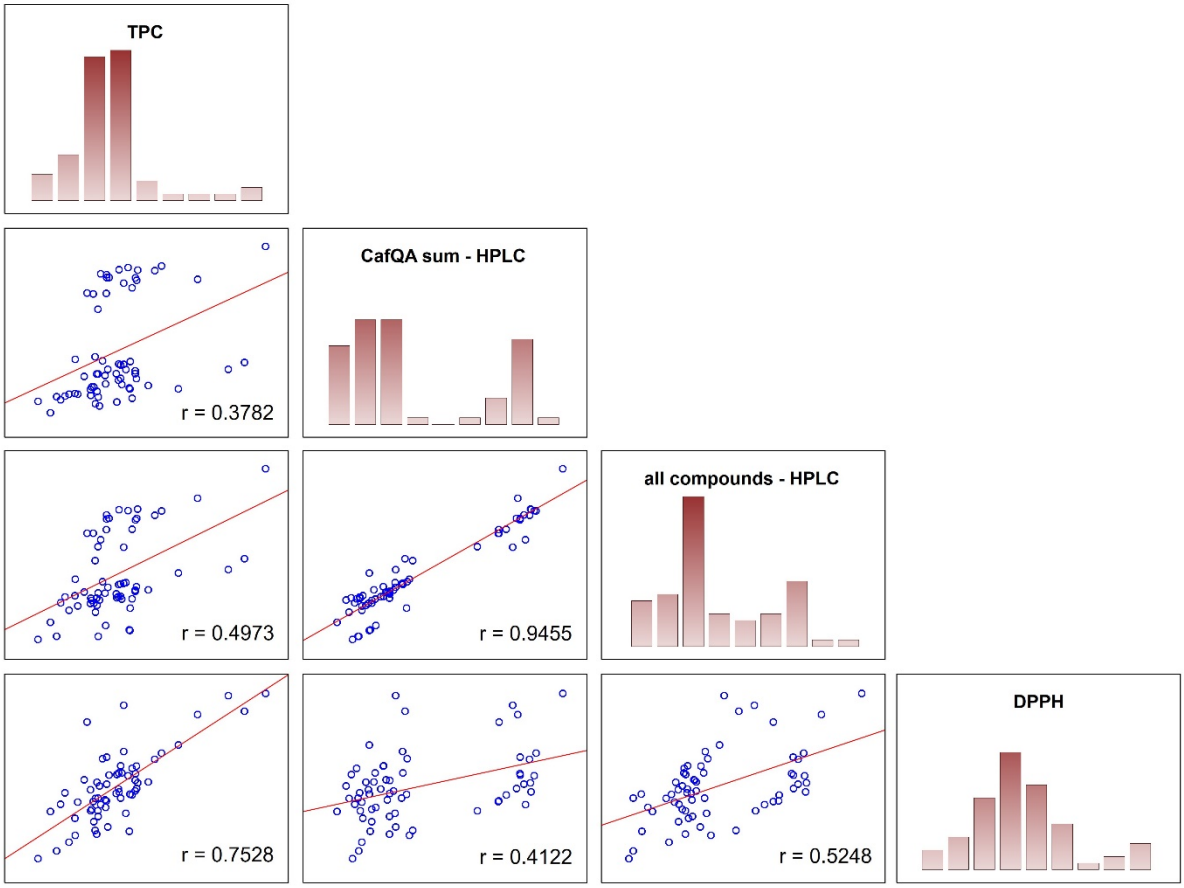

Fig. 2 Correlation of total phenolic content (TPC) and antioxidant capacity (determined by the DPPH method) with the sum of caffeoylquinic acid isomers (determined using HPLC) as well as the sum of all chromatographically analysed compounds (including caffeine) [32]

The corresponding correlation coefficient (*r*) is given.

Conclusions

Spectrophotometric determination of antioxidant capacity and total phenolic content using the DPPH and Folin-Ciocalteu methods, respectively, has been employed to assess the potential impact of coffee beans processing (roasting and decaffeination process). First, both analytical methods needed to be optimized in terms of volumes of reagents and reaction time. Then, the optimised procedures were successfully applied to a large set of 64 regular, as well as decaffeinated (un)roasted coffee bean samples, including commercially available coffee brands plus coffee beans obtained from various local roasteries. It has been proven that the Robusta variety offers a higher antioxidant capacity compared to that of Arabica coffee beans. With respect to sample processing, decaffeination had a much greater negative impact on the antioxidant capacity of the coffee brew than roasting. The total phenolic content was comparable for all the samples, indicating that the processing did not fundamentally affect the sum of compounds with antioxidant properties.

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