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ORIGINAL PAPER

Electrochemical, photometric, and chromatographic methods for the evaluation of organic matter and bioactive compounds in coffee brews

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Abstract

Sensitive electrochemical techniques were combined with photometric and chromatographic assays to characterize the organic matter, determine selected bioactive compounds, and evaluate antioxidant properties of popular coffee brews. Physicochemical properties of organic material released in Greek/Turkish, espresso, filter, and instant coffee brews were investigated measuring copper-complexing ligands (L_T), surface active substances (SAS), and catalytically active compounds (CAC). In addition, organic carbon, total and individual phenolics, and caffeine were measured. The content of Maillard reaction products was estimated. Antioxidant potential was primarily assessed measuring DPPH-radical scavenging capacity and ferric-reducing antioxidant power. The potential inhibition of human serum lipoprotein oxidation in vitro was also investigated. The effect of fast or slow sipping of Greek/Turkish coffee was investigated as regards organic matter release and antioxidant activity. Coffee brews release Cu complexing ligands at different concentrations (74.6 and 364 mM in filter and Greek/Turkish coffees, respectively). Coffee brews contained 50.1–445 mg caffeoylquinic acids (CQAs) per 100 mL, the 47–53% being chlorogenic acid. Among coffee brews, espresso contained more CAC, SAS, copper-complexing ligands, caffeine, browned compounds, and phenolics. In addition, espresso exhibited higher antiradical activity and reducing power, while Greek/Turkish coffees higher resistance to serum lipid oxidation.

Keywords Coffee brews · Organic matter · Copper complexation · Phenolic compounds · Caffeine · Antioxidant activity

Introduction

Coffee is commonly consumed worldwide [1]. Coffee consumption is related to many health benefits [2, 3] owed to its antioxidant activity [4]. Apart from caffeine, roasted coffee contains also phenolic compounds, niacin and its precursor trigonelline, soluble fiber, nicotinic acid, and melanoidins; the latter, produced according to the intensity of roasting [5], are responsible for coffee's color and partly for its antioxidant and metal-chelating properties [2, 6–8]. The antioxidant potential of coffee is mostly linked to phenolics [9, 10].

The antioxidant activity of coffee is attributed to organic compounds that scavenge free radicals and chelate redox active metals, which are considered significant exogenous sources of Reactive Oxygen and Nitrogen Species (ROS, RNS) [11].

The antioxidant activity of coffee brews involves chromogen compounds of a radical nature which stimulate reductive oxygen species [12]. Herein, radical scavenging, reducing

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power, and copper complexing were evaluated in espresso, filter, instant, and Greek/Turkish coffee brews. In addition, caffeine, total, and individual phenolics were measured. For first, to our knowledge, the organic matter content (TOC) and its physicochemical characterization measuring surface active substances (SAS) and catalytically active compounds (CAC) were assessed. Total serum lipoprotein resistance to oxidation was applied as a biologically relevant assay representing human antioxidant capacity. Based on the hypothesis that the contact of Greek/Turkish brew with solids in cup may be involved in a continuing release of organic matter and bioactive microconstituents, resulting in different antioxidant capacities, the influence of sipping rate was also investigated.

Materials and methods

A detailed list of reagents and chemicals is provided in the Supplemental material (Table S2).

Samples

Four types of commercial grounded coffee samples, namely, espresso (ESP), filter (FIL), instant (INS), and Greek/Turkish (G) style, were purchased from local market. All samples were vacuum-packed and—according to their labels—medium roasted and far from their expiration dates. With the exception of espresso, which was 100% Arabica, the rest of samples were blends of Arabica and Robusta.

Preparation of coffee brews

Coffee brews were prepared applying common practices; the quantity of coffee and water used is shown in Table S1. Espresso was prepared in a commercial machine using 14 g of powder with steam flow under 15 atm. Filter coffee was prepared using 6 g of grounded coffee in a coffee maker with 300 mL of water. Instant coffee was prepared adding 3 g of coffee in 250 mL of boiling-hot water. Greek/Turkish coffee was prepared in a traditional pot called "briki" adding 4 g of grounded coffee in 70 mL of water, followed by heating slowly to gentle boiling until a thick foam was formed. Greek/Turkish style coffee was further investigated relatively to sipping rate, since it is traditionally believed that slow sipping provides full flavor. To obtain samples of slow and fast sipping, coffee brews were decanted in glass beakers: 10 mL aliquots at 5 min intervals for "fast" and 10 mL aliquots at 10 min intervals for "slow" sipping, the samples named GF and GS, respectively. Fifteen replicates were prepared for individual samples. All brews were lyophilised (HetoLyolab 3000, Heto-Holten, Allerod, Denmark) and dry residues were weighed. The 15 replicates for individual coffee type were pooled in 3 composite samples stored at − 20 °C.

Analytical methods

Electrochemical measurements

For the determination of copper-complexing capacity (L_T), solutions of 50 mg/L were prepared dissolving lyophilised brews in Milli-Q water (18.2 MΩ cm; Millipore). Following the addition of 5 drops of 3M NaCl (Merck, Darmstadt, Germany), samples were immediately subjected to copper-complexing capacity (L_T) and apparent stability constant (K_{app}) determinations. For surface active substances (SAS), solutions of 10 mg/L were prepared dissolving lyophilised brews in Milli-Q water and were measured in 0.55M NaCl. For catalytically active compounds (CAC), solutions of 10 mg/L concentrations were prepared in 0.55M NaCl + 0.5M NaAc (sodium acetate buffer, pH 5.1) and organic-free (UV-irradiated) seawater (pH 8.2). Samples were measured immediately after preparation. Electrochemical measurements were performed using a μAutolab type III (Eco-Chemie, Utrecht, The Netherlands) instrument connected to a three-electrode cell (663 VA Stand, Metrohm, Herisau, Switzerland) with a static mercury drop electrode (SMDE) as the working electrode. The reference electrode was an Ag/AgCl (3M KCl). A carbon-rod electrode served as the auxiliary electrode. Determination of copper-complexing capacity (L_T) was conducted according to Plavšić et al. [13], of surface active substances (SAS) according to Ćosović [14] and of catalytically active compounds (CAC) according to Strmečki et al. [15] and Strmečki and Plavšić [16]. To our knowledge, these electrochemical measurements are applied in coffee brews for first. Detailed description of the analytical methodologies is provided in Table S3.

Chemical determinations

Total organic carbon (TOC) was determined by high-temperature catalytic oxidation employing a TOC-5000A Shimadzu analyzer. Copper determination was performed following wet digestion by graphite furnace atomic absorption spectrometry with Zeeman background correction (SpectrAA 640Z; Varian, Mulgrave, Victoria, Australia) [17, 18] (Table S3).

Products of Maillard reaction were quantitated spectrophotometrically in aliquots of coffee brews by measuring their absorbance at 420 nm, as described by Ludwig et al. [19].

For caffeine determination, freeze-dried brews (5 mg each) were dissolved in deionised water (1 mL) and extracted with chloroform (3 × 3 mL); caffeine was measured in the extract by GC/MS (see supplementary material).

Phenolic compounds were extracted from freeze-dried brews with 3 × 2 mL MeOH. Aliquots of the methanolic extracts were evaporated to dryness by centrifugal evaporator and were silylated by reaction with BSTFA for 20 min at 70 °C. Simple phenolics were quantified in the form of their trimethylsilyl derivatives by selective ion monitoring GC/MS [20] (for more details and for the target and qualifier ions used, see supplementary material); chlorogenic acid (5-O-CQA), and neochlorogenic acid (3-O-CQA) were quantified by means of pure reference compounds, while quantification of cryptochlorogenic acid (4-O-CQA) was based on the 5-O-CQA response factors.

Total phenolic content was assayed in the methanolic extracts of freeze-dried brews (5 mg/mL MeOH) by the Folin–Ciocalteu assay, using caffeic acid as the reference standard [21].

Evaluation of antioxidant activity

Antiradical activity and reducing power were carried out according to [21] in methanolic extracts of lyophilised coffee brews (Table S3). The antioxidant activity of the extracts was additionally evaluated by the kinetics of copper-induced lipid oxidation in total human serum, employing lag time as a criterion for antioxidative power [22] (Table S3).

Statistical analysis

All analyses were performed in triplicate except otherwise indicated. Results were expressed as mean values ± standard deviation. Statistical significance of differences between means was evaluated with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Herndon, VA, USA) by performing analysis of variance (ANOVA) and employing Duncan's multiple range test. A value of $p < 0.05$ (95% confidence level) was considered to indicate a significant difference of the statistical analysis of the data.

Results and discussion

Total and suspended solids

Total solids varied from 805 to 4497 mg per 100 mL in filter coffee and espresso, respectively (Table 1b), in decreasing order espresso > Greek/Turkish > instant > filter. Correlations of total solids with TOC, browned compounds, caffeine, SAS, antiradical activity, reducing power, *p*-OH benzoic acid, and vanillic acid were observed ($p < 0.05$).

Suspended solids were higher in Greek/Turkish coffees (119–124 mg per 100 mL) compared to 10.2–23.1 mg per

Table 1 Total and suspended solids, total organic carbon, caffeine, total phenolics, sum of caffeoylquinic acids, and antioxidant activity of the coffee brews

	ESP	FIL	GF	GS	INS
(a) Per g of coffee					
Total solids (mg)	194 ± 5[a]	362 ± 3[b]	327 ± 12[b]	298 ± 3[b]	870 ± 32[c]
Total organic carbon (mg)	49.9 ± 1.3[a]	90.6 ± 5.8[b]	109 ± 7[b]	108 ± 4[b]	242 ± 36[c]
Caffeine (mg)	8.01 ± 1.02[a]	28.2 ± 0.9[c]	18.8 ± 2.1[b]	17.8 ± 0.6[b]	40.8 ± 4.7[d]
Total phenolics (mg CAE)	22.6 ± 2.8[a]	37.8 ± 3.2[b]	54.2 ± 1.4[c]	51.3 ± 0.9[c]	60.1 ± 6.5[d]
Sum of caffeoylquinic acids (mg)[#]	19.1 ± 3.2[a]	25.6 ± 3.4[a]	48.2 ± 5.6[b]	46.3 ± 5.8[b]	41.7 ± 3.8[b]
Radical scavenging capacity (mg TE)	7.98 ± 0.68[a]	10.3 ± 1.2[b]	14.2 ± 1.1[c]	12.7 ± 0.6[c]	21.7 ± 1.7[d]
Reducing power (mg AAE)	3.47 ± 0.30[a]	6.27 ± 0.45[b]	6.19 ± 0.33[b]	5.88 ± 0.48[b]	9.16 ± 0.83[c]
(b) Per 100 mL coffee brew					
Total solids (mg)	4497 ± 126[d]	805 ± 6.7[a]	1987 ± 76[c]	1862 ± 15[c]	1041 ± 46[b]
Suspended solids (mg)	21.0 ± 2.0[a]	10.2 ± 2.8[a]	124 ± 8.0[b]	119 ± 6.9[b]	23.1 ± 2.0[a]
Total organic carbon (mg)	1160 ± 30[d]	201 ± 13.2[a]	622 ± 43.3[c]	616 ± 21[c]	289 ± 43[b]
Caffeine (mg)	186 ± 23[c]	62.5 ± 1.7[a]	116 ± 12.8[b]	108 ± 3.3[b]	48.6 ± 5.6[a]
Total phenolics (mg CAE)	526 ± 65[c]	84.3 ± 7.0[a]	333 ± 8.3[b]	312 ± 5.0[b]	72.0 ± 7.8[a]
Sum of caffeoylquinic acids (mg)[A]	445 ± 35[c]	57.0 ± 7.2[a]	290 ± 23[b]	282 ± 23[b]	50.1 ± 4.2[a]
Radical scavenging capacity (mg TE)	187 ± 15[c]	31.3 ± 2.7[a]	87.0 ± 7.1[b]	79.7 ± 4.0[b]	28.1 ± 2.0[a]
Reducing power (mg AAE)	81.1 ± 7.1[c]	14.0 ± 1.0[a]	38.2 ± 2.0[b]	36.9 ± 2.9[b]	10.9 ± 1.1[a]

Results are expressed (a) per g of coffee and (b) per 100 mL of coffee brew

ESP espresso, *FIL* filter, *GF* Greek/Turkish, fast sipping, *GS* Greek/Turkish, slow sipping, *INS* instant

[A]Sum of 3-O-CQA (neochlorogenic acid), 4-O-CQA (cryptochlorogenic acid) and 5-O-CQA (chlorogenic acid), *CAE* caffeic acid equivalents, *TE* Trolox equivalents, *AAE* ascorbic acid equivalents

Data are provided as mean ± SD of triplicate analyses; values in the same raw not sharing lower case letters are significantly different ($p < 0.05$, Duncan multiple range test)

100 mL in filter, espresso and instant coffees (Table 1b), representing 0.5% of total solids in espresso up to 6.2–6.4% in Greek/Turkish coffees. Elevated suspended solids in Greek/Turkish coffees are expected, since the constant contact of brew and grounds may result in resuspension during sipping.

Total organic carbon

Total organic carbon (TOC) content ranged between 201 and 1160 mg per 100 mL in filter and espresso, respectively (Table 1b). TOC represented 25–28% w/w of dry matter in instant, filter and espresso and 31–33% of dry matter in Greek/Turkish coffee brews. Correlations of TOC with total solids, SAS, total phenolics, sum of CQAs, caffeine, antiradical activity, and reducing power were reported ($p < 0.05$).

Organic matter characterization employing electrochemical techniques

Reported here for the first time are the concentrations of catalytically active compounds (CAC) at pH 5.1 and 8.2 in coffee brews. The pH values are quoted, since it has been shown that the proximity of the dissociation constants of buffer and catalyst is a precondition for the occurrence of catalytic activity [23]. Such a consideration, accompanied by the fact that the catalytic activity, i.e., occurrence of "peak H", is related to the presence of S, N, O, and P atoms in the organic molecule [24] may lead to the conclusion that, at pH 8.2, N-containing organic material could be detected, since dissociation constants of the majority of N-containing groups in organic molecules are close to this pH [15]. For this reason, human serum albumin (HSA) with 15.7% nitrogen content was selected as a calibration compound at pH 8.2. At pH 5.1, the sulphate and/or carboxylic groups present in organic molecules could be presumed to dissociate, causing the catalytic activity for H ions. Compounds like polysaccharides with sulphate and or carboxylic groups could be catalytically active at pH 5.1. However, since the method employed is relatively new [15, 16, 25] and not all classes of compounds have been tested so far, we could hypothesize that other catalytically active compounds also dissociate at this pH. All coffee brews tested demonstrated the presence of CAC at pH 5.1 (Fig. 1a), with mean values varying between 6759 mg/L eq. xanthan in instant coffee and 226,836 mg/L eq. xanthan in espresso. The respective mean concentrations of CAC at pH 8.2 ranged from 2.8 mg/L eq. HSA in instant coffee to 81 mg/L eq. HSA in espresso (Fig. 1b).

CAC at pH 8.2 correlated well ($p < 0.05$) with TOC, SAS, total phenolics content, sum of CQAs, antiradical activity, and reducing power, while CAC at pH 5.1 did not correlate with any of the parameters studied.

The concentrations of surface active substances (SAS) were also measured for the first time in coffee brews. The

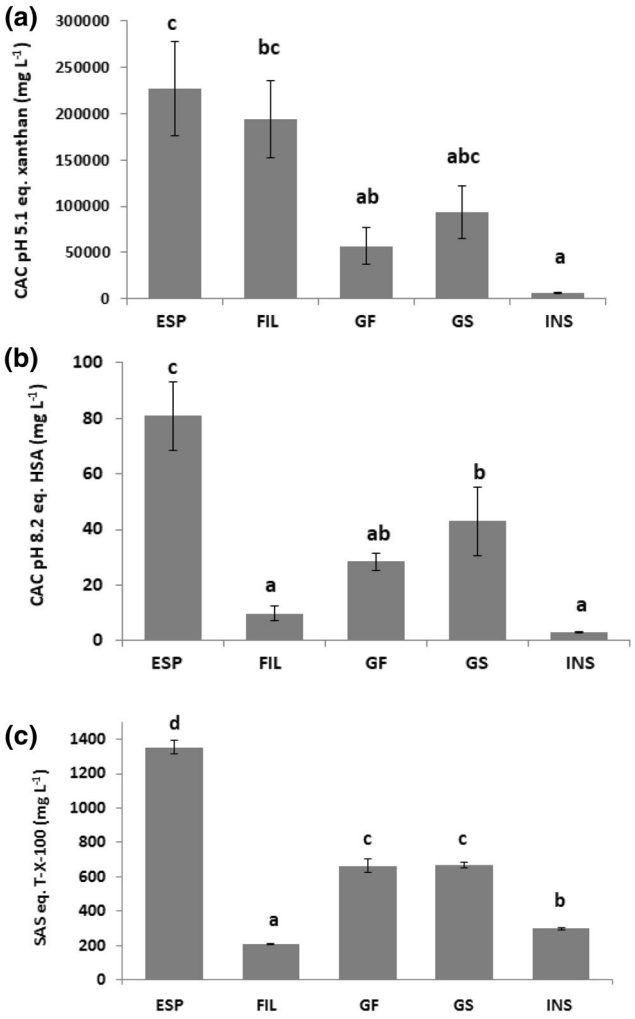

Fig. 1 Concentrations of catalytically active compounds (CAC) at pH 5.1 and 8.2 and surface active substances (SAS) in coffee brews. Different letters indicate significant differences between samples ($p < 0.05$); abbreviations as in Table 1

brews examined contain SAS at mean levels varying from 206 mg/L eq. Triton-X-100 (T-X-100) in filter coffee to 1354 mg/L in espresso (Fig. 1c). SAS were found to correlate ($p < 0.05$) with total solids, TOC, CAC at pH 8.2, caffeine, total phenolics, sum of CQAs, antiradical activity, reducing power, *p*-OH benzoic acid, vanillic acid, and protocatechuic acid.

SAS and TOC concentrations were correlated to those of organic model substances with different functional groups and hydrophobic properties, to test which one of them better resembles the adsorption characteristics in coffee brews [26]. The correlation of SAS data for model substances with the corresponding TOC values is presented in Fig. 2. As shown, coffee brews examined demonstrated adsorption characteristics similar to those of dextran and xanthan, which are both polysaccharides of high molecular mass (5×10^5 and 2×10^6,

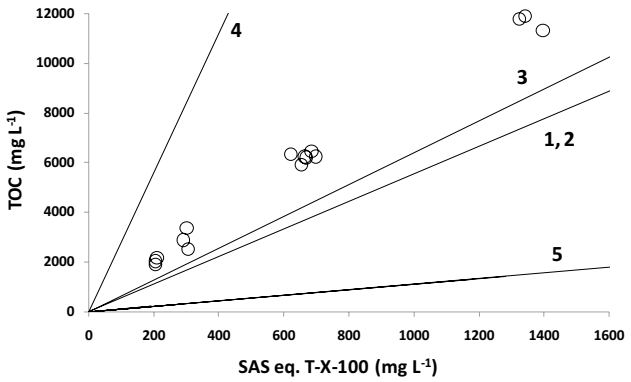

Fig. 2 Correlation of surface active substances (SAS) concentrations eq. to Triton-X-100 (T-X-100) and total organic carbon (TOC) values in coffee beverages. Numbered lines correspond to different model substances: No 1 to protein–albumin No 2 to fulvic acid No 3 to dextran No 4 to xanthan and No 5 to Triton-X-100

respectively). The alike adsorption data of the polysaccharides pseudomelanoidins and of melanoidins have been previously reported [27]. Because the content of melanoidins in coffee brews is as high as 25% of the beverage's dry matter, and based on the common adsorption characteristics of melanoidins with pseudomelanoidins, it is hypothesized that adsorption characteristics for coffee brews are owed to the contained melanoidins.

Browned compounds and caffeine

Browned compounds—recorded as absorbance at 420 nm [19]—decreased in the order: espresso (0.631) > Greek fast sipping (0.191) ≈ instant (0.188) ≈ Greek slow sipping (0.185) > filter (0.132) and exhibited good correlations ($p < 0.05$) with total solids, TOC, caffeine, SAS, antiradical activity, reducing power, *p*-OH-benzoic acid, vanillic, and protocatechuic acids. Ludwig et al. [19] reported also higher browned compounds in espresso compared to filter coffee.

Caffeine was higher in espresso (186 mg per 100 mL) and lower in instant and filter coffees (48.6–62.5 mg per 100 mL) (Table 1b), in accordance with the previous studies [19, 28].

Fast sipping of Greek/Turkish coffee resulted in insignificant differences in caffeine content compared to slow sipping (116 vs 108 mg per 100 mL, and 18.8 vs 17.8 mg/g of ground coffee, respectively, $p < 0.05$).

Simple and total phenolics, chlorogenic acids

Concentrations of simple phenolics in coffee brews are given in Table 2. Caffeoylquinic acids (CQAs) predominated, being 50–57 mg per 100 mL in instant and filter, 282–290 mg per 100 mL in Greek/Turkish and 445 mg per 100 mL in espresso. Fast sipping Greek coffee exhibited higher CQAs compared to slow sipping. Chlorogenic acids represent the main phenolics in coffee, consisting mainly of CQAs [27]. Hereby, 5-O-CQA (chlorogenic acid) predominated, followed by 4-O-CQA (cryptochlorogenic acid) and 3-O-CQA (neochlorogenic acid), comprising 47–53, 27–31, and 14–23% of CQAs, in agreement with the previous studies [19, 28, 29]. Likewise, 47–53% of total CQAs in espresso, Greek/Turkish, and filter coffees corresponded to 5-O-CQA, while in instant coffee, 38.2% corresponded to 5-O-CQA [29].

According to Ludwig et al. [29], total CQAs values range as 244–306 mg per serving in light roast espresso, 119–160 mg per serving in medium roast and 75–96 mg per serving in dark roast espresso. Since caffeine is more stable than CQAs during usual roasting, the caffeine/total CQAs ratio has been suggested as a marker of roasting degree in coffee beans. Total CQAs are the sum of 5-O-CQA, 4-O-CQA, and 3-O-CQA, while ratio values equal to 0.7, 1.3 and 2.6 correspond to light, medium, and dark roast, respectively [29]. The caffeine/total CQA ratios herein were 0.38–0.39 in Greek/Turkish coffee, 0.42 in espresso, and 0.98–1.10 in instant and filter coffee, suggesting that light-to-medium roasting.

In the cases of Greek/Turkish, espresso, and filter coffee brews, the extraction efficiency of preparation techniques was expressed as total CQAs per g of coffee brewed. Efficiency ranged from 19.1 mg/g in espresso to 46.3 or 48.2 mg/g of coffee brewed in Greek/Turkish type coffees (Table 1a).

Apart from CQAs, coffee brews contained hydroxycinnamic acids caffeic, *p*-coumaric, cinnamic, ferulic and sinapic, the phenolic acids syringic, vanillic, *p*-hydroxybenzoic and protocatechuic, the phenol vanillin, and the flavonoids chrysin, naringenin, and quercetin (Table 2). In addition, trace amounts of oleanolic and ursolic acids were detected, in all brews studied.

Total phenolic content (TPC) was expressed in mg caffeic acid equivalents (CAE) per 100 mL. TPC decreased in the order: espresso > Greek/Turkish > filter > instant, as in the case of CQAs (Table 1b). In terms of TPC and CQA contents, in some studies, instant coffee predominates [31], while in others, filter [28, 32] or espresso [19] predominates.

When expressing TPC in terms of extraction efficiency, i.e., as mg CAE per g of coffee brewed, the order is: Greek/Turkish > filter > espresso (Table 1a). The higher extraction of CQAs in filter compared to espresso coffees herein is in agreement with existing data [19, 33]. Short brewing time and high coffee-to-water ratio (Table S1) are probably responsible for lower extraction of phenolics in espresso compared to filter and Greek/Turkish coffees.

Table 2 Simple phenolics (mg per 100 mL) in coffee brews

Compound	ESP	FIL	GF	GS	INS
Phenolic acids					
p-Hydroxybenzoic acid	0.040 ± 0.003^{b}	0.009 ± 0.001^{a}	0.022 ± 0.002^{a}	0.019 ± 0.001^{a}	0.016 ± 0.001^{a}
Syringic acid	0.002 ± 0.000^{ab}	0.001 ± 0.000^{a}	0.005 ± 0.000^{ab}	0.003 ± 0.000^{ab}	0.009 ± 0.000^{b}
Vanillic acid	0.025 ± 0.003^{b}	0.004 ± 0.0001^{a}	0.007 ± 0.001^{a}	0.006 ± 0.001^{a}	0.005 ± 0.001^{a}
Protocatechuic acid	0.074 ± 0.005^{b}	0.004 ± 0.0001^{a}	0.009 ± 0.001^{a}	0.007 ± 0.001^{a}	0.010 ± 0.001^{a}
Phenols					
Vanillin	0.008 ± 0.001^{a}	0.005 ± 0.001^{a}	0.007 ± 0.001^{a}	0.007 ± 0.001^{a}	0.007 ± 0.001^{a}
Cinnamic acid	0.011 ± 0.001^{a}	0.006 ± 0.001^{a}	0.013 ± 0.001^{a}	0.011 ± 0.001^{a}	0.009 ± 0.001^{a}
Flavonoids					
Chrysin	0.032 ± 0.002^{a}	0.013 ± 0.001^{a}	0.037 ± 0.003^{a}	0.033 ± 0.002^{a}	0.011 ± 0.001^{a}
Naringenin	0.007 ± 0.001^{a}	0.010 ± 0.001^{a}	0.022 ± 0.003^{a}	0.011 ± 0.001^{a}	0.022 ± 0.003^{a}
Quercetin	0.030 ± 0.003^{a}	0.011 ± 0.001^{a}	0.030 ± 0.003^{a}	0.031 ± 0.003^{a}	0.028 ± 0.002^{a}
Hydroxycinnamic acids					
Caffeic acid	0.331 ± 0.020^{b}	0.041 ± 0.002^{a}	0.311 ± 0.019^{b}	0.281 ± 0.031^{b}	0.136 ± 0.008^{a}
p-Coumaric acid	0.008 ± 0.001^{ab}	0.004 ± 0.000^{a}	0.013 ± 0.002^{ab}	0.007 ± 0.001^{ab}	0.025 ± 0.003^{b}
Ferulic acid	0.011 ± 0.001^{a}	0.010 ± 0.001^{a}	0.034 ± 0.003^{a}	0.031 ± 0.003^{a}	0.027 ± 0.003^{a}
Sinapic acid	0.001 ± 0.000^{a}	0.001 ± 0.000^{a}	0.003 ± 0.000^{a}	0.002 ± 0.000^{a}	0.002 ± 0.000^{a}
5-O-CQA* (chlorogenic acid)	140 ± 13^{c}	16.0 ± 1.4^{a}	92.7 ± 8.3^{b}	90.1 ± 8.1^{b}	15.2 ± 1.4^{a}
4-O- CQA (cryptochlorogenic acid)	75.9 ± 6.1^{c}	10.4 ± 0.8^{a}	46.4 ± 3.7b	45.2 ± 3.6^{b}	8.7 ± 0.7^{a}
3-O- CQA (neochlorogenic acid)	51.0 ± 5.6^{c}	7.80 ± 0.86^{a}	34.9 ± 3.8bc	34.1 ± 3.8^{b}	6.18 ± 0.68^{a}
Sum of CQA	267 ± 21^{c}	34.2 ± 2.7^{a}	174 ± 14b	169 ± 14^{b}	30.1 ± 2.5^{a}

Data are the mean ± SD of triplicate analyses; values in the same row not sharing lowercase letters are significantly different ($p < 0.05$, Duncan multiple range test)

**CQA* caffeoylquinic acid; other abbreviations are as in Table 1; for caffeoylquinic acids, the preferred IUPAC numbering was followed [30]

Antioxidant properties of coffee brews

Radical scavenging and reducing power

All coffee brews exhibited antiradical activity, instant, and filter coffees demonstrating the lower activity (28.1–31.3 mg TE per 100 mL) and espresso the highest (187 mg TE per 100 mL) (Table 1b). Antiradical activity of coffee brews correlated ($p < 0.01$) with CQAs, caffeine, total solids, organic carbon, and surface active substances (SAS). In a relevant study, 5-O-CQA exhibited the highest activity, both in-vitro and ex-vivo, followed by caffeine [34].

Similarly, ferric-reducing antioxidant power (FRAP) was lower in instant and filter coffee (10.9–14.0 mg AAE per 100 mL), followed by Greek/Turkish coffee brews (36.9–38.2 mg AAE per 100 mL); the higher FRAP was reported in espresso (81.1 mg AAE per 100 mL) (Table 1b). When considering the antioxidant properties per gram of brewed coffee, instant coffee demonstrated the higher antioxidant potential, whereas espresso the lower (Table 1a).

Copper-complexing properties of organic matter in coffee brews

All coffee types release ligands in their brews, demonstrated by L_T concentrations. The mean L_T values varied significantly, being highest in the Greek/Turkish fast sipping (364 μM) and espresso (361 μM), followed by Greek/Turkish slow sipping (234 μM), instant (167 μM), and filter coffees (74.6 μM) (Fig. 3a). The significant metal-chelating properties of coffee are well established and are related to high chlorogenic and caffeic acids reported to be the strongest chelators among phenolic acids [35]. Hereby, L_T concentrations correlated well ($p < 0.05$) with the sum of CQAs, cinnamic and caffeic acids.

The mean values of $\log K_{app}$, expressing the stabilities of Cu–organic complexes, ranged from 6.6 to 8.9 for espresso and Greek/Turkish slow sipping, respectively (Fig. 3b). The relatively small variation of $\log K_{app}$ values suggests that they are independent of the amount of organic material released. Furthermore, this variation indicates that ligands deriving from the various coffee brew types are likely to share similar binding sites for Cu ions.

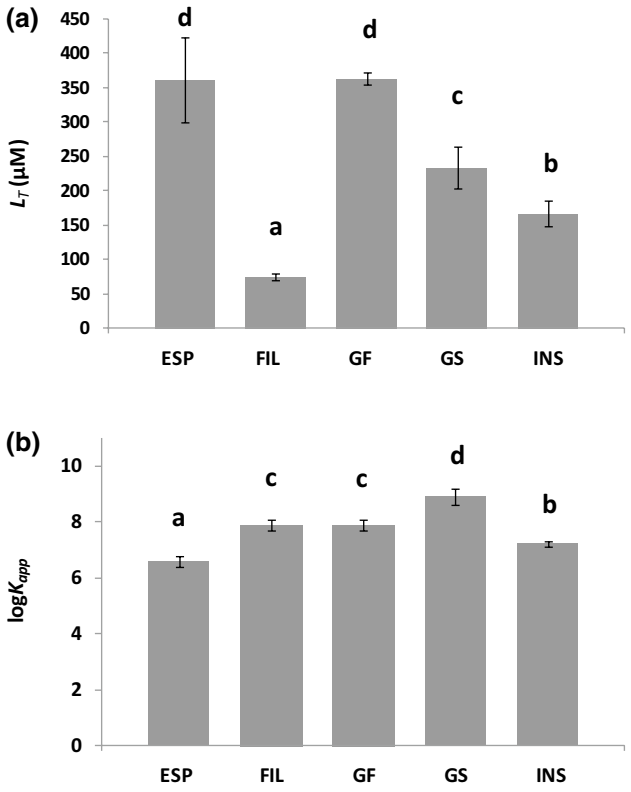

Fig. 3 Copper-complexing capacity (L_T) concentrations and log of apparent stability constant (logK_{app}) values of coffee brews. Different letters indicate significant differences between samples ($p < 0.05$); abbreviations as in Table 1

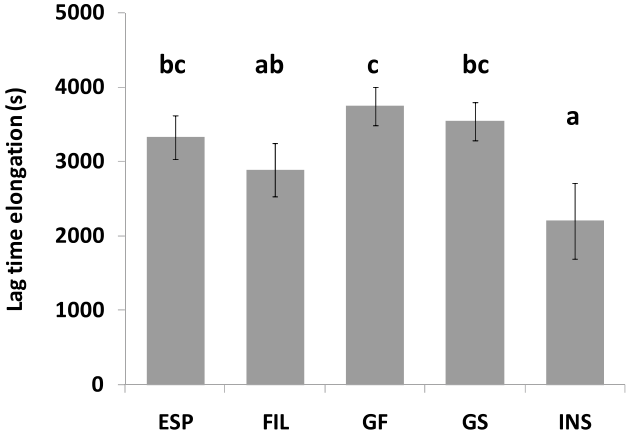

Fig. 4 Increase in lag time of total serum lipoproteins oxidized in vitro by copper sulphate in the presence of methanolic extracts from coffee brews compared to serum lipoproteins oxidized by copper sulphate (control). Results are expressed as mean values (±SD) of four independent experiments. Different letters indicate significant differences in elongation time between samples ($p < 0.05$); abbreviations as in Table 1

Coffee contains melanoidins which are responsible for its color, being additionally known as very good complexing ligands for copper ions [36] and characterized by logK_{app} values similar to the ones determined hereby. This suggests that melanoidins are potentially the most significant complexing ligands for Cu ions in coffee brews.

Mean total copper concentrations (TCu) varied significantly among coffee beverages, ranging from 0.2 μM in instant coffee, 0.3 μM in filter, 0.8 μM in Greek/Turkish fast sipping and espresso, to 1.6 μM in Greek/Turkish slow sipping. In any case, TCu did not exceed the corresponding L_T concentrations, demonstrating that all coffee brews examined contain fully complexed Cu.

Since the coffee brews studied contained different quantities of organic matter (Table 1), a normalization of L_T concentrations as for organic carbon concentrations was carried out. The normalized values for the coffee brews ranged between 31 and 59 nmol Cu/mg C, their relatively limited range indicating the chemical similarity of organic ligands of Cu ions, irrespectively of differences in brews preparation methods. Similar calculations have reported values laying between 0.91 and 7.0 nmol Cu/mg C for Greek beers [37] and 16–128 nmol Cu/mg C for herbal infusions [38].

Inhibition of serum oxidation

Significant elongation of lag time and subsequent increased defense of lipid particles against oxidation was observed with the addition of extracts from all coffee brews, compared with the control (control lag time: 15412.5 ± 254.8 s). The degree of elongation was found to increase as: instant < filter < espresso < Greek/Turkish slow < Greek/Turkish fast (Fig. 4). Elongation was significantly lower in instant coffee compared to Greek/Turkish of slow and fast sipping and to espresso ($p < 0.05$). Similarly, elongation was significantly lower in filter compared to Greek/Turkish of slow and fast sipping and to espresso ($p < 0.05$). Lag time elongation correlated well ($p < 0.05$) with chrysin and coffee-to-water ratio.

Greek/Turkish coffees exhibited higher resistance to serum oxidation compared to that of espresso, although the latter contained more total phenolics, chlorogenic acids, and caffeine, demonstrating higher antiradical activity and reducing power. This is most likely attributed to the presence of other compounds acting synergistically, not assayed here, such as melanoidins [39]. The aforementioned are also reflected by the slightly higher logK_{app} values determined for Greek/Turkish slow sipping coffee, indicating the presence of stronger binding sites for Cu ions in ligands deriving from this coffee brew. Antioxidant activity was weaker in the case of instant and filter coffees and higher in espresso and Greek/Turkish coffees.

Overall, the differences in parameters examined herein could be attributed to a concert of different preparation conditions of coffee brews. The brewing method (espresso or filter or Turkish) has been reported to decisively affect

several physicochemical parameters as well as the amount per milliliter of total phenols, caffeine, and antioxidants [40]. Instant coffee brews have been shown to possess the highest total phenolic and total flavonoid contents, as well as the highest antioxidant capacity, while filter coffee brews show the lowest content of polyphenols and the lowest antioxidant capacity in the study of Niseteo et al. [31]. Higher total phenolics and CQA have been found in espresso compared to filter [19]. As such, brewing time or brewing temperature are important parameters affecting the antioxidant content in coffee brews [41]. Ludwig and co-workers [19] have shown that in espresso coffee, more than 70% of the antioxidants of a coffee brew are extracted during the first 8 s, while in filter coffee, a U-shape extraction profile occurs, starting in different brewing timepoints, probably due to different wettability. Increased extraction efficiency mostly in the less polar diCQA is dependent on higher turbulence and longer contact time [19]. Roasting time and temperature of coffee beans may as well be key factors in antioxidant content of coffee brews. It has been shown that the content of chlorogenic acid is lowered most significantly by varying the roasting time/temperature curve and the total chlorogenic acid in regular brewed coffees is higher in light compared with dark or very dark roasting conditions [42]. In addition to the above technological conditions, when comparing the CQA content and profile in different brewing processes, including homemade brews—boiled, filter, French, and mocha coffee—and commercial brewed coffee, the brewing mechanisms were found to have a profound effect on the amount of CQA delivered per cup [43]. The mocha coffeemaker has been shown to have the highest yield per gram of ground roasted coffee in coffee antioxidant extraction, whereas espresso coffee is richest in terms of antioxidant intake per milliliter of coffee brew [33]. In espresso coffee brew, except for the chemoprofile, also the aromatic profile is significantly affected by the extraction time and the grinding grade of coffee powder, the majority of organic acids, solids, and caffeine contained into the coffee ground being extracted during the first 8 s of percolation [44]. All in all, different preparation conditions are responsible for the final coffee product consumers drink, but above all the serving size is an essential parameter to be considered when appraising the nutritional value and health benefit of coffee brews.

Conclusions

Significant amounts of copper-complexing ligands, nitrogen containing organic matter, and polysaccharide-like compounds with sulphate and/or carboxylic groups occur in commonly consumed coffee brews. The considerable copper-complexing capacity reported in coffee brews is due to their content in chlorogenic and caffeic acids, but also due to melanoidins. We observed differences in organic matter that may be linked to coffee-to-water ratio. Coffee-to-water ratio along with brewing time may be linked to different concentrations of phenolics that were, however, significant in all coffee brews. The DPPH-radical scavenging and reducing power revealed a high antioxidant activity in espresso brew that still did not exhibit a high ability to protect serum lipids from oxidation. The sipping rate of Greek/Turkish coffee brews did not affect significantly bioactive compounds and antioxidant potential; however, fast sipping brew exhibited higher resistance to serum lipid oxidation, higher release of copper ligands, and lower release of nitrogen containing organic matter and polysaccharide-like compounds with sulphate and/or carboxylic groups (CAC at pH 8.2 and 5.1, respectively).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with Ethics requirements All procedures performed in studies involving humanparticipants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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