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Usage of Capillary Isotachophoresis and Antioxidant Capacity Measurement in Analysis of Changes in Coffee Properties After Roasting, Steaming and Decaffeination

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Abstract The aim of the study was to optimise and validate the method for quantitation of short-chained organic acids in coffee brews by capillary isotachophoresis (ITP). The linearity of the method was satisfactory with R^2 from 0.9924 for lactic acid to 0.9998 for acetic acid. The limit of detection (LOD) was from 4.9 μmol L^{-1} for acetic acid to 24.8 μmol L^{-1} for lactic acid. Precision of the method was from 0.20 to 2.69 %. This method was successfully applied to determine six organic acids (tartaric, formic, citric, malic, lactic and quinic) in Arabica and Robusta green and roasted coffee brews. The roasting as well as steaming and decaffeination processes of beans influenced degradation of acids (citric and malic) and their formation (quinic, tartaric, lactic and formic) in coffee brews. The influence of coffee processing on the antioxidant capacity of coffee brews was also tested by using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu and chelating Fe(II) assays. The roasted coffee brews possessed higher antioxidant capacity than unprocessed coffee brews, excluding chelating activity.

Keywords Capillary isotachophoresis · ABTS assay · DPPH assay · Coffee brew · Chelating activity · Folin-Ciocalteu assay

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Introduction

The unique aroma and flavour of coffee make it one of the most popular beverages all over the world. Species of *Coffea* genus *Coffea arabica* and *Coffea canephora* var. robusta belong to Rubiaceae family. Arabica usually comes from Brazil and other Southern American countries, but it also comes from the uplands and mountain areas of East Africa—Kenya or Congo. The main places of Robusta origin are Vietnam and the lowlands of Central and West Africa as well as South Asia and South America (ICO 2013).

Green coffee brews contain the complex of antioxidants, mainly chlorogenic acids (caffeoylquinic acids), and they show antioxidant properties in vitro (Jeszka-Skowron et al. 2016a, b). Green coffee extracts show a hypotensive effect on rats (Igho et al. 2011), reduce visceral fat and body weight (Shimoda et al. 2006; Suzuki et al. 2002), and chlorogenic acid (5-caffeoylquinic acid) can prevent from neurodegenerative diseases such as ischemic stroke (Mikami and Yamazawa 2015). Coffee brew including this from roasted beans also contains chlorogenic acids and caffeine and other bioactive compounds with antioxidant capacity such as theophylline and theobromine, tocopherols, cafestol, kahweol and trigonelline as well as products of Maillard reaction (Budryn and Nebesny 2008; Jeszka-Skowron et al. 2015; Perrone et al. 2008).

The short-chain organic acids are also found in coffee brews. Their presence imparts the taste and flavour of the beverages such as tea or coffee. These acids can be determined by using chromatographic techniques mainly gas chromatography and liquid chromatography (Jeszka-Skowron and Zgoła-Grześkowiak 2014; Jham et al. 2002; Kampmann and Maier 1982; Maiser and Engelhardt 1985; Rodrigues et al. 2007). GC technique is very tedious and not eco-friendly due to complicated sample preparation causing significant

losses of some organic acids after purification (Jham et al. 2002; Kampmann and Maier 1982). On the other hand, HPLC and CE techniques were found to be simple and faster than GC and were successfully used to determine organic acids with good separation (Galli and Barbas 2004; Jham et al. 2002). Capillary isotachophoresis (ITP) seems to be a good alternative; however, only some of these acids were so far determined by ITP (Kvasnicka 2000; Maiser and Engelhardt 1985; Soltze and Mayer 1982). This technique is quick and inexpensive, and it fits to green chemistry trend due to usage of eco-friendly solvents.

The main goals of the study were to compare the content of short-chain organic acids and antioxidant capacity of green and roasted coffee brews (Arabica and Robusta) depending on the place of origin and method of bean preparation (decaffeinated, steamed and roasted Vietnam coffee beans). Short-chained organic acids were determined with the use of isotachophoresis analysis including the optimisation and validation of the method. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, and chelating activity assays were used to evaluate the antioxidant properties of coffee brews.

Materials and Methods

Reagents and Standard Solutions

ABTS, DPPH, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt (ferrozine), Folin-Ciocalteu reagent and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). The short-chained acids (acetic, ascorbic, aspartic, citric, formic, gallic, glutamic, lactic, malic, oxalic, succinic, propionic, quinic and tartaric) were obtained from the same supplier. MS-grade acetonitrile was from POCH (Gliwice, Poland), and MS-grade formic acid was from Sigma-Aldrich. Water was prepared by reverse osmosis in a Demiwa system from Watek (Ledec nad Sazavou, Czech Republic), followed by double distillation from a quartz apparatus. Potassium persulfate (di-potassium peroxdisulfate), hydroxyethylcellulose (HEC), Triton X-100, poly(vinyl alcohol) (PVA) and Mohr's salt were obtained from POCH.

Sixteen green and roasted coffee bean samples from different regions of the world were provided by Strauss Café Poland (Swadzim/Poznań, Poland): Robusta beans from Vietnam (green and roasted bean Gr2, decaffeinated green and roasted Gr2, steamed green and roasted Gr2) and Arabica coffee beans: Brazil (TG), Congo, Guatemala (SGH), Honduras (HG) and Peru (HB). The roasting process of green coffee beans was performed at temperature 170–190 °C in 8 min (Jeszka-Skowron et al. 2016a).

Extraction Process

Briefly, 0.5 g of milled beans of coffee was extracted by 50 mL of distilled water at temperature 95 °C. Then, the solution was cooled to room temperature and filtered through 0.45-μm polytetrafluoroethylene syringe filter from Agilent Technologies (Santa Clara, CA, USA) and finally diluted to proper volume with distilled water. The coffee solution was prepared before analysis, and pH value of each sample was immediately measured.

Equipment

Isotachophoretic separations were performed by using the Electrophoretic Analyser EA 100 (Villa Labeco, Slovak Republic) equipped with a column coupling system consisting of two capillaries made of fluorinated ethylene propylene copolymer. The first preseparation capillary (160 × 0.8 mm ID) was connected to the analytical capillary (160 × 0.3 mm ID) via the bifurcation block. The analyser was equipped with a sample valve of 30 μL fixed volume, and conductivity detectors placed on both columns 40 mm from the outlet ends. Separations were performed at ambient temperature. The isotachopherograms were evaluated by a personal computer software package supplied with the analyser.

The leading electrolyte was 10 mmol L^{-1} hydrochloric acid containing 1 % Triton X-100 and adjusted with β-alanine to pH 3.0. The terminating electrolyte was 5 mmol L^{-1} propionic acid (pH 3.5). The driving current in the preseparation capillary was 250 μA. The initial driving current in the analytical capillary was 50 μA.

All spectrophotometric determinations connected with antioxidant capacity were performed with the use of Beckman UV-Vis Spectrophotometer 7500DU (Brea, CA, USA) with glass cuvettes of 1-cm optical length. Spectra were recorded in the range from 380 to 800 nm. All determinations were carried out in triplicate.

Quantification and Validation

Qualitative information in the isotachopherogram was obtained from the relative step heights (RSHs—counted as the ratio of the step height of the analyte to the step height of the terminator), while the zone lengths give quantitative information. The concentrations can be calculated by comparing the step length of a compound with the calibration curve of standard solutions. Calibration curves were plotted by using zone length responses of individual standards against six different concentration levels (0.025, 0.05, 0.08, 0.1, 0.2, 0.4 mmol L^{-1}). The method performance was validated in

terms of linearity, limits of detection (LOD) and quantification (LOQ) and precision according to the US Food and Drug Administration Guideline (FDA 1996).

Antioxidant Capacity of Coffee Brews

The ability of coffee brews to scavenge ABTS radicals was studied according to the method of Re et al. (1999) and modified by Jeszka-Skowron et al. (2016b). The results were expressed as millimole of Trolox per litre of the brew.

The ability of coffee brews to scavenge DPPH radicals was determined according to the method of Jeszka-Skowron and Zgoła-Grześkowiak (2014). The absorbance of samples was measured at 516 nm. The results were expressed as millimole of Trolox per litre of the brew.

Reducing ability of coffee brews was analysed by using Folin-Ciocalteu's reagent according to the method of Everette et al. (2010) modified by Jeszka-Skowron et al. (2016b). The absorbance of samples against a reagent blank was measured at 754 nm. The results were expressed as millimole of gallic acid equivalent (GAE) per litre of the brew.

Chelating activity of coffee brews was analysed according to the method of Tang et al. (2002). The absorbance of samples (without further dilution) against a reagent blank was measured at 562 nm after 20 min of incubation. The ability of coffee brews to chelate ferrous ions was calculated as chelating activity (CA) in %:

$$\mathrm{CA\%} = \left(1 - A_{\mathrm{sample}} / A_{\mathrm{control}}\right) \times 100$$

Statistical Analysis

Results were 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 %. The experimental data were analysed by using Statistica 12.5 program.

Results and Discussion

Optimisation and Validation of ITP Method

To optimise the most efficient isotachophoretic separation of 12 organic acids, 3 different anticonvection substances were tested in the leading buffer. HEC and PVP in 0.05 and 0.1 % concentrations did not enable to separate acids with very similar pKa: citric and malic acids, as well as glutamic, ascorbic and acetic acids. To separate these acids, 0.1 % of Triton X-100 was chosen (Fig. 1a).

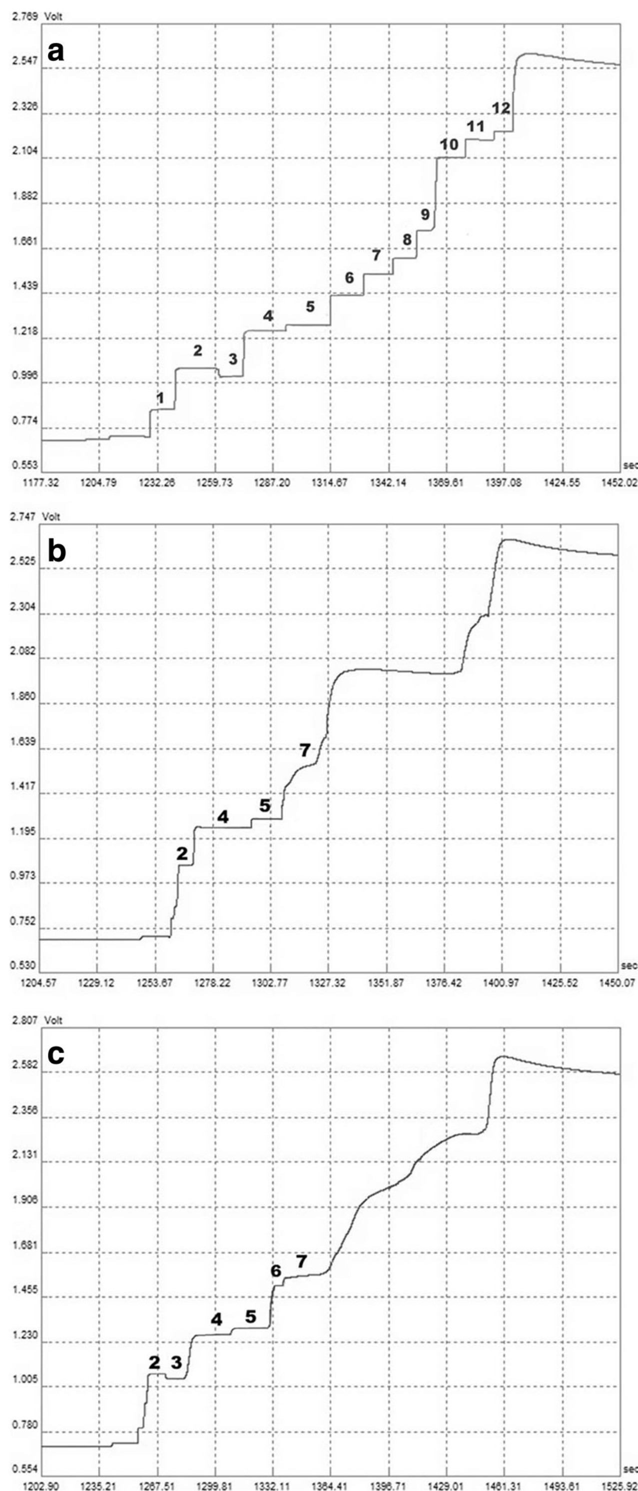

Fig. 1 The isotachopherograms of organic acids: **a** mix of 12 standard compounds, **b** coffee brew from green beans (Brazil, Arabica) and **c** coffee brew from roasted beans (Brazil, Arabica). Oxalic acid (*1*), tartaric acid (*2*), formic acid (*3*), citric acid (*4*), malic acid (*5*), lactic acid (*6*), quinic acid (*7*), aspartic acid (*8*), succinic acid (*9*), glutamic acid (*10*), ascorbic acid (*11*) and acetic acid (*12*)

The method performance was validated in terms of linearity (correlation coefficient—R^2), LOD and LOQ and precision

as a RSH of six different measurements (Table 1). LOD was determined as 3.3 $\Delta b/a$ and LOQ as 10 $\Delta b/a$ (FDA 1996) where a is the slope of the regression line and Δb is the standard error of the y-intercept of the regression line.

Isotachophoretic Analysis

The short-chain organic acids were analysed with the use of isotachophoretic analysis. The method was found to be useful for analysis of selected organic acids in coffee samples (Fig. 1b, c).

The major organic acid in green Arabica and Robusta coffee brews was citric acid (80.3–119.7 mg L^{-1}) (Table 2). However, the major acid in all roasted coffees was quinic acid quantified from 107.4 to 166 mg L^{-1}. Quinic acid as a part of chlorogenic acids in coffee brew was determined at 2–4-fold higher level in brews made from roasted beans (also decaff. and steamed beans) than from green coffee beans. The roasting of coffee beans influenced tartaric acid concentration which was from 0.3 to 3-fold higher in roasted coffee beans. The highest level of tartaric acid was determined in Vietnam Robusta roasted coffee—87.3 mg L^{-1}. On the other hand, decaffeination process or steaming had little impact on the formation of tartaric acid in coffee brews. Formic acid was determined in all roasted coffee brews also in steamed coffee, especially in Arabica coffee brews (17–38.4 mg L^{-1}). The roasting process had an impact on the formation of lactic acid in Arabica and Robusta coffees. The concentration of malic acid was about 5–10 % lower after roasting process in both Robusta and Arabica coffees.

The degradation of the citric and/or malic acids and the formation of lactic and formic acids in roasted coffee beans were reported previously (Galli and Barbas 2004; Rodrigues et al. 2007). The main organic acids in Columbian roasted coffees were citric and quinic acid (Ginz et al. 2000) as well as in *C. arabica* immature beans and cherry beans (Jham et al. 2002). In other reports, the content of acetic, citric and succinic acids in brewed coffee samples was the highest and decreased with longer roasting of coffee beans (Rodrigues et al. 2007). According to presented results, also malic acid was determined in brewed coffee samples at relatively low levels—below 6 mg L^{-1} of brew (Rodrigues et al. 2007). There were no reports about the determination of tartaric acid as the main acid in roasted coffee brews.

Another important parameter of coffee is its acidity influencing the taste. pH of coffee brew was lower for roasted coffee brews as well as steamed coffee brews than green coffee brews (Table 2). Roasting process has an impact on analysed acids especially in higher level of quinic, tartaric, formic and lactic acids in coffees—even 2-fold higher than in green coffees. On the other hand, this process affects the polyphenols, especially chlorogenic acids which undergo chemical modification or degradation during roasting of beans, and more than 60 % of chlorogenic acid present in green coffee is degraded upon roasting (Nebesny and Budryn 2003). The degree of roasting also influences pH of coffee brew, especially in Arabica coffee samples (Daglia et al. 2000).

Antioxidant Capacity of Green and Roasted Coffee Brews

Antioxidant capacity of coffee brews was analysed with the use of ABTS and DPPH in in vitro radical scavenging assays and is presented in Table 3. These methods are usually used

Table 1 Parameters of merit

Acid	pK_a	RSH	CV (%)	Slope of the regression line (a)	y-intercept	R^2	LOD[a] [μmol L^{-1}]	LOQ[a] [μmol L^{-1}]	LOD [mg L^{-1}]	LOQ [mg L^{-1}]	Δb[b]
Oxalic	1.27	0.083	2.31	100,067	1.677	0.9974	15.9	48.3	1.43	4.35	0.48
Tartaric	2.89	0.188	1.57	182,509	1.082	0.9976	15.2	46.1	2.28	6.92	0.84
Formic	3.77	0.167	2.69	97,828	0.918	0.9948	11.8	35.7	0.54	1.64	0.35
Citric	3.13	0.277	0.69	179,586	1.738	0.9972	11.0	33.3	2.11	6.41	0.60
Malic	3.40	0.282	1.05	177,755	1.120	0.9945	15.4	46.6	2.06	6.25	0.83
Lactic	3.86	0.366	1.18	40,860	5.977	0.9924	24.8	75.1	2.23	6.77	0.31
Quinic	3.58	0.425	0.98	221,567	−1.677	0.9961	19.7	59.8	3.79	11.49	1.32
Aspartic	3.90	0.465	1.03	154,460	0.299	0.9993	8.00	24.2	1.06	3.23	0.37
Succinic	4.20	0.543	0.30	148,138	0.676	0.9989	10.2	31.1	1.21	3.67	0.46
Glutamic	4.07	0.742	0.64	144,873	1.799	0.9991	9.26	28.1	1.36	4.13	0.41
Ascorbic	4.10	0.772	0.20	156,960	2.583	0.9986	11.6	35.3	2.05	6.21	0.55
Acetic	4.76	0.781	1.03	127,833	1.000	0.9998	4.88	14.8	0.29	0.89	0.19

RSH relative step height, *CV* coefficient of variation for six different measurements of RSH, *LOD* limit of detection, *LOQ* limit of quantification

[a] LOD and LOQ are instrumental parameters

[b] Δb is the standard error of the y-intercept of the regression line

Table 2 Organic acids in coffee brew measured by isotachopheretic analysis (mg L^{-1})

Coffee type/origin	Coffee origin/processed	pH	Tartaric	Formic	Citric	Malic	Lactic	Quinic
Robusta Vietnam Gr2	Green	5.92	25.40 ± 0.92	<LOQ	85.22 ± 1.41	29.91 ± 1.13	<LOD	38.75 ± 1.24
	Roasted	5.69	87.31 ± 1.26	19.72 ± 0.61	101.64 ± 2.32	25.72 ± 1.04	21.41 ± 1.21	148.64 ± 2.16
	Decaff. green	5.85	28.09 ± 0.54	<LOQ	85.22 ± 1.52	25.65 ± 0.79	<LOD	73.88 ± 1.19
	Decaff. roasted	5.55	43.28 ± 1.23	19.91 ± 0.53	99.82 ± 1.97	23.15 ± 1.02	<LOQ	108.44 ± 1.62
	Steamed	5.03	50.48 ± 1.74	20.33 ± 0.45	86.13 ± 1.01	45.67 ± 1.31	7.96 ± 0.29	113.78 ± 1.79
	Steamed roasted	5.22	51.72 ± 2.02	18.40 ± 0.31	70.83 ± 1.21	44.92 ± 1.01	7.96 ± 0.21	123.53 ± 1.98
Arabica	Brazil green	6.01	16.93 ± 0.82	<LOQ	119.72 ± 1.98	36.06 ± 1.32	<LOD	55.49 ± 1.01
	Brazil roasted	5.27	25.40 ± 1.35	16.99 ± 0.55	106.24 ± 1.69	31.98 ± 1.13	6.67 ± 0.15	119.24 ± 1.34
	Congo green	5.87	13.56 ± 1.57	<LOQ	93.56 ± 1.99	36.73 ± 1.75	<LOD	51.24 ± 1.02
	Congo roasted	4.88	26.47 ± 1.40	38.49 ± 0.96	105.49 ± 1.91	36.24 ± 1.86	<LOQ	107.40 ± 1.17
	Guatemala green	5.92	12.65 ± 0.87	<LOQ	80.35 ± 1.57	34.66 ± 1.74	<LOD	40.83 ± 1.21
	Guatemala roasted	5.11	30.50 ± 1.54	27.58 ± 0.59	95.81 ± 1.87	24.51 ± 0.84	<LOD	166.03 ± 2.52
	Honduras green	5.92	18.57 ± 0.21	<LOQ	118.27 ± 1.81	31.15 ± 1.41	<LOD	48.38 ± 1.69
	Honduras roasted	5.08	31.48 ± 1.14	34.60 ± 0.88	101.75 ± 1.76	22.40 ± 1.16	7.85 ± 0.10	108.49 ± 1.64
	Peru green	5.95	20.79 ± 1.24	<LOQ	85.38 ± 1.41	37.07 ± 1.21	<LOD	47.43 ± 1.02
	Peru roasted	5.34	30.99 ± 1.71	33.60 ± 0.99	109.08 ± 1.59	27.27 ± 1.23	<LOQ	131.60 ± 1.86

due to their simplicity and rapidity (Pyrzyńska and Pękal 2013; Van der Werf et al. 2014). The Folin-Ciocalteu assay was also used as a method to measure the concentration of reducing compounds in the sample (Singleton et al. 1999). The ferrous ion-chelating assay was used which measures the ability of secondary antioxidants to chelate metal ions. Antioxidants in brew act indirectly by preventing the formation of free radicals through the Fenton's reaction. Only more than two assays are sufficient to investigate the activity of antioxidants in a complex system (Prior et al. 2005). The abovementioned methods show the total antioxidant capacity of coffee brew.

Robusta Vietnam coffee brews (green, roasted, steamed, steamed roasted, decaffeinated and decaffeinated roasted beans) possessed higher antioxidant capacity in ABTS, DPPH and Folin-Ciocalteu assays than Arabica coffees (Table 3). It was found that roasting increases antioxidant capacity. The highest change was obtained for Arabica Peru

Table 3 Antioxidant capacity of coffee brews

Coffee type/origin	Coffee origin/ processed	ABTS (mmol Trolox L^{-1})	DPPH (mmol Trolox L^{-1})	Folin-Ciocalteu (mmol GAE L^{-1})	Chelating activity[a] (%)
Robusta Vietnam Gr2	Green	2.37 ± 0.16	3.35 ± 0.21	2.69 ± 0.04	71.38 ± 0.36
	Roasted	2.97 ± 0.10	3.02 ± 0.17	2.31 ± 0.04	52.60 ± 0.96
	Decaff. green	3.15 ± 0.23	3.35 ± 0.10	2.53 ± 0.21	67.25 ± 0.07
	Decaff. roasted	2.69 ± 0.03	3.15 ± 0.27	2.36 ± 0.08	48.94 ± 0.49
	Steamed	3.41 ± 0.04	3.30 ± 0.26	2.73 ± 0.03	31.52 ± 0.70
	Steamed Roasted	2.85 ± 0.14	3.34 ± 0.17	2.65 ± 0.04	41.39 ± 2.82
Arabica	Brazil green	1.59 ± 0.05	2.04 ± 0.15	1.57 ± 0.06	78.20 ± 1.39
	Brazil roasted	2.20 ± 0.20	2.33 ± 0.21	1.79 ± 0.03	55.99 ± 0.65
	Congo green	1.38 ± 0.06	1.93 ± 0.18	1.42 ± 0.04	73.85 ± 1.43
	Congo roasted	2.53 ± 0.21	2.86 ± 0.02	2.18 ± 0.03	51.45 ± 0.59
	Guatemala green	1.11 ± 0.04	1.86 ± 0.09	1.39 ± 0.10	80.47 ± 0.40
	Guatemala roasted	1.69 ± 0.05	2.26 ± 0.07	1.62 ± 0.04	47.04 ± 2.16
	Honduras green	1.33 ± 0.09	2.10 ± 0.17	1.47 ± 0.01	66.77 ± 3.08
	Honduras roasted	2.36 ± 0.20	2.99 ± 0.07	1.86 ± 0.06	56.64 ± 0.77
	Peru green	0.93 ± 0.31	2.03 ± 0.20	1.27 ± 0.02	81.31 ± 2.02
	Peru roasted	2.01 ± 0.03	2.50 ± 0.12	1.80 ± 0.09	66.25 ± 0.80

[a] Chelating activity of Fe(II) ions of coffee brew (without dillution)—0.5 g in 50 mL of water

Table 4 Corelation coefficient (R^2) between pH and antioxidant capacity of coffee brews

	pH	ABTS assay	DPPH assay	Folin-Ciocalteu assay
ABTS assay	0.239			
DPPH assay	0.147	0.845		
Folin-Ciocalteu assay	0.114	0.860	0.924	
Chelating activity	0.650	0.546	0.385	0.399

roasted coffee in ABTS assay. Similar results of higher antioxidant activity in DPPH assay in green Robusta coffees than in roasted coffees were obtained by Nebesny and Budryn (2003).

On the other hand, Arabica coffee brews had a little stronger chelating activity (51–81 %) on Fe^{2+} ions than Vietnam Robusta coffees (31–71 %). Green coffee brews showed higher activity than roasted (also steamed and decaffeinated) coffees. This chelating activity has a different mechanism of antioxidants in this assay in comparison to radical scavenger assays. Similar effect was observed by Tang et al. (2002) for green tea extracts (containing 86 % of dietary tea catechins) which possessed limited chelating effects.

According to all assays, the roasting process had a positive influence on antioxidant capacity of Arabica and Robusta coffee brews with the exception of chelating activity. The brews from roasted coffee beans had a higher antioxidant activity than green coffee brews probably due to melanoidin content increasing after roasting (Perez-Hernandez et al. 2012). Different results were obtained by Perez-Hernandez et al. (2012) with the exception of Torrefacto coffee and instant coffee as well as by Van der Werf et al. (2014) for ABTS-HPLC assay. Chu et al. (2009) also found that roasted coffee brew with chlorogenic acid lactones had higher neuroprotective properties than green coffee brew.

Correlation between pH and antioxidant activity of coffee brews was tested. Nevertheless, the Pearson's correlation coefficients between these parameters were low (Table 4). The high positive correlation was obtained for ABTS and DPPH (0.845) and ABTS and Folin-Ciocalteu (0.860), and the highest was determined for DPPH and Folin-Ciocalteu (0.924). On the other hand, negative correlation was obtained for chelating activity and pH and chelating activity and ABTS.

Conclusions

The ITP method was optimised and validated to achieve the efficient separation of 12 organic acids in coffee brews, out of which 6 were quantified. Increase of tartaric acid content after roasting of coffee beans was found which to the best of authors' knowledge was never reported previously.

It was found that the steaming process of beans enlarged antioxidant capacity of the brews more than the roasting process. Moreover, brews from the roasted coffee beans (Arabica and Robusta) had higher antioxidant activity measured by ABTS, DPPH and Folin-Ciocalteu assays than those from the green coffee beans (excluding chelating activity). The higher antioxidant activity of roasted coffee results from the presence of Maillard reaction products, and these compounds do not have the ability to chelate iron ions. In this case, better properties exhibit green coffee, which has higher amounts of chlorogenic acids having chelating properties.

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Compliance with Ethical Standards

Conflict of Interest Magdalena Jeszka-Skowron declares that she has no conflict of interest. Agnieszka Zgoła-Grześkowiak declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable

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