

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SIMPLE AND FLAVOURED TURKISH COFFEE

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Abstract: The aim of our paper was the evaluation of phenolic content and antioxidant activity of several Turkish coffee samples (regular simple coffee or flavoured with mastic/carob and decaffeinated coffee flavoured with cardamom/pistachio). Phenolic compounds analysis was based on qualitative (specific chemical reactions and thin layer chromatography - TLC) and quantitative assays. The antioxidant capacity was determined based on the scavenger activity towards DPPH, ABTS·+ free radicals and ferric reducing power. TLC analysis revealed the presence of chlorogenic acid for simple regular coffee and carob/mastic flavoured samples. The highest total phenolic and tannins contents were observed for simple and mastic regular coffee samples. We have found a weak (or at least medium) correlation between the antioxidant activity and the total phenolic content of analysed samples.

Keywords: Arabica coffee, chlorogenic acid, antioxidant capacity, mastic, decaffeination.

INTRODUCTION

Coffee is one of the most popular drinks worldwide and it has been consumed for centuries due to its special taste and stimulant properties (Dos Santos Scholtz et al., 2016; Shateri et al., 2016). Two important coffee species are cultivated worldwide on commercial scale: *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* Pierre ex Froehner (robusta coffee) (Dos Santos Scholtz et al., 2016; Adepoju et al., 2017). The quality of coffee species varies with its geographical region, pedoclimatic conditions, cultivars and processing (Dong et al., 2015; Ribeiro et al., 2016). The coffee process begins with removal of the external components (skin, pulp/husk, silver skin) of coffee cherries by wet methods (for Arabica coffee) or dry methods (for robusta coffee), leaving only green coffee beans (Janissen et al., 2018). Roasting, brewing or decaffeination process of coffee beans significantly influence the phenolic, caffeine and fiber contents (Janissen et al., 2018). Coffee seeds are rich in alkaloids (caffeine – 1.2-2.85%; trigonelline – 0.8-1.24%) and their concentration is usually reduced during roasting (Patay et al., 2016; Cheng et al., 2016; Rezk et al., 2018; Dong et al., 2015). Coffee seeds also contain phenolcarboxylic acids (4-14%) (chlorogenic acid, caffeic acid, ferulic acid, sinapic acid etc); flavones (quercetin and kaempferol glycosides) and tannins (4.5-9.3%) (Patay et al., 2016; Janissen et al., 2018; Cheng et al., 2016). Moreover, the total lipid content of coffee cultivars varies between 8-12%, with major fatty acids being linoleic acid, palmitic acid, oleic acid, arachic acid and to a less extent linolenic acid (Dong et al., 2015). Essential aminoacids, volatile flavor compounds, minerals and diterpenes (cafestol, kahweol) have been also identified in coffee seeds (Dong et al., 2015; Şemen et al., 2017; Shateri et al., 2016).

Regarding the therapeutic effects of coffee, besides its stimulant properties, coffee consumption has been associated with a lower risk of type 2 diabetes and obesity (Natella et al., 2012; Costabile et al., 2018; Buchanan et al., 2013; Raouf et al., 2017); antioxidant, antiinflammatory, immunomodulatory and hepatoprotective effects (Jung et al., 2017; Hall et al., 2015; Sharif et al., 2017; Wadhawan et al., 2016). On addition, coffee has chemopreventive properties towards different types of cancer (colorectal or bladder carcinomas) (Monteiro et al., 2019). Moreover, coffee components, like chlorogenic acid and its metabolite (dihydrocaffeic acid) have shown neuroprotective effects (Lee et al., 2015); pyridinium compounds (formed from trigonelline upon coffee roasting) have antithrombotic properties (Kalaska et al., 2014; Toci et al., 2018), while coffee beans volatile compounds exert anti-anxiety-like effects in mice (Hayashi et al., 2012). Coffee diterpenes, kahweol, prevents osteoclastogenesis (Fumimoto et al., 2012).

However, detrimental effects of excessive coffee consumption include insomnia, hypertension, cardiovascular risk, gastric ulcer and risk of miscarriage (Monteiro et al., 2019).

Recently, consumption of coffee enriched with herbal products or spices has become popular among coffee drinkers, mainly due to increased flavour characteristics (Febrianto et al., 2016).

Taking into consideration the scientific data and coffee drinkers preferences, the aim of our study was the evaluation of phenolic content and antioxidant activity of several Turkish coffee samples. We have analysed both regular (simple Arabica coffee and flavoured with mastic - the resin obtained from *Pistachio lenticus* or carob pods, from *Ceratonia siliqua* L.) and decaffeinated (flavoured with cardamom pods, from *Elletaria cardamomum* (L.)

Maton or pistachio seeds, from *Pistachia vera* L.) coffee samples.

MATERIALS AND METHODS

Materials

Simple and flavoured coffee samples (regular and decaffeinated) were acquired from Misir Carsisi (Spice market) from Istanbul, Turkey in September 2018.

Samples were encoded as follows: simple regular Arabica coffee (**CS**), regular Arabica coffee flavoured with carob (**CRO**), regular Arabica coffee flavoured with mastic (**CR**), decaffeinated coffee flavoured with cardamom (**CC**) and decaffeinated coffee flavoured with pistachio (**CF**).

Reagents and solvents

All reagents and solvents were purchased from Karl Roth (Germany) unless otherwise stated. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺ free radical) were from Sigma-Aldrich (Germany). Trichloroacetic acid was acquired from Merck (Germany).

Phytochemical screening

Qualitative assays consisted in specific chemical reactions for phenolcarboxylic acids, flavones, tannins, proanthocyanins (Gird et al., 2010) and thin layer chromatography (TLC) for phenolcarboxylic acids (PCAs) and flavones.

Preparation of samples for qualitative/ quantitative assays and antioxidant activity: 3 g of each analysed coffee sample (CS, CRO, CR, CC, CF) were heated with 100 mL water for 5 min. After cooling, the solutions were filtered and brought in a 100 mL volumetric flask for further analysis. The solutions were encoded as follows: **CSS** (simple coffee), **CROS** (carob coffee), **CRS** (mastic coffee), **CCS** (cardamom coffee) and **CFS** (pistachio coffee).

Preparation of samples for thin layer chromatography: 2 mL of each aqueous solution (CSS, CROS, CRS, CCS, CFS) were evaporated on a water bath (Raypa, Spain) and brought to residue. Each residue was dissolved in 2 mL ethanol 96%. The ethanolic solutions were encoded: CSE, CROE, CRE, CCE and CFE respectively.

Thin layer chromatography analysis (TLC)

Thin layer chromatography was used for phenolcarboxylic acids (PCAs) and flavones identification. It was performed on an aluminium-coated TLC plate (20x20cm, Merck, Germany), which was kept for one hour at 105°C, before use. A mixture of ethyl acetate: formic acid: water = 80:8:12 (v/v/v) was used as eluent system (Wichtl et al., 2002). The plate was spotted with CSE, CROE, CRE, CCE and CFE solutions. Rutin, caffeic acid and chlorogenic acid (0.1 mg/mL methanolic solutions) were used as standard references. The plate was developed over a path of 16 cm, air dried and sprayed with a 10 g/L diphenylboriloxylethylendiamine (DFBOA) methanolic solution. The plate was examined in UV light ($\lambda = 366$

nm), before and after spraying with the detection reagent.

Spectrophotometric and volumetric assays

The total phenolic content (expressed as gallic acid equivalents) was determined according to Singleton et al., 1965 method, using Folin-Ciocalteu reagent (Singleton et al., 1965). Tannins content (expressed as gallic acid equivalents) was determined as previously described (Costea et al., 2016). Phenolcarboxylic acids (PCAs) were evaluated based on the formation of oxymes in the presence of sodium nitrite and sodium hydroxide (Costea et al., 2016). Calibration curves of: gallic acid (1.22-7.22 $\mu\text{g/mL}$, $R^2 = 0.9989$, $n = 6$) and chlorogenic acid (0.01131-0.05278 mg/mL , $R^2 = 0.9998$, $n = 6$) were used to determine the content of active compounds. All spectrophotometric determinations were performed using a Jasco V-530 spectrophotometer (Jasco, Japan).

Antioxidant activity

The antioxidant capacity was evaluated by means of scavenger activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) free radicals and ferric reducing power.

• DPPH free radical scavenging capacity

Briefly, 0.5 mL of CSS, CROS, CRS, CCS, CFS solutions (0.24 - 3 mg/mL) were treated with 3 mL of a 0.1 mM ethanolic solution of DPPH. The mixture was kept in the dark, at room temperature and the absorbance was measured at $\lambda = 516$ nm, before (A_{start}) and 30 min. after adding the samples (A_{end}). Ethanol was used as a blank (Brand-Williams et al., 1995).

The ability to scavenge the DPPH free radical was calculated using the following formula: DPPH radical scavenging activity (%) = $(A_{\text{start}} - A_{\text{end}}) / A_{\text{start}} \times 100$ (1).

• ABTS⁺ free radical scavenging capacity

Briefly, the ABTS⁺ radical cation was generated by incubation of ABTS diammonium salt (7 mM) with potassium persulphate (2.45 mM) in the dark, at room temperature for 16 hours. The absorbance of the ABTS⁺ radical solution was equilibrated to a value of 0.700 ± 0.02 , at $\lambda = 734$ nm after dilution with ethanol. Afterwards, 0.5 mL of CSS, CROS, CRS, CCS, CFS solutions (0.12 - 1.2 mg/mL) were mixed with 3 mL reagent and the absorbance was measured at $\lambda = 734$ nm, before (A_{start}) and 6 min. after adding the samples (A_{end}) (Re et al., 1999). The ABTS⁺ scavenging activity was determined according to formula (1).

• Ferric reducing power

The reducing power assay was determined according to Oyaizu method (Oyaizu, 1986). Briefly, CSS, CROS, CRS, CCS, CFS solutions (0.24 - 3 mg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. Samples were kept at 50°C in a water bath (Raypa, Spain) for 20 min. After, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 2500 rpm for 5 min. (Universal 16

centrifuge). The upper layer (2.5 mL) was mixed with 2.5 mL water and 0.5 mL of a 0.1% ferric chloride solution. The absorbance was measured at $\lambda = 700$ nm, after 10 min., against a blank that contained all reagents except for samples.

Antioxidant activity was assessed by means of trolox equivalents (mM trolox/g), which were determined based on trolox calibration curves, as previously described (Costea et al., 2019).

Statistical analysis

Results for spectrophotometric and antioxidant assays represent the average of three replicates, from three independent determinations. Results are presented as mean \pm standard deviation (SD) and were statistically analysed using GraphPad Prism 8 software (GraphPad, USA), by means of one-way ANOVA followed by Tukey's multiple comparisons test. Pearson coefficient (r) has been used to evaluate the correlation between antioxidant assays and the phenolic content of analysed coffee samples. A value of $p < 0.05$ was considered the threshold for a statistically significant difference.

RESULTS AND DISCUSSION

The macroscopic exam for analysed coffee samples is presented in fig. 1. All samples had a specific taste

and aroma of pure coffee or of herbs/spices that were used in combination.

According to our qualitative assays (table I) all coffee samples are a source of phenolcarboxylic acids (PCAs), flavones, and tannins. Our results regarding the phytochemical screening are similar to other authors, that have also reported the presence of phenolic compounds in coffee samples (Da Silveira et al., 2020, Patay et al., 2016; Janissen et al., 2018; Cheng et al., 2016; Bampouli et al., 2015). Still, the chemical reactions for flavones and phenolcarboxylic acids were weaker for CF and CC samples, while proanthocyanidins were not identified in CF. We assume that these results are the consequence of the decaffeination process. According to recent studies, the decaffeination process is responsible for almost 10% loss in polyphenols content (Jezka-Skowron et al., 2016; Vicente et al., 2014).

Besides, according to scientific literature pistachio seeds are mainly a source of lipophilic compounds (phytosterols, fatty acids, tocopherols and carotenoids), while polar substances (gallotannins and flavonoids) are found in much smaller amounts (Grace et al., 2016). Cardamom fruits are a source of essential oil, while polyphenols (catechin, kaempferol, quercetin) are not the main constituents (Teneva et al., 2016).



Fig. 1 Macroscopic exam of analyzed coffee samples. A – CS, B – CF, C – CC, D – CR, E – CRO. CS – simple regular Arabica coffee, CF – decaffeinated coffee flavoured with pistachio, CC – decaffeinated coffee flavoured with cardamom, CR – regular Arabica coffee flavoured with mastic, CRO – regular Arabica coffee flavoured with carob.

Regarding TLC analysis, chlorogenic acid was identified in CS, CR and CRO coffee samples (fig. 2) and our results are in agreement with other authors research (Patay et al, 2016; Janissen et al, 2018). The spots corresponding to chlorogenic acid for CR and

CRO have an intense fluorescence, since carob pulp is an important source of caffeic acid, ferulic acid, sinapic acid and syringic acid (Stavrou et al, 2018), while mastic contains galloyl quinic acids (Bampouli et al, 2015).

Qualitative analysis of analyzed coffee samples

Active substance	CS	CR	CRO	CC	CF
Phenolcarboxylic acids (PCAs)	++	++	+	±	±
Flavones	+	+	+	±	±
Tannins	+	+	+	+	+
Proanthocyanidins	+	+	+	-	-

"+" – positive reaction, "++" – intense positive reaction, "±" – weak positive reaction, "-" – negative reaction

Legend: CS – simple regular Arabica coffee, CF – decaffeinated coffee flavoured with pistachio, CC – decaffeinated coffee flavoured with cardamom, CR – regular Arabica coffee flavoured with mastic, CRO – regular Arabica coffee flavoured with carob.

As shown in fig. 2, we haven't observed any spots corresponding to PCAs (with a blue fluorescence) for CC and CF samples. We assume that our results are probably the consequence of the decaffeination process or the chromatographic conditions (Vicente et al., 2014; Polak et al., 2019). Analysing fig.2, one can note

other spots with a blue fluorescence, corresponding to other PCAs, that were not identified, due to lack of standard references. Although, scientific data foresees the presence of flavones in coffee samples (Patay et al., 2016), these compounds were not identified in the current chromatographic conditions.

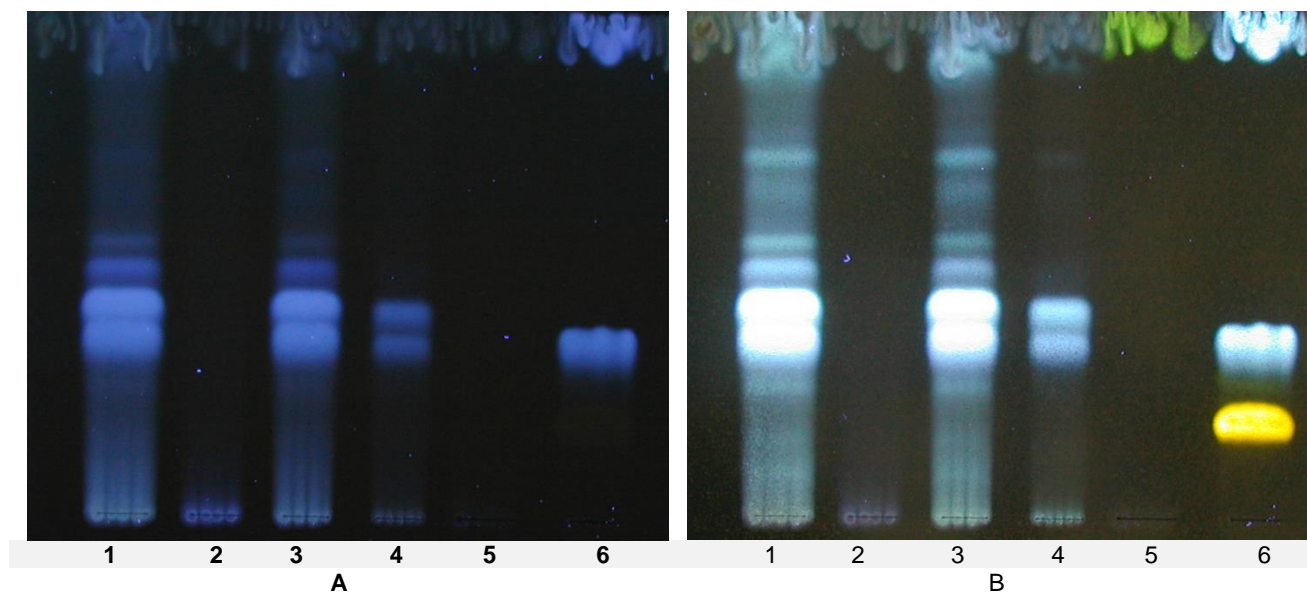


Fig. 2 TLC analysis of coffee samples (A – UV light 366 nm before spraying with DFBOA, B – UV light 366 nm after spraying with DFBOA). 1 – CS, 2 – CC, 3 – CR, 4 – CRO, 5 – CF, 6 – mixture of rutin, chlorogenic acid, caffeic acid (from bottom to top). CS – simple regular Arabica coffee, CF – decaffeinated coffee flavoured with pistachio, CC – decaffeinated coffee flavoured with cardamom, CR – regular Arabica coffee flavoured with mastic, CRO – regular Arabica coffee flavoured with carob.

According to our spectrophotometric results the highest phenolic content (fig. 3A) was determined for CS (3.89 g%) and CR (3.92 g%) coffee samples. Significant differences were found between all analysed coffee samples ($p < 0.0001$), except for CS/CR ($p > 0.05$). Tannins content decreased as follows: CS (2.27 g%) > CR (2.03 g%) > CRO (1.11 g%) > CF (0.93 g%) > CC (0.092 g%) (fig. 3B).

Significant differences, regarding tannins content, have been found between simple and flavoured coffee samples (fig. 3B). We have also found significant differences between all flavoured coffee samples (data not shown). CS, CRO, CF and CR had a similar

content of phenolcarboxylic acids (0.64-0.84 g%). For coffee flavoured with cardamom pods, the phenolcarboxylic acid content was not determined; since the amount was under the detection limit of the method (fig. 3C).

Regarding the total phenolic content of simple coffee our results are similar to other authors (Hećimović et al., 2011), that found 1.7-3.9 g polyphenols (expressed as gallic acid equivalents)/100 g Arabica coffee varieties. Still, our results are much lower compared to Daniel et al. (2017), that analysed different brand of Ethiopian coffee and found a concentration between 5-8 g polyphenols (expressed as

gallic acid equivalents)/100 g (Daniel et al., 2017). Our results regarding PCAs content (for regular simple coffee) are also similar to other authors (Król et al., 2020), that found 0.6-0.8 g phenolcarboxylic acids (expressed as chlorogenic acid) for several Brazilian coffee varieties.

According to recent studies (Alshammari et al., 2017) the association between regular coffee and other spices/herbal products leads to a stronger antioxidant activity, due to a higher phenolic content. For our Turkish samples, CS and CR have a similar phenolic profile, while CRO showed a lower phenolic content. A possible explanation for our results might be the different coffee:herbal products ratio, between analysed samples, or the interaction of Folin-Ciocalteu reagent with other compounds (aminoacids, sugars etc) (Bastalo et al., 2017).

Our results regarding *in vitro* antioxidant activity (fig. 4 A, B, C) showed that CS and CR samples had

the highest scavenger activity towards DPPH and ABTS^{•+} + free radicals and the best ferric reducing power. As shown in fig. 4A, the scavenger activity towards DPPH free radical at the highest concentration (3 mg/mL) was 83.97 % (for CS), 77.45% (for CRO), 81.64 % (for CR), 44.53 % (for CF) and only 14.14% (for CC). Analysed coffee samples have also scavenged ABTS^{•+} + free radical in a dose-dependent manner (fig. 4B). The samples displayed scavenging activity with values ranging from 94.55% (for CS), 98.14% (for CR), 68.31% (for CRO), 53.19 % (for CF) and only 29.66 % (for CC) at the highest concentration (1.2 mg/mL). Concerning ferric reducing power (fig. 4C) at 3 mg/mL the absorbance was 1.8779 (for CS), 2.0730 (for CR), 1.066 (for CRO), 0.5524 (for CF) and only 0.1424 (for CC).

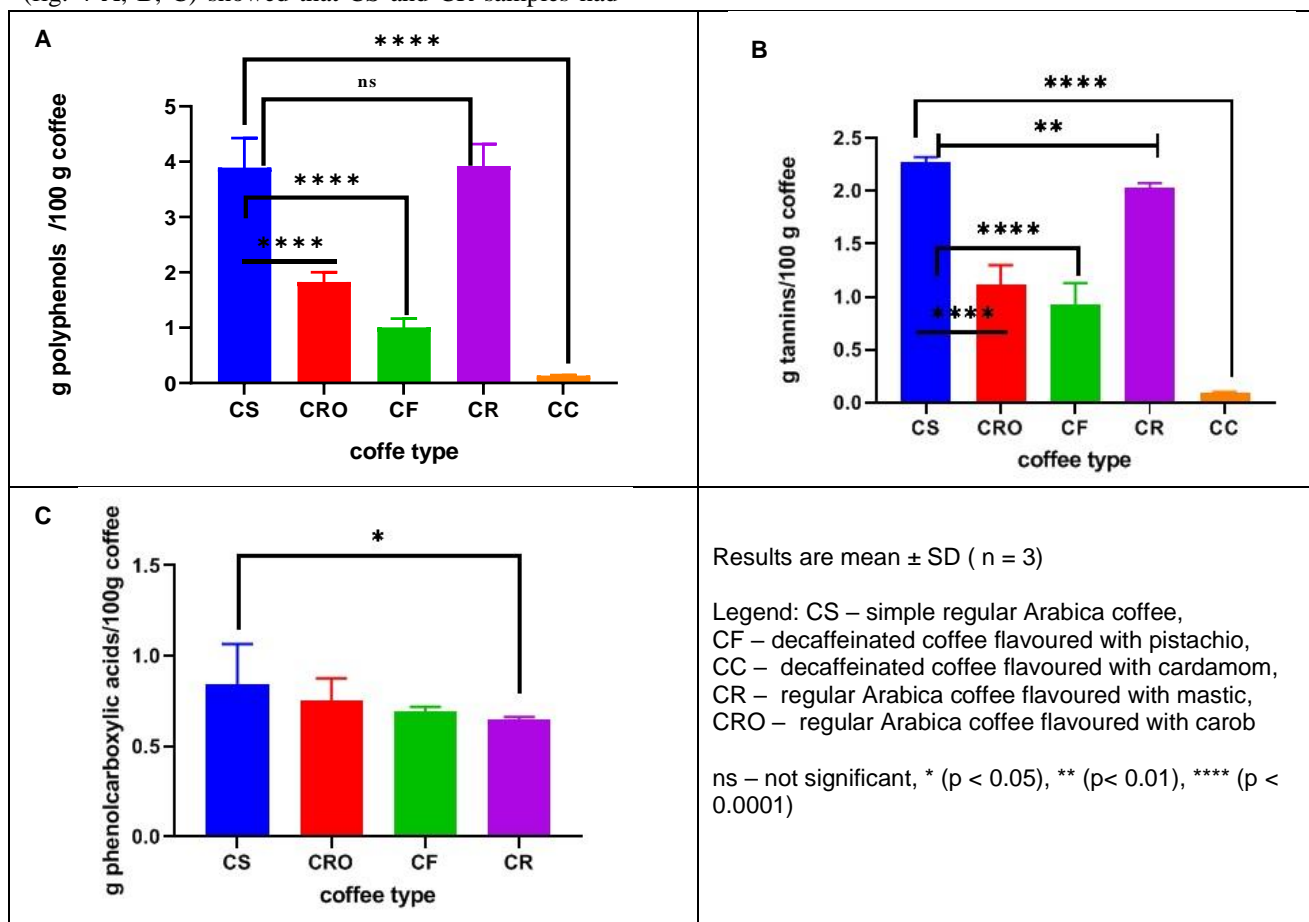


Fig. 3 Phenolic content of analysed coffee samples. A – total phenolic content, B – tannins content, C – PCAs (phenolcarboxylic acids) content.

We have found significant differences between analysed coffee samples in terms of trolox equivalents (table II). According to our results, CR and CS have a similar antioxidant activity by means of DPPH and ferric reducing power assays, while a stronger antioxidant activity was observed by means of ABTS^{•+} + method. In fact, for all coffee samples the scavenger

activity towards ABTS^{•+} + free radical was higher compared to DPPH, which is in agreement with other authors reports (Jung et al., 2017).

We assume that these differences are the consequence of a different mechanism of action, since ABTS^{•+} + assay is used to determine the antioxidant activity of both lipophylic and hydrophilic compounds,

while DPPH method is mainly used for polar compounds (Dudonné et al., 2009).

According to scientific literature coffee is also a source of much more lipophilic compounds (sterols, diterpenes) that can contribute to a better scavenger activity towards ABTS^{•+} + free radical (Dong et al., 2017; Shateri et al., 2016). In terms of CR sample, mastic also contains triterpenic compounds (moronic acid, masticadienonic acid, isomasticadienonic acid), that might be responsible for the better scavenger activity towards ABTS^{•+} + free radical (Xynos et al., 2018). CRO sample showed a much lower antioxidant capacity compared to CS and CR and this is in agreement with our spectrophotometric results (regarding the total phenolic content) and with other authors reports (Sekeroglu et al., 2012). Studies performed on traditional herbal coffees consumed in Anatolia (Turkey), revealed that the association between carob and regular Arabica coffee had a weaker antioxidant activity by means of DPPH and DMPD⁺ (N,N-dimethyl-p-phenylenediamine) assays (Sekeroglu et al., 2012). Similar results for the association between

regular coffee and spices were also found by Al Doghaier et al. (2017), who reported a lower DPPH scavenger activity for a mixture of coffee and cloves compared to simple Arabica coffee. A possible explanation for these results might be the polymerization or interaction between phenolic compounds (Al Doghaier et al., 2017).

However, the ferric reducing power of CS, CR and CRO samples are higher compared to Daniel et al. (2017) that analysed different brands of Ethiopian coffee (prepared in a similar manner) (Daniel et al., 2017).

Our results regarding CF and CC antioxidant activities, are similar to other authors, that also confirmed a lower antioxidant activity for decaffeinated coffee samples (Jezka-Skowron et al., 2016; Vicente et al., 2014). In fact for CC sample we were not able to evaluate the antioxidant activity by means of DPPH and ferric reducing power methods, since the absorbances did not fall within trolox calibration curves.

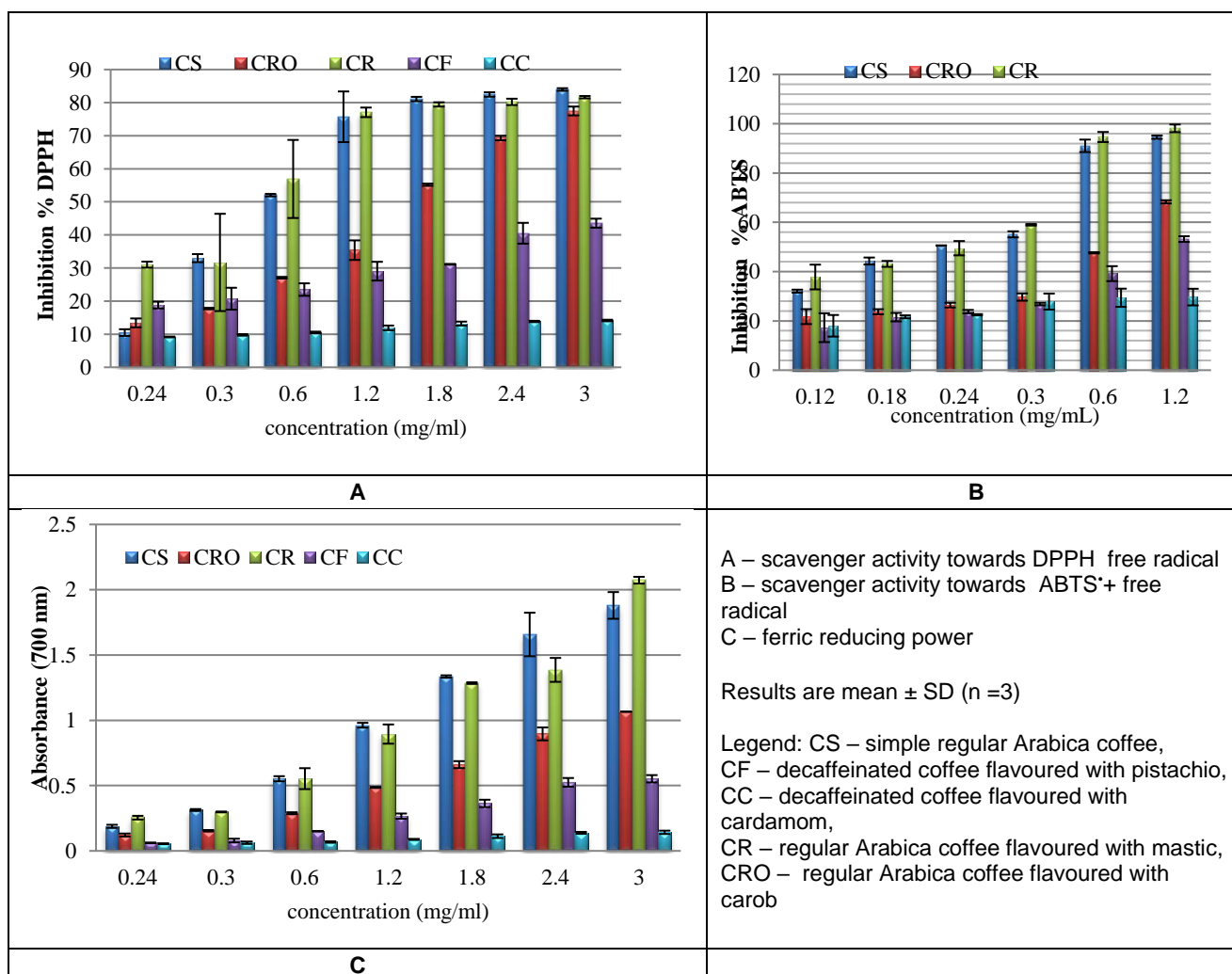


Fig. 4 Antioxidant activity of coffee samples.

Table 2
Antioxidant activity of coffee samples

SAMPLE	mM TROLOX/g		
	DPPH assay	ABTS'+ assay	Ferric reducing power
CS	34.5321 ± 1.4102 ^{ac*/ad**}	54.3175 ± 2.6586 ^{ac**/ad**/ab*/ae**}	104.6625 ± 2.0445 ^{ac****/ad****}
CR	38.1071 ± 1.6426 ^b	78.44 ± 1.2974 ^{be****}	107.095 ± 1.7262 ^b
CRO	16.7820 ± 1.6074 ^{cb**}	27.1214 ± 0.9897 ^{bc****}	46.845 ± 6.4389 ^{cb****}
CF	11.5183 ± 5.0660 ^{db***}	23.6160 ± 1.7971 ^{db****}	33.3260 ± 2.0316 ^{db****}
CC	nd	24.3483 ± 1.089 ^e	nd

Results are mean ± SD (n = 3), nd – not determined

Different letters in the same column means significant differences between coffee samples - * (p < 0.05), ** (p < 0.001), *** (p < 0.001), **** (p < 0.0001)

Legend: CS – simple regular Arabica coffee, CF – decaffeinated coffee flavoured with pistachio, CC – decaffeinated coffee flavoured with cardamom, CR – regular Arabica coffee flavoured with mastic, CRO – regular Arabica coffee flavoured with carob.

Regarding the correlation between the phenolic contents and antioxidant methods, the Pearson coefficients revealed a weak (r < 0.2) or medium (r = 0.3-0.4) correlation, which was not significant (table III). Nevertheless a strong, positive and significant correlation (r = 0.7287, p < 0.001) was found between tannins content and DPPH assay (table III). It is well known that tannins are a class of phenolic compounds, which are highly soluble in polar solvents (including water) and DPPH is a suitable method for evaluation of hydrophilic compounds antioxidant activity (Serrano et al., 2009; Dudonné et al., 2009). As for ABTS'+ assay and the total phenolic content, our results showed a negative correlation (table III), which is plausible since

this method is also suitable for lipophilic compounds (Dudonné et al., 2009). Furthermore, we have also found a positive and significant correlation (r = 0.4673, p < 0.05) between DPPH and ABTS'+ antioxidant assays (table III).

We assume that the weak (or at least medium) correlation between the phenolic contents and antioxidant assays is the consequence of other active substances (alkaloids, glycosidic forms of sterols/diterpens, mineral elements etc.) involvement in the overall antioxidant capacity of coffee samples (Patay et al., 2016; Janissen et al., 2018; Cheng et al., 2016; Şemen et al., 2017).

Table 3
Pearson correlation coefficients (r)

	PCAs	Tannins	DPPH	ABTS'+	Ferric reducing power
Total phenolic content	-0.04251 (p = 0.8758) ^{ns}	-0.1258 (p = 0.6303) ^{ns}	0.3219 (p = 0.1250) ^{ns}	-0.1908 (p = 0.3950) ^{ns}	0.3977 (p = 0.0918) ^{ns}
PCAs		0.2477 (p = 0.3611) ^{ns}	-0.1128 (p = 0.6775) ^{ns}	-0.1810 (p = 0.5024) ^{ns}	0.1225 (p = 0.7360) ^{ns}
Tannins			0.7287 (p = 0.0009) ***	0.2525 (p = 0.3282) ^{ns}	-0.4669 (p = 0.1476) ^{ns}
DPPH				0.4673* (p = 0.0283) *	-0.1840 (p = 0.4647) ^{ns}
ABTS'+					-0.2387 (p = 0.3732) ^{ns}

Legend: * (p < 0.05), *** (p < 0.001), ns – not significant, PCAs – phenolcarboxylic acids.

CONCLUSIONS

The analysed coffee samples are a source of phenolic compounds with antioxidant activity. Among Turkish coffee samples, regular Arabica coffee (simple or flavoured with mastic or carob) have shown the highest total phenolic content and the best *in vitro* antioxidant capacity. The forthcoming research will focus on evaluation of the antioxidant activity by means of other methods, with a higher physiological relevance (such as ORAC, superoxide dismutase method, lipid

peroxidation assay etc). On addition our research will continue with other coffee brands analysis.

AUTHORS CONTRIBUTION

Conceptualization: T. Costea.; Methodology: T. Costea, A. Sevde; Data collection: T. Costea, A. Sevde; Data validation: T. Costea; Data processing: T. Costea; Writing - original draft preparation, T. Costea; writing - review and editing: T. Costea, A. Sevde.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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