ORIGINAL ARTICLE

Antioxidant efficiency of polyphenols from coffee and coffee substitutes-electrochemical versus spectrophotometric approach

Stanislava Gorjanović[1] · Draženka Komes[2] · Jovanka Laličić-Petronijević[3] ·
Ferenc T. Pastor[4] · Ana Belščak-Cvitanović[2] · Mile Veljović[3] · Lato Pezo[1] ·
Desanka Ž. Sužnjević[1]

Abstract Antioxidant (AO) capacity of instant, espresso, filter and Turkish/Greek coffee brews, coffee substitutes (roasted chicory root, barley, pea, chickpea, carob and dried fig) and individual compounds (phenolic acids, flavonoids, methylxanthines, N-methyl pyridinium and HMW melanoidins) was assessed using DC polarographic assay based on decrease of anodic current originating from hydroxo-perhydroxo mercury complex formed in alkaline solutions of H_2O_2 at potential of mercury dissolution, as well as three spectrophotometric assays (DPPH, ABTS and FRAP). A large difference between applied assays ability to recognize various types of individual AOs was noticed. Only according to DC polarographic assay significant AO activity was ascribed to methylxanthines and N-methyl pyridinum. The total content of phenolics (TPC) present in complex samples was determined by FC assay. The highest TPC was ascribed to instant coffees and coffee substitutes while the lowest to decaffeinated filter coffee. Complex samples were grouped based on principal components analysis, phenolics AO coefficient, calculated as the ratio between AO capacity and TPC, and relative AO capacity index (RACI), calculated by assigning equal weight to all applied assays including FC. The highest values of RACI were ascribed to instant coffee brews, followed by substitutes while the lowest to the decaffeinated espresso coffee.

✉ Jovanka Laličić-Petronijević
jovankal@agrif.bg.ac.rs

✉ Lato Pezo
latopezo@yahoo.co.uk

[1] Institute of General and Physical Chemistry,
P. O. Box 45, Belgrade 118 11158, Serbia

[2] Faculty of Food Technology and Biotechnology, University
of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia

[3] Faculty of Agriculture, University of Belgrade, Nemanjina 6,
Belgrade 11081, Serbia

[4] Faculty of Chemistry, University of Belgrade, Studentski Trg
12-16, Belgrade 11001, Serbia

Introduction

Coffee is the third most consumed beverage in the world (Wang and Ho 2009). Antioxidant (AO) capacity of coffee was investigated by mostly using spectrophotometric assays. In comparison to other polyphenolic beverages, coffee possesses superior AO activity (Fukushima et al. 2009; Carlsen et al. 2010). Origin, blending, roasting degree and grinding of coffee beans as well as the brewing influence AO capacity of coffee beverage (Ludwig et al. 2012). In order to imitate coffee and provide caffeine-free beverages without the adverse psychoactive effects of caffeine, certain grains and fruits are roasted and brewed in the same manner as coffee AO capacities of coffee substitutes and their ingredients such as carob (Sahin et al. 2009; Custodio et al. 2011), chicory (Jurgonski et al. 2011), chickpea (Segev et al. 2012), roasted barley (Omwamba and Hu 2010) and fig (Vinson et al. 2005) were reported.

Electrochemical assays were recently applied to determine AO capacity of coffee. Until now, methods based on the cyclic and square wave voltammetry at modified titanium electrodes (El Qouatli et al. 2011), adsorptive transfer stripping voltammetry at a boron-doped diamond electrode (Yardim, 2012), differential pulse voltammetry at multi-walled carbon nanotube-modified glassy carbon electrode

(Ziyatdinova et al. 2013) and carbon paste electrode (Oliveira-Neto et al. 2016) were employed.

Here, dropping mercury electrode has been used for rapid measurement of AO activity of 24 different coffee brews (Turkish, instant, espresso and filter) and 6 coffee substitutes. A direct current (DC) polarographic assay based on the decrease of anodic current originating from hydroxo-perhydroxo mercury complex (HPMC) formed in alkaline solutions of H_2O_2 at potential of mercury dissolution has been applied in parallel with common spectrophotometric AO assays. The AO capacity of coffees and substitutes, as well as individual compounds present (phenolic acids, flavonoids, methylxanthines, N-methylpyridinium (NMP) and HMW melanoidins) has been compared to their scavenging activity against artificial radicals (ABTS and DPPH) and total reducing power (FRAP). Complex samples have been grouped based on relative AO capacity index (RACI) calculated by assigning equal weight to all applied assays, including FC, phenolics AO coefficient (PAC) calculated as the ratio between AO capacity determined by each AO assay and total phenolic content (TPC), and principal components analysis (PCA).

Materials and methods

Chemicals

Folin-Ciocalteu reagent, ammonium peroxodisulphate, sodium carbonate, sodium acetate trihydrate, acetic acid, hydrochloric acid, ferric chloride hexahydrate and ferric sulphate heptahydrateof analytical grade were supplied by Kemika (Zagreb, Croatia). DPPH (2.2-diphenyl-1-picrylhydrazyl) was supplied by Fluka (Buchs, Switzerland) and methanol (HPLC grade) was purchased from J.T.Baker (Deventer, Netherlands). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), TPTZ (2,4,6- tripyridyl-S-triazine), ABTS (2.2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt), and gallic acid (GA) were obtained from Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany).

Hydrogen peroxide, medical grade, 35% (v/v) solution and boric acid, potassium chloride and sodium hydroxide (analytical grade) of Merck were used.

Quercetin, rutin, chlorogenic and caffeic acid, caffeine, theophylline and theobromine were acquired from Sigma (St. Louis, MO, USA). Working standard solutions (2.0 mmol/L) were prepared daily in ethanol or water.

N-Me-Pyridinium iodide was prepared following literature procedure (Carlsson et al. 2012). 1.115 g of pure product was obtained (90%). M.p. 117–119 °C (Et₂O). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.01 (2H, d,

$J = 5.62$ Hz), 8.59 (1H, t, $J = 7.86$ Hz), 8.14 (2H, t, $J = 6.74$ Hz), 4.37 (3H, s). ^{13}C (50 MHz, DMSO-d_6) δ: 145.56 (b), 145.13, 127.72, 48.03. NMR spectra were recorded in DMSO-d_6 on Varian Gemini 2000 (200/50 MHz) instrument. Chemical shifts were referenced with respect to solvent signal. Melting point was determined on Stuart SMPT-10 apparatus in an open capillary tube and was uncorrected. Concentration of working solution was 4 mmol/L.

HMW melanoidins were isolated from coffee brew by 48 h dialysis against distilled water in a cellulose dialysis tubing (cut off 2.4 kDa, Sigma). The contribution of HMW fraction to the total AO capacity of coffee was calculated based on a comparison between the activity of a whole (undialyzed) coffee sample and a dialyzed one.

Coffee and coffee surrogate samples

All coffee samples were commercially available and were purchased from local markets. The samples of Turkish, espresso and filter coffee were obtained in the form of medium roasted coffee beans, while instant coffee samples were in lyophilized form i.e. powder/granules. As for the coffee surrogates, carob and chicory were acquired in local bio-shops in roasted and ground form. Barley, chickpea and pea were purchased raw and roasted in a laboratory oven (DeLonghi EO 12001.W, Italy) at 200 °C for 15 min, while dried fig was further dried at 140 °C for 10 min.

Brews preparation

Turkish coffee samples were ground into a fine powder in an old traditional electric mill (Borac, Serbia) just before brewing. Brewing was conducted using a traditional Turkish coffee pot, prepared with 7 g of ground coffee and 50 mL of cold tap water. The brew was heated until it had foamed twice, allowed to settle for 5 min, and then decanted for analysis. Grinding of surrogate samples and preparation of beverages were conducted in the same manner as for Turkish coffee.

Roasted filter coffee beans were ground in the same mill as Turkish coffee, which was set to coarse grinding, and the samples were prepared by infusion method, employing filter coffee maker (Bartscher Regina, Germany). 7.5 g of ground coffee was used for every 125 mL of water. Extraction took about 5 min at 90 °C.

Roasted beans for espresso coffee were ground in an espresso grinder (LA Cimbali, model Cadet, Italy). Brews were made by pressure method in an espresso machine (LA Cimbali, M29 Select, Italy), using 6.5 g of ground coffee and hot water (T = 90 °C, p = 9 bar), for volume of 40 mL.

Instant coffee beverages were made by pouring 200 mL of hot water over 6 g of instant coffee and stirred until dissolved.

Antioxidant capacity by DC polarographic assay

Measurements were performed using Polarographic Analyzer PAR (Princeton Applied Research) model 174A coupled with X–Y recorder (Houston Instruments, Omnigraphic 2000). Three-electrode electrolytic cell was used. The cell volume was 30 mL. A dropping mercury electrode (DME) was the working electrode. Capillary constant of DME was $m = 2.5$ mg s^{-1} at mercury reservoir height of 75 cm. A programmed drop time of DME was 1 s. Current oscillations of DME were filtered with low pass filter of instrument positioned at 3 s. The saturated calomel electrode (SCE) and the platinum foil were used as the reference and the counter electrode, respectively.

The supporting electrolyte used was Clarc and Lubs (CL) buffer (pH 9.8), prepared by mixing 25 mL of 0.4 M H_3BO_3, 25 mL of 0.4 M KCl and 40.8 mL of 0.2 M NaOH. The volume of the supporting electrolyte in the cell was 19.9 mL. 0.100 mL of 1.00 M hydrogen peroxide was directly added in supporting electrolyte. The initial concentration of H_2O_2 was 5.0 mmol/L. The polarographic current–potential (i–E) curves with, or without, the analysed extracts were recorded starting from 0.1 V versus SCE towards negative potentials, with a sweep rate of 10 mV/s. In order to remove dissolved oxygen, the supporting electrolyte in the electrolytic cell was purged with pure nitrogen (>99.995%, Messer, Serbia) for 2 min before H_2O_2 addition, and 30 s after addition of each samples. The atmosphere above the cell solution was kept inert during polarographic curve recording by a continuous flow of nitrogen.

Brews were gradually added into the cell solution in aliquots of 50 µL. Instant coffee brews were diluted 5 times. Gradual addition of brews into the buffered H_2O_2 solution caused uniform decrease of initial anodic limiting current, i_{p0}. The relative decrease of i_{p0} upon each addition (Δi_p) was calculated according to the following equation:

$$\Delta i_P(\%) = \left(1 - \frac{i_p}{i_{p0}}\right) \times 100$$

where: Δi_p (%) represents a relative decrease of i_{p0} upon addition of brews, while i_p is the remaining part of i_{p0} after sample addition. Percentage of decrease was plotted versus volume of samples added. The slope of the linear part of obtained plots was used as a measure of AO capacity.

DPPH radical scavenging

The antioxidant capacity of the coffee and coffee substitute brews was determined using the DPPH radical scavenging assay (Brand-Williams et al. 1995), with some modifications. Antioxidant capacity was expressed as mmol Trolox equivalents (TE)/L, using the calibration curve of Trolox (0–1000 µM), a water soluble vitamin E analogue.

ABTS radical cation

The Trolox equivalent antioxidant capacity (TEAC) was estimated by the ABTS radical cation decolorization assay (Re et al. 1999). The results, obtained from triplicate analyses, were expressed as TE and derived from a calibration curve determined for this standard (100–1000 µM).

Ferric reducing/antioxidant power (FRAP)

The ferric reducing/antioxidant power (FRAP) assay was carried out according to standard procedure (Benzie and Strain 1996).All measurements were performed in triplicate. Aqueous solutions of $FeSO_4 \times 7H_2O$ (100–1000 µM) were used for the calibration and the results expressed as mmol Fe(II)/L, while results for individual samples as mmol TE/mol.

Relative antioxidant capacity index (RACI)

A standard score was calculated according to the following equation:

$$\text{Standard score} = (x - \mu)/\sigma$$

where: x was the raw data, μ was the mean, and σ was the standard deviation The standard scores of a sample for different assays when averaged gave a single unitless value, termed as RACI.

Comparison of results and statistical evaluation

Descriptive statistical analyses for calculating the means and the standard error of the mean were performed using Microsoft Excel 2007 software. The results were correlated separately for coffee brews and coffee substitute brews using regression analysis and statistically evaluated using analysis of variance (ANOVA), Brown–Forsythe test and PCA (Principal Components Analysis). Post-hoc Tukey's HSD test was calculated to confirm statistically significant differences between different samples. Accuracy of DC polarographic assay in comparison with other assays applied in parallel was tested based on coefficients of variation.

Results and discussion

Antioxidant capacity of coffees and coffee substitutes' brews

In order to obtain information regarding the AO activity of coffee brews (Turkish/Greek, instant, espresso and filter) and coffee substitutes (roasted chicory root, barley, pea, chickpea, carob and dried fig), multiple AO assays were performed. AO capacity of coffee and coffee substitute ingredients measured by the DC polarographic (HPMC) assay, DPPH and ABTS scavenging activity, FRAP and TPC are shown in Table 1. Results have been given following descending order of AO capacity determined by DC polarography.

No significant difference between the results of the DC polarographic (HPMC) assay and FC, FRAP, ABTS and DPPH assays were observed. Results of spectrophotometric assays have been found similar to DC polarographic ones at $p < 0.01$ significance level ($F_{FC} = 13741$, $F_{FRAP} = 9012$, $F_{ABTS} = 6517$, and $F_{DPPH} = 41113$, with Fcrit = 2.059). FC has been found to be the most influential variable for the final AO result, while HPMC has been found more influential than FRAP, ABTS and DPPH assay. However, according to Brown–Forsythe test of homogeneity of variances, DC polarographic assay was more influential compared to spectrophotometric AO assays.

Considered coffees represent a rich source of phenolic compounds. The highest TPC was observed for instant coffees and coffee substitutes. Coffee substitutes were better source of phenolics than Turkish, espresso and filter coffees. The effect of the brewing method on TPC of differently prepared coffee brews has been found in accordance with previous results (Hečimović et al. 2011; Niseteo et al. 2012). Decaffeinated instant coffees exhibited lower TPC (up to 7.727 g GAE/L) than their regular coffee counterparts (up to 9.614 g GAE/L). The lowest TPC among 24 coffee samples has been ascribed to decaffeinated filter coffee.

Coffee brew AO capacity determined by DC polarography has been found superior in comparison to substitutes' brews. Amongst the substitutes, the highest content of TPC has been found in carob. This substitute possesses superior AO activity according to all assays applied. The high AO activity of carob observed previously was explained by the presence of catechin and gallic acid (Custodio et al. 2011). Increasing of AO activity with roasting temperature and duration was reported earlier (Sahin et al. 2009). This increase suggested significant contribution of melanoidins. Roasted barley has been found to be the second substitute according to both TPC and AO activity determined by all four AO assays. HMW melanoidinic component was found to be a prevalent contributor to its AO activity (Papetti et al. 2006). Increase of AO activity of chickpea (*Cicer arietinum* L.) during roasting was also related to Maillard reaction products formation (Segev et al. 2012). The dried and roasted root of chicory (*Cichorium intybus* L.) contains both phenolic AOs and melanoidins (Jurgonski et al. 2011). Dried fig (*Ficus carica L*) has been considered a rich source of AOs (Vinson et al. 2005).

Relative antioxidant capacity index and phenolics antioxidant coefficients

In order to get better insight into differences of samples' AO capacity, relative antioxidant capacity index (RACI) has been calculated by assigning equal weight to AO assays applied, including FC as a measure of total reducing activity. Also, phenolic AO coefficients (PAC) have been calculated as the ratio between particular AO activity and total phenolic content (expressed in g GAE/L). As seen in Fig. 1, the highest values of RACI have been ascribed to instant coffee brews, followed by substitutes. The lowest values among instant coffees have been ascribed to decaffeinated samples. On the other side, decaffeinated instant and espresso coffee possess the highest PAC_{HPMC} in comparison to regular coffee. Positive values of RACI have been ascribed only to instant coffees and substitutes (1.27–1.73), while all other samples of coffees have negative RACI (from −1.03 to −0.36). The lowest value of RACI belongs to the decaffeinated espresso coffee (−1.03). RACI ascribed to substitutes have been found lower in comparison to instant coffees, while much higher than Turkish, espresso and filter coffee brews. Chickpea and chicory have the highest PAC_{HPMC} among coffee substitutes. Descending order of HPMC has been found in good corroboration with RACI for all three recognized groups of samples, particularly for substitutes.

PAC values calculated based on DC polarographic and spectrophotometric assays were found to be significantly different. Possibility to group samples based on PAC_{ABTS} and PAC_{FRAP} has not been observed. According to PAC_{FRAP} and PAC_{ABTS} similar level of phenolics efficiency for coffees and coffee substitutes has been noticed. According to PAC_{HPMC}, instant coffees can be easily recognized as a specific group of samples. According to PAC_{HPMC}, the efficiency of phenolics present in substitutes has been found the lowest while according to PAC_{DPPH} the highest amongst analysed samples. An almost equal value of RACI has been obtained for carob and decaffeinated instant coffee, while a large difference in their PAC_{HPMC} has been found.

Table 1 Total phenolic content (TPC) and AO activity of coffees and coffee substitutes brews as determined using DC polarographic (HPMC), DPPH, FRAP and ABTS assays

Sample no.	HPMC (%/mL)	TPC (g GAE/L)	DPPH (mM TE/L)	FRAP (mM Fe(II)/L)	ABTS (mM TE/L)
Coffees					
1. Instant	2391 ± 94f	9.614 ± 0.205o	12.66 ± 0.57n	91.88 ± 0.70l	44.73 ± 0.42o
2. Instant	2300 ± 100ef	9.386 ± 0.114n	13.24 ± 0.06o	94.13 ± 0.61m	47.41 ± 0.33q
3. Instant	2247 ± 114e	9.159 ± 0.023m	10.58 ± 0.47l	92.26 ± 0.42lm	46.50 ± 0.40p
4. Instant*	2229 ± 42e	7.727 ± 0.000l	10.75 ± 0.23l	76.39 ± 0.28j	37.51 ± 0.42m
5. Instant*	2197 ± 127e	7.182 ± 0.227k	11.57 ± 0.15m	83.69 ± 0.56k	40.56 ± 0.62n
6. Turkish	635 ± 36d	3.523 ± 0.023ef	6.40 ± 0.15j	43.20 ± 0.33i	21.40 ± 0.46kl
7. Turkish	618 ± 38cd	3.795 ± 0.114g	6.86 ± 0.54k	44.51 ± 0.51i	20.71 ± 0.26jk
8. Espresso	603 ± 22cd	4.432 ± 0.205j	7.03 ± 0.54k	42.64 ± 0.80i	20.12 ± 0.40j
9. Turkish	598 ± 19cd	4.000 ± 0.182h	6.99 ± 0.06k	40.10 ± 0.27h	22.15 ± 0.52l
10. Espresso	589 ± 30cd	3.614 ± 0.023ef	6.43 ± 0.32j	36.18 ± 0.61g	18.08 ± 0.33gh
11. Turkish	578 ± 20cd	4.227 ± 0.045i	5.60 ± 0.27gh	32.81 ± 0.70f	19.10 ± 0.52i
12. Turkish	571 ± 29cd	3.795 ± 0.023g	5.73 ± 0.26h	38.05 ± 0.33gh	17.66 ± 0.47fg
13. Filter	551 ± 26bcd	3.500 ± 0.0 91ef	4.91 ± 0.31d	36.60 ± 2.34g	15.62 ± 0.33c
14. Turkish	547 ± 14bcd	3.591 ± 0.455ef	5.44 ± 0.12fg	29.96 ± 0.19e	18.25 ± 0.20gh
15. Turkish	543 ± 17bcd	3.295 ± 0.295cd	6.01 ± 0.13i	36.28 ± 0.70g	16.69 ± 0.13de
16. Filter	542 ± 32bcd	3.205 ± 0.295bc	6.89 ± 0.15k	31.36 ± 0.28ef	18.35 ± 0.20ghi
17. Turkish	538 ± 25bcd	3.455 ± 0.364de	3.72 ± 0.46b	24.12 ± 0.23c	18.57 ± 0.67hi
18. Filter	527 ± 32bcd	3.182 ± 0.182bc	3.99 ± 0.11c	27.12 ± 0.92d	16.53 ± 0.26de
19. Turkish	525 ± 39bcd	3.205 ± 0.068bc	5.19 ± 0.39e	30.27 ± 0.45e	16.91 ± 0.20ef
20. Turkish	498 ± 29bcd	3.636 ± 0.045fg	2.58 ± 0.12a	20.75 ± 0.49b	8.56 ± 0.40a
21. Turkish	494 ± 16bc	3.545 ± 0.182ef	5.72 ± 0.23h	37.17 ± 0.75g	16.10 ± 0.72cd
22. Turkish	483 ± 13bc	3.455 ± 0.045de	5.32 ± 0.25ef	33.00 ± 0.61f	17.60 ± 0.20fg
23. Turkish	413 ± 22a	3.068 ± 0.114b	5.80 ± 0.13hi	32.89 ± 0.27f	15.73 ± 0.35c
24. Filter*	401 ± 16a	2.295 ± 0.114a	4.20 ± 0.25c	16.15 ± 0.23a	10.97 ± 0.27b
Ingredients for coffee substitutes					
1. Carob	517 ± 42f	7.827 ± 0.091f	44.82 ± 3.59g	73.52 ± 3.67f	37.58 ± 0.39g
2. Barley	457 ± 24e	6.609 ± 0.036d	36.96 ± 0.63f	66.65 ± 0.28d	29.36 ± 0.99e
3. Chickpea	437 ± 15d	5.382 ± 0.039b	34.14 ± 1.85d	62.87 ± 6.89c	25.77 ± 1.15c
4. Chicory	432 ± 8c	5.309 ± 0.040a	31.07 ± 1.21b	51.58 ± 7.06b	23.88 ± 2.26b
5. Pea	395 ± 15b	5.091 ± 0.037c	31.29 ± 0.73c	50.24 ± 0.65a	23.33 ± 0.12a
6. Fig	367 ± 10a	5.291 ± 0.020a	29.01 ± 0.40a	50.05 ± 3.09a	27.39 ± 1.07d
CV	6.74	7.33	6.36	7.65	9.27

Data represent the means of a triplicate experiment ± standard deviation

CV coefficient of variation

* Decaffeinated samples

a–m, Values with the same letter in column, are not statistically different at the $p < 0.05$ level, 95% confidence limit, according to Tukey's HSD test

Correlations between applied assays

Results of all applied assays and RACI have been correlated (Table 2). Coffees HPMC show good agreement with FC, FRAP, ABTS and DPPH. Low correlations have been obtained between HPMC and other AO assays and FC for coffee substitues.

Principal component analysis

The PCA allows detection of structure in the relationship between measured parameters and different varieties of tested brews that give complimentary information. For visualizing the data trends and the discriminating efficiency of the used descriptors a scatter plot of samples using the
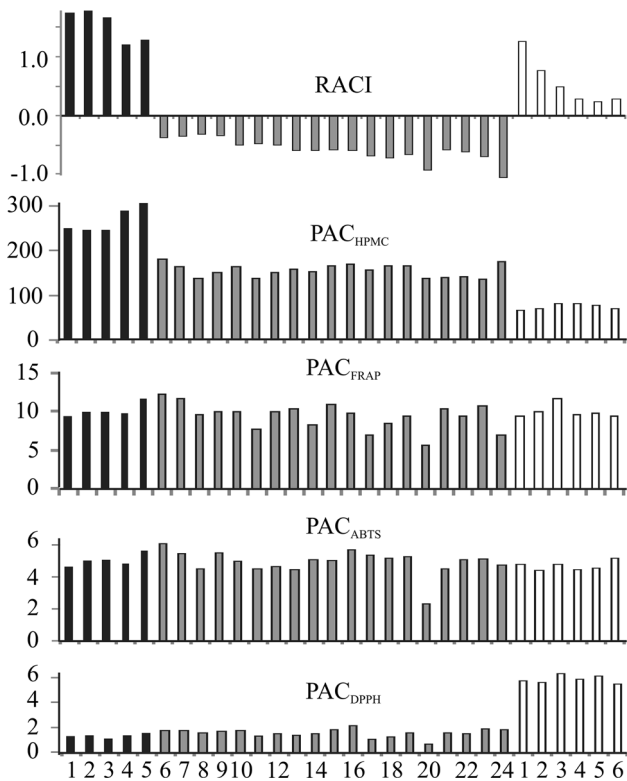
Fig. 1 Relative antioxidant capacity index (RACI) and phenolic antioxidant coefficients (PAC) for coffee and coffee substitute brews (*black bar*—instant coffees, *grey bar*—Turkish, filter and espresso coffees, *white bar*—substitutes)

Table 2 Correlation coefficients between HPMC and FC GAE, FRAP, ABTS and DPPH, as well as with RACI for coffee and coffee surrogates brews (at 95% confidence limit)

	HPMC	FRAP	ABTS	DPPH	RACI
Coffee brews					
FC GAE	0.969	0.969	0.970	0.920	0.987
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
RACI	0.984	0.992	0.991	0.957	
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	
DPPH	0.918	0.965	0.956		
	$p < 0.01$	$p < 0.01$	$p < 0.01$		
ABTS	0.961	0.982			
	$p < 0.01$	$p < 0.01$			
FRAP	0.961				
	$p < 0.01$				
Coffee substitutes brews					
FC GAE	0.885	0.896	0.957	0.957	0.975
	$p = 0.02$	$p = 0.02$	$p < 0.01$	$p < 0.01$	$p < 0.01$
RACI	0.922	0.963	0.938	0.987	
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	
DPPH	0.950	0.949	0.894		
	$p < 0.01$	$p < 0.01$	$p = 0.02$		
ABTS	0.773	0.834			
	$p = 0.08$	$p = 0.04$			
FRAP	0.910				
	$p < 0.01$				

first two principal components (PCs) issued from PCA of the data matrix is obtained (Fig. 2). FC (24.7%), FRAP (24.9%) and ABTS (24.8%) are the dominant variables in the first, while the DPPH (70.3%) and HPMC (29.5) in the second principle component. The first two principal components (98.27% of the total variability) are sufficient for data representation. Coffee substitutes are most pronounced in DPPH values, while instant coffees (samples 1–5) have the highest values of HPMC. Coffee samples no. 6 to 24 have correlated well with FC, FRAP and ABTS.

AO capacity of individual coffee constituents

In order to achieve a better understanding of variations in the total AO activity of brews, AO activity of individual compounds present in non roasted (NRC) (phenolic acids, flavonoids and methylxanthines) as well as melanoidins and N-methyl pyridinum present in roasted coffee (RC) has been determined (Table 3). A large difference between applied assays ability to recognize various types of AOs has been noticed. According to DC polarographic assay, significant AO activity has been ascribed to methylxanthines and N-methyl pyridinum, while according to
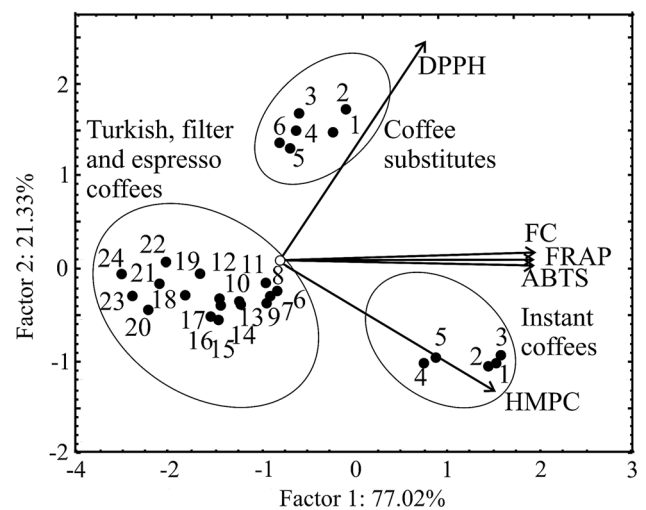

Fig. 2 Biplot for AO activity of coffee and coffee substitutes brews

spectrophotometric assays these compounds show no AO activity.

In conclusion, high accuracy of the DC polarographic assay was clearly shown by correlation analysis, ANOVA and F-test, as well as Brown–Forsythe's test. The accuracy

Table 3 Individual compounds AO activity as determined by DC polarographic (HPMC), DPPH, ABST and FRAP assays

	DPPH (mM TE/mol)	FRAP (mM TE/mol)	ABTS (mM TE/mol)	HPMC (%/μmol)
Chlorogenic acid	1430 ± 5	1805 ± 50	3190 ± 31	14.96 ± 0.45
Caffeic acid	1070 ± 33	1180 ± 23	1515 ± 35	12.65 ± 0.75
Caffeine	12 ± 3	0	0	4.35 ± 0.23
Theophylline	17 ± 15	30 ± 10	8 ± 8	22.30 ± 0.95
Theobromine	27 ± 3	33 ± 11	12 ± 6	15.49 ± 0.68
N-methylpyridinium	50 ± 0	8 ± 3	28 ± 5	7.78 ± 0.50
Catechin	1402 ± 17	1903 ± 42	2169 ± 65	47.57 ± 3.28
Quercetin	3088 ± 36	4188 ± 72	5410 ± 59	37.37 ± 5.24
Rutin	2432 ± 99	2772 ± 102	4930 ± 46	24.65 ± 2.16

The data represents the means of a triplicate experiment ± standard deviation

of the assay was expressed by its low coefficient of variation. In comparison to spectrophotometric assays, DC polarographic assay enabled a specific insight into the AO activity of complex and individual samples analysed within the scope of this study. In contrast to negligible scavenging activity against DPPH and ABTS, as well as reducing power (FRAP), substantial AO capacity of physiologically active compounds (methylxanthines and N-methyl pyridinum) present in complex samples was established using the DC polarographic (HPMC) assay.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

References

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of free radical method to evaluate antioxidant activity. LWT 28:25–30

Carlsen MH, Halvorsen BL, Holte K, Bohn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, Barikmo I, Berhe N, Willett WC, Phillips KM, Jacobs DR, Blomhoff R (2010) The total antioxidant content of more than 3100 foods beverages spices herbs and supplements used worldwide. Nutr J 9:3

Carlsson ACC, Grafenstein J, Budnjo A, Laurila JL, Bergquist J, Karim A, Kleinmaier R, Brath U, Erdelyi M (2012) Symmetric halogen bonding is preferred in solution. J Am Chem Soc 134:5706–5715

Custodio L, Fernandes E, Escapa AL, Fajardo A, Aligue R, Albericio F, Neng NR, Nogueira JMF, Romano A (2011) Antioxidant and cytotoxic activities of carob tree fruit pulps are strongly influenced by gender and cultivar. J Agric Food Chem 59:7005–7012

El Qouatli SE, Rosie NT, Valery HG, Oubenamar H, Hissou H, Najih R, Chtaini A (2011) Electrochemical analysis of the antioxidant capacity of coffee. Bulletin of the Catalysis Society of India 10(3):15–20

Fukushima Y, Ohie T, Yonekawa Y, Yonemoto K, Aizawa H, Mori Y, Watanabe M, Takeuchi M, Hasegawa M, Taguchi C, Kondo K (2009) Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. J Agric Food Chem 57:1253–1259

Hečimović I, Belščak-Cvitanović A, Horžić D, Komes D (2011) Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chem 129:991–1000

Jurgonski A, Milala J, Juskiewicz J, Zdunczyk Z, Krol B (2011) Composition of chicory root peel seed and leaf ethanol extracts and biological properties of their non-inulin fractions. Food Technol Biotech 49:40–47

Ludwig IA, Sanchez L, Caemmerer B, Kroh LW, Paz de Pena M, Cid C (2012) Extraction of coffee antioxidants: impact of brewing time and method. Food Res Int 48:57–64

Niseteo T, Komes D, Belščak-Cvitanović A, Horžić D, Budeč M (2012) Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition. Food Chem 134:1870–1877

Oliveira-Neto JR, Garcia Rezende S, de Fatima Reis C, Benjamin SR, Rocha MV, de Souza Gil E (2016) Electrochemical behavior and determination of major phenolic antioxidants in selected coffee samples. Food Chem 190:506–512

Omwamba M, Hu Q (2010) Antioxidant activity in barley (*Hordeum Vulgare* L) grains roasted in a microwave oven under conditions optimized using response surface methodology. J Food Sci 75:C66–C73

Papetti A, Daglia M, Aceti C, Quaglia M, Gregotti C, Gazzani G (2006) Isolation of an in vitro and ex vivo antiradical melanoidin from roasted barley. J Agric Food Chem 54:1209–1216

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Bio Med 26:1231–1237

Sahin H, Topuz A, Pischetsrieder M, Ozdemir F (2009) Effect of roasting process on phenolic antioxidant and browning properties of carob powder. Eur Food Res Technol 230:155–161

Segev A, Badani H, Galili L, Hovav R, Kapulnik Y, Shomer I, Galili S (2012) Effects of baking roasting and frying on total

polyphenols and antioxidant activity in colored chickpea seeds. Food Nutr Sci 3:369–376

Vinson JA, Zubik L, Bose P, Samman N, Proch J (2005) Dried fruits: excellent in vitro and in vivo antioxidants. J Am Coll Nutr 24:44–50

Wang Y, Ho CT (2009) Polyphenolic chemistry of tea and coffee: a century of progress. J Agric Food Chem 57:8109–8114

Yardim Y (2012) Electrochemical behavior of chlorogenic acid at a boron-doped diamond electrode and estimation of the antioxidant capacity in the coffee samples based on its oxidation peak. J Food Sci 77:C408–C413

Ziyatdinova G, Aytuganova I, Nizamova A, Budnikov H (2013) Differential pulse voltammetric assay of coffee antioxidant capacity with MWNT-modified electrode food. Anal Method 6:1629–1638