

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Interactions between major chlorogenic acid isomers and chemical changes in coffee brew that affect antioxidant activities



Ningjian Liang^a, Wei Xue^b, Pierre Kennepohl^b, David D. Kitts^{a,*}

^a Department of Food, Nutrition, and Health, Faculty of Land and Food Systems, University of British Columbia, 2205 East Mall, Vancouver, British Columbia, Canada

^b Department of Chemistry, Faculty of Science, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada

ARTICLE INFO

Article history:

Received 20 April 2016

Received in revised form 9 June 2016

Accepted 14 June 2016

Available online 16 June 2016

Keywords:

Coffee

Chlorogenic acid isomer

Antioxidant

Principle component analysis

ABSTRACT

Coffee bean source and roasting conditions significantly ($p < 0.05$) affected the content of chlorogenic acid (CGA) isomers, several indices of browning and subsequent antioxidant values. Principal component analysis was used to interpret the correlations between physiochemical and antioxidant parameters of coffee. CGA isomer content was positively correlated ($p < 0.001$) to capacity of coffee to reduce nitric oxide and scavenge Frémy's salt. Indices of browning in roasted coffee were positively correlated ($p < 0.001$) to ABTS and TEMPO radical scavenging capacity, respectively. Only the CGA content of coffee corresponded to intracellular antioxidant capacity measured in Caco-2 intestinal cells. This study concluded that the intracellular antioxidant capacity that best describes potential health benefits of coffee positively corresponds best with CGA content.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Coffee is consumed globally and recent epidemiological studies have observed that coffee consumption may reduce the risk of type 2 diabetes (Ding, Bhupathiraju, Chen, van Dam, & Hu, 2014), colorectal cancer (Li, Ma, Zhang, Zheng, & Wang, 2012), and death attributed to cardiovascular disease (Ding et al., 2015). The physiological chemistry that underlies these claims often involves several bioactive compounds that exhibit antioxidant activity. Such activity in brewed coffee is attributed to phenolic compounds that are originally present in green coffee beans and to products derived from browning that arise from advanced phenol-nitrogen condensation reactions, or Maillard reaction products (MRP), generated during roasting (Liu & Kitts, 2011). Chlorogenic acids (CGAs), which include many different isomeric forms, are the

predominant phenolic compounds in coffee beans. The major CGA isomers found in coffee include as 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) (Farah, Monteiro, Donangelo, & Lafay, 2008). Various factors that significantly affect CGA isomer profiles in green coffee beans include coffee plant variety, geographic location and the conditions under which the beans are roasted to produce consumable brews (Anthony, Clifford, & Noiro, 1993; Campa, Doubeau, Dussert, Hamon, & Noiro, 2005; Ky et al., 1999). Some MRP derivatives contribute to flavor and aroma, whereas others are incorporated into high molecular weight coffee melanoidins that provide color and antioxidant activity (Bekedam, Schols, Van Boekel, & Smit, 2008; Liu & Kitts, 2011; Wijewickreme & Kitts, 1998).

Comparing the relevance of antioxidant activity in coffee across studies is challenging because various types of chemical assays produce inconsistent results. This is due to the complex composition of coffee components that are specific to the brews tested in several studies (Liang & Kitts, 2014). The antioxidant capacity of coffee has also been assessed using oxygen radical scavenging assay (ORAC) assays. Nitric oxide (NO) assay is a reducing assay that can be measured chemically *in vitro* using Griess reagent. Electron paramagnetic resonance (EPR) spectroscopy is also used to measure the antioxidant capacity of coffee brew through by selectively quenching persistent stable radicals such

Abbreviations: CGA, chlorogenic acid; 3-CQA, 3-caffeoylquinic acid; 4-CQA, 4-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; 3,4-diCQA, 3,4-dicaffeoylquinic acid; 3,5-diCQA, 3,5-dicaffeoylquinic acid; 4,5-diCQA, 4,5-dicaffeoylquinic acid; ORAC, oxygen radical absorbance capacity; ABTS, 2,2'-azino-bis(3-ethylbenzothiazol-6-sulphonate); DPPH, 2,2-diphenyl-1-picrylhydrazyl; NO, nitric oxide; DCFH-DA, dichlorofluorescein diacetate; Trolox, 2,2,6,6-tetramethylpiperidine-1-oxyl; EPR, electron paramagnetic resonance; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MEM, minimum essential medium; HPLC, high performance liquid chromatography; HMW, high molecular weight; MRP, Maillard reaction products.

* Corresponding author.

E-mail address: david.kitts@ubc.ca (D.D. Kitts).

<http://dx.doi.org/10.1016/j.foodchem.2016.06.041>

0308-8146/© 2016 The Authors. Published by Elsevier Ltd.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

as 4-hydroxy-2,2,6,6-tetra-methylpiperidine,N-oxyl (TEMPO) or Frémy's salt (Brezová, Šlebodová, & Staško, 2009). In summary, although such chemical assays are valuable in determining the antioxidant activity of coffee, the radical species involved and methods of redox quantitation must be considered when interpreting the results in reference to human health potential.

Isomer of CGA are easily altered under coffee roasting conditions, and exhibit a range of free radical scavenging capacities when transformed to derivatives that arise during high-temperature processing. Thus, the antioxidant capacity of brewed coffee will largely depend on the overall sensitivity of all relevant coffee molecules that can react in the defined mechanism(s) involved in the antioxidant assays used to quantify activity. Hence, the varying composition of CGA isomers among coffee brews and the involvement of coffee browning components added are responsible for the wide range of antioxidant activities found in coffee preparations.

The present study aimed to determine the effects of geographic regions from which *Arabica* coffee beans are sourced, of processing factors, on profiles of CGA isomers. We also aimed to apply PCA to interpret the relationship between the composition of CGA isomers as well as the production of browning products and the antioxidant activity of various coffee brews assessed using specific chemical and biological assays. The latter provided a context for the antioxidant activity of brewed coffee that included potential limitations that influence cellular uptake and responses.

2. Materials and methods

2.1. Materials

We purchased the following from Sigma (St. Louis, MO, USA): ABTS, 2,2'-azobis(2-methylpropanamidinedihydrochloride) (AAPH), dichlorofluorescein diacetate (DCFH-DA), 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein, sulphanylamine, naphthyl ethylenediamine dichloride, sodium nitroprusside, TEMPO, Frémy's salt; NaNO_2 , 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), minimum essential medium (MEM), hydrochloric acid, citric acid, phosphoric acid, methanol (HPLC grade), and sodium dodecyl sulfate. Fetal bovine serum (FBS), penicillin, streptomycin were purchased from Gibco® (Grand Island, NY, USA) and CGA isomers, 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA were obtained from Cerilliant Corporation (Round Rock, TX, USA) and Chengdu Must Bio-Technology Co., Ltd (Chengdu, Sichuan, China). Quartz EPR tubes were purchased from Wilmad Lab-Glass (Vineland, NJ, USA).

2.2. Coffee brew preparation

Arabica green coffee beans obtained from Sumatra, Dominican, Peru, Ethiopia, and Papua New Guinea (PNG) were roasted at 210 °C for 12 min (light roast), 223 °C for 14 min (medium roast), and 235 °C for 15 min (dark roast) in a commercial roaster (Nep-tune, Probat Burns, Vernon Hills, IL, USA) to achieve specific time-temperature thermal processing conditions for each roast. Coffee brews were prepared by solid-liquid extraction with deionized water as described with modifications (Anese & Nicoli, 2003). Briefly, coffee beans were ground to a powder and passed through a sieve (diameter <0.5 mm). The brewing conditions were: 10 min contact of ground coffee with boiling water (100 °C) at a coffee to water ratio of 1:20 (w:v), employing a drip system. The brew was cooled on ice and filtered through a membrane (Whalman No. 4) under a vacuum. Final brews were freeze-dried and stored at -80 °C.

2.3. Measurement of color and browning of coffee brews

Color of freeze-dried coffee brew extracts was analyzed using a Color Quest XE spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA). Color characteristics were expressed in Hunter *L* (lightness), *a* (red-green component) and *b* (yellow-blue component) color scales. Freeze-dried extracts of brewed coffee were dissolved in deionized water to a final concentration of 1 mg/ml and absorbance was measured at 420 nm using a Lambda 25 UV-vis spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

2.4. Determination of high molecular weight (HMW) MRP (>10 kDa) content in coffee brew extracts

Freeze-dried extracts of brewed coffee were dissolved in deionized water to a concentration of 2 mg/ml and then fractionated by ultrafiltration (Millipore, Billerica, MA, USA) through a membrane with a 10 kDa nominal molecular mass cut-off membrane as described (Delgado-Andrade, Rufian-Henares, & Morales, 2005). Samples were ultrafiltered under nitrogen (pressure of 30 psi) and residues were lyophilized to calculate the HMW MRP content in coffee brew extracts.

2.5. Determination of CGA isomers and caffeine contents in coffee brew extracts by high performance liquid chromatography (HPLC)

CGA isomers in extracts of coffee brews were quantified by HPLC according the method developed by Fujioka and Shibamoto (Fujioka & Shibamoto, 2008) using an Agilent 1100 instrument (Agilent Technologies Inc., Palo Alto, CA, USA). CGA isomers were separated by C-18 column (250 mm × 4.6 mm i.d., 5 μm particle size) using a mobile phase that comprising 10 mM citric acid (A) and 100% methanol (B) at a flow rate of 1 ml/min. Injected samples (5 μL) were eluted with a gradient consisting of 85% A from 0 to 5 min, 85%-60% A from 5 to 40 min, maintain at 60% A from 40 to 85 min, 60%-85% A from 85 to 90 min. The detector was set at 325 nm and 276 nm for CGA isomers and caffeine, respectively. The limit of quantification was defined as the minimum concentration at the signal-to-noise ratio (S/N) of 10:1. All samples were analyzed in triplicate.

2.6. ABTS assay

The ABTS assays proceeded as described with modifications (Almeida et al., 2011). Radical ABTS cations were generated by mixing ABTS (7 mM) with potassium persulfate (2.45 mM) in distilled water. Serial concentrations of Trolox (0 ~ 0.0125 mg/mL) or coffee brew extracts (0.01 ~ 0.5 mg/ml) mixed with ABTS radical working solution (180 μL; absorbance, 0.70 ± 0.02 at 734 nm) were incubated for 10 min at room temperature and then absorbance was measured at 734 nm. Blank wells contained only distilled water and the negative control comprised 20 μL water and 180 μL ABTS working solution. The% inhibition of ABTS by samples was defined as:

$$1 - (\text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{negative control}}) \times 100$$

The ratio between the slopes of the regression equations for the coffee samples and Trolox was defined as coffee antioxidant capacity. The results are expressed as mmol equivalents of Trolox/g of coffee brew extract (dry weight).

2.7. ORAC_{FL} assay

The antioxidant capacity of coffee brew extracts was determined using oxygen radical absorbance capacity fluorescein (ORAC_{FL}) assays as described by Kitts and Hu (Kitts & Hu, 2005). Briefly, 100 µL serial concentrations of Trolox (0 ~ 0.001 mg/mL) or coffee brew extract (0.001 ~ 0.02 mg/mL) in 75 mM phosphate buffer (pH 7.0) or Trolox standards (final concentration, 0–6.0 mM) and 60 µL of fluorescein (Sigma-Aldrich, St. Louis, MO, USA) with a final concentration of 60 nM, were added to 96-well black-walled plates and incubated at 37 °C for 10 min. Thereafter, AAPH (60 µL; 12 mM) was added and fluorescence depletion was monitored every minute for 60 min at excitation and emission wavelengths of 485 and 527 nm, respectively. The antioxidant activity of coffee brew extracts is expressed as the ratio between the regression equations slopes for coffee and Trolox. ORAC values are expressed as mmol Trolox Equivalent/g coffee brew extract.

2.8. Nitric oxide radical scavenging assay

The affinity of coffee brew extracts to scavenge nitric oxide radicals (NO[•]) was determined as previously described (Kang, Yokozawa, Kim, & Park, 2006). Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.4) was incubated with s Trolox (0 ~ 0.0025 mg/mL) or coffee brew extracts (0.05 ~ 0.5 mg/mL) at 25 °C for 150 min. The amount of NO[•] was quantified by measuring nitrite using the Griess reagent (1% sulphanimide, 0.1% naphthylethylenediamine dichloride in 3% phosphoric acid). The absorbance of the chromophore formed during nitrite diazotization with the Griess reagent was read at 546 nm. The negative control contained water instead of test samples. All analyses were repeated three times in triplicate. The % inhibition of nitric oxide radicals was defined as:

$$1 - (\text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{negative control}}) \times 100.$$

The ratio between the regression equation slopes for coffee samples and for Trolox was defined as nitric oxide radical-scavenging ability and is expressed as mmol equivalents of Trolox/g of coffee brew extract (dry weight).

2.9. Study antioxidant activity by EPR spectroscopy

The antioxidant activities of coffee brew extracts added to known oxidants were assessed using EPR spectroscopy. To quench the TEMPO radicals, coffee extracts (5–20 mg/ml) were mixed with an equal volume of TEMPO (1 mM in distilled water) and the reaction was monitored for 40 min using an Elexsys E500 series continuous wave EPR spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). Samples were analyzed at a frequency of 9.40 GHz (X-band), 100 kHz field modulation, 5 gauss modulation amplitude, 1.28 ms time constant, 5.12 ms conversion time and 0.64 mW microwave power. The ability of coffee extracts to inhibit TEMPO radicals was calculated as: % Inhibition = $(I_0 - I/I_0) \times 100\%$, where I_0 is the intensity of the EPR spectrum of TEMPO at $t = 0$ min mixing; I is the intensity of the EPR spectrum of TEMPO at 40 min after mixing with coffee extract. The % inhibition vs. coffee extract concentration was plotted to obtain a regression equation, with Trolox being the reference standard to express the antioxidant activity of various extracts of coffee brews. Trolox (0.0125 ~ 0.125 mg/mL) or coffee brew extract (5 ~ 20 mg/mL) was reacted with an equal volume of TEMPO (1 mM) and a regression equation was determined for Trolox/sample concentration vs. % of inhibition. The ratio between the regression equation slopes for coffee extracts and Trolox, defined the antioxidant capacity of individual extracts of coffee

brew. The results are expressed as mmol equivalents of Trolox/g of coffee brew extract (dry matter).

The antioxidant activity of coffee brew extracts towards inorganic radicals was also assessed using Frémy's salt. Coffee extracts (0.075 ~ 2.0 mg/mL) were reacted with an equal volume of Frémy's salt (1 mM in 50 mM phosphate buffer; pH 7.4) and then free-radical intensity was monitored immediately ($t = 0$) and at 30 ($t = 30$) min thereafter. The EPR spectrometer operating conditions comprised: field modulation, 100 kHz; modulation amplitude, 3 gauss; time constant, 2.56 ms; conversion time, 10.49 ms and attenuation, 10 dB. The reference was Trolox (0.006–0.05 mg/mL) reacted with an equal volume of Frémy's salt (1 mM) in phosphate buffer for 30 min. The antioxidant activity of coffee brew extract determined using Frémy's salt is expressed as mmol equivalents of Trolox/g of coffee brew extract (dry weight).

2.10. Cell culture

Caco-2 cells (ATCC, Manassas, VA, USA) were maintained in MEM supplemented with 10% FBS, penicillin (100 kU/L) and streptomycin (100 g/L) at 37 °C under a 5% CO₂ atmosphere in a humidified incubator. After reaching confluence, the cells were subcultured and maintained with medium changes every 2–3 days.

2.11. Assessment of cell viability

The viability of Caco-2 cells (3.2×10^5 /mL) incubated with coffee brew extracts in 96-well plates was determined using MTT assays. After 21 days, the cells were incubated for 24 h at 37 °C with medium alone (negative control) or supplemented with coffee brew extract (0.125–1 mg/mL). The cells were incubated with medium containing MTT (0.5 mg/mL) for 4 h in the dark at 37 °C. Formazan was determined by measuring absorbance at 540 nm using a Multiscan Spectrum microplate reader (ThermoFisher Scientific, Waltham, MA, USA).

2.12. Intracellular oxidative assay in Caco-2 cells

The effect of coffee brew extracts on peroxy radical-initiated intracellular oxidation was assessed as described by Hu, Kwok and Kitts (Hu, Kwok, & Kitts, 2005). Caco-2 cells were seeded in 96-well plates at a density of 3.2×10^5 /mL in MEM with medium changes every 2–3 days. The cells were incubated after 21 days with control medium or medium containing coffee brew extract (0.5 and 1 mg/mL) for 24 h at 37 °C, rinsed with PBS (pH 7.2) and incubated with a DCFH-DA probe (5 µM) in PBS at 37 °C for 30 min. After rinsing with fresh PBS, intracellular oxidation was initiated by adding 100 µL of 1 mM AAPH. Fluorescence emission was measured using a Fluoroskan Ascent™ FL luminometer (Thermo Fisher Scientific, Waltham, MA, USA) at excitation and emission wavelengths of 485 and 527 nm, respectively, one hour after adding AAPH. The positive and negative controls comprised the DCFH-DA probe with and without AAPH, respectively. The % fluorescence inhibition is expressed as: $(F_{pc} - F_{\text{coffee brew}}) / (F_{pc} - F_{nc}) \times 100\%$, where F_{pc} , $F_{\text{coffee brew}}$ and F_{nc} represent the fluorescence intensity of the positive control, coffee brew extract and the negative control, respectively.

2.13. Statistics

All data were analyzed using Origin, version 9.2 (OriginLab Corporation, Northampton, MA, USA) and are expressed as means ± standard deviation (SD). Post-hoc Bonferroni tests were performed to investigate if samples were significantly different among each other (Granato, de Araújo Calado, & Jarvis, 2014). We considered p values <0.05 as being statistically significant

unless otherwise indicated. PCA proceeded on a data matrix of 60 lines (samples) and 16 variables comprising nine physiochemical parameters and six antioxidant values of coffee brew extracts to determine correlations between physiochemical characteristics and the antioxidant activities of coffee brew extracts. The data was standardized by subtracting the means of values for each variable, from each variable value and dividing the result by the standard deviation of the values for each variable (Hossain, Patras, Barry-Ryan, Martin-Diana, & Brunton, 2011). The purpose of standardization was to make sure that each parameter carried an equal weight in the principal component calculation.

3. Results and discussion

3.1. Composition of coffee brews along with assessment of browning

Fig. 1 shows the composition of six CGA isomers in brew extracts made from *Arabica* coffee beans processed under green, light, medium, and dark roasting conditions and derived from

Sumatra, Dominican, Peru, Ethiopia, and PNG. The most abundant CGA isomer was 5-CQA, which accounted for 69–74% of total CGA in all coffee brew extracts, especially those from green beans. The range of differences among samples sourced from five regions was relatively small. The 5-CQA content decreased >85% in coffee brews prepared from dark roasted beans compared with those prepared from non-roasted green beans derived from the same region. For example, the total CGA content in coffee brew extracts of light-, medium- and dark-roasted Sumatran beans decreased to 35.60%, 62.91%, and 80.60%, respectively, compared with the original CGA content in green beans. The 3-CQA and 4-CQA contents were higher in brews prepared from light-roasted, compared with green beans. This has been attributed to acyl migration via the formation of an *ortho*-ester intermediate (Deshpande, Jaiswal, Matei, & Kuhnert, 2014; Moon, Yoo, & Shibamoto, 2009). The content of CGA isomers in coffee brew prepared from roasted beans decreased in the order of 5-CQA > 4-CQA > 3-CQA > 3,4-diCQA > 4,5-diCQA > 3,5-diCQA. This is comparable mostly to the findings of a previous study in which the relative isomer content

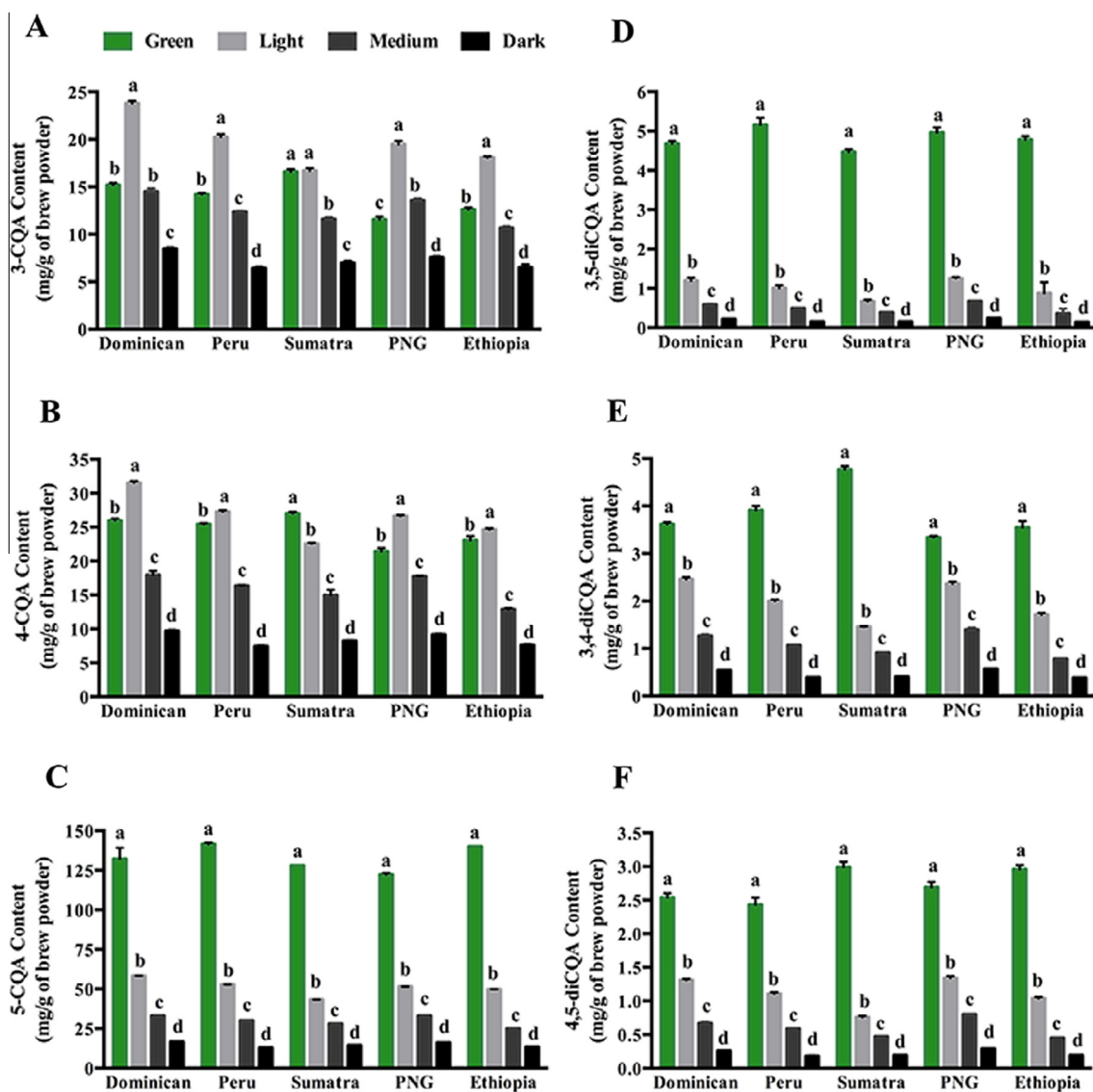


Fig. 1. (A) 3-CQA, (B) 4-CQA, (C) 5-CQA, (D) 3,5-diCQA, (E) 3,4-diCQA, and (F) 4,5-diCQA (mg) in freeze-dried coffee brew extract (g) prepared from coffee beans derived from 5 different regions (Dominican, Peru, Sumatra, PNG, Ethiopia) with different roasting conditions (Green, Light, Medium, and Dark roasted beans). Data were expressed as Mean \pm SD, $n = 3$. ^{a-d}Denoted significant ($p < 0.05$) differences in CGA isomer content among coffee brew extracts derived from the beans with different roasting degree within the same geographic region by using Bonferroni post-tests. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in commercially blended and brewed coffee (Fujioka & Shibamoto, 2008).

Table 1 presents the total CGAs and caffeine contents, along with browning parameters that included color characteristics (E), absorbance at 420 nm and the recovery of HMW MRPs (MW >10 kDa) in brew extracts of coffee beans. The impact of the geographic region where the beans were sourced and the degree of roasting significantly affected the CGA content in *Arabica* coffee brews, which partially explains the relative changes in CGA content among various samples. We previously reported that CGA losses are the greatest in dark-roasted coffee (Liu & Kitts, 2012), as have others who also attributed this fact to the conversion of CGA isomers to precursors incorporated in melanoidins (Perrone, Farah, & Donangelo, 2012). Although this appears to be true of roasted coffee in general, the magnitude of the CGA disappearance in medium and dark roasted coffee brews did not correspond to a relative difference in various parameter measures of MRP browning (namely, E , 420 nm absorbance and >10 kDa MRP) in the same brews. One explanation for this might be the absence of specific differences in the amount of MRP fractions with MW >10 kDa in medium to dark coffee brews, despite parallel significant decreases in all CGA isomers. Since our coffee brews represented only water soluble MRP, we might have underestimated the complete transformation to HMW melanoidins by not fully recovering all HMW MRP.

3.2. Antioxidant activity of coffee brew extract evaluated by ABTS, ORAC, and NO assays

Table 2 shows the antioxidant activities of various coffee brews evaluated by chemical methods. Coffee brew extracts tested for chemical based antioxidant activity exhibited a relatively higher Trolox equivalent in ORAC, than in ABTS or NO inhibition assays, respectively. Although absolute antioxidant values varied depending on the chemical assay, trends were similar between green coffee and roasted coffee brews using ORAC and ABTS assays, whereas free radical scavenging capacity was actually lower ($p < 0.05$) in green bean brews compared with roasted coffee brews. Results from ABTS and ORAC assays also showed that coffee brews derived

from medium and dark roasts, respectively, were less effective at scavenging radicals compared with light-roast brews ($p < 0.05$). Others examining the effect of roasting conditions on the antioxidant activity of coffee brews using Folin-Ciocalteu and ABTS assays also reported maximum antioxidant activity in light to medium-roasted coffee brews (Smrke, Opitz, Vovk, & Yeretizian, 2013). The antioxidant activity in light-roast coffee brews determined using both ABTS assay and ORAC assays was maximal in coffee from Dominica and Peru, but not from Sumatra, PNG and Ethiopia, indicating the existence of some varietal differences. Of interest was the fact that these particular varieties also exhibited modest differences in the generation of MRP parameters. We previously studied the relative contributions of CGA and MRP in dark roasted coffee brews measured using ABTS and ORAC. The results indicated that a major source of antioxidant activity could be sub-structures of MRP that are not components of the chromophore (Liu & Kitts, 2011). This conclusion explains how changes in MRP parameters, such as in the color and recovery of MW >10 kDa MRPs do not always follow predicted antioxidant activities. Others have also reported that degradation products of CGA isomers in brewed coffee that are not involved in MRP, have antioxidant activity (Kamiyama, Moon, Jang, & Shibamoto, 2015). Moreover, we discount the influence of caffeine as a factor in the overall difference observed in antioxidant capacity of different brews, since caffeine concentrations were not affected by our roasting conditions, and they are relatively more stable during the brewing process than CGA isomers (Crozier, Stalmach, Lean, & Crozier, 2012; Liu & Kitts, 2012).

The mechanisms of interaction between coffee components and free radicals will also depend on a combination of differences in the molecular structures of antioxidant components and the chemical characteristics of free radicals that are involved in the assay system. Free radicals vary in terms of relative affinity for interacting with atoms that have the highest electron density. The peroxy radical used for ORAC testing interacts with chlorogenic acid at hydroxyl groups attached to the carbon atom with the largest electronic density in the benzene ring. This is different to the ABTS radical, for which interaction with chlorogenic acid depends on the degree of ionization with carboxyl and phenolic hydroxyl groups.

Table 1

Total chlorogenic acids and caffeine content with color characteristics, browning and HMW MRPs in coffee brew extracts.¹

Region	Roasting degree	Total CGAs content (mg/g)	Caffeine (mg/g)	E	Absorbance at 420 nm	HMW MRPs (g/100 g)
Dominican	Green	184.18 ± 7.96 ^a	37.38 ± 0.98 ^a	4045.24 ± 12.45 ^a	0.22 ± 0.01 ^a	ND
	Light	118.61 ± 0.76 ^b	36.89 ± 0.34 ^a	1482.56 ± 15.46 ^b	0.55 ± 0.01 ^b	40.04 ± 0.97 ^a
	Medium	68.32 ± 1.30 ^c	37.04 ± 0.44 ^a	944.05 ± 12.68 ^c	0.59 ± 0.00 ^c	37.41 ± 1.27 ^b
	Dark	35.74 ± 1.43 ^d	37.58 ± 1.05 ^a	576.88 ± 13.57 ^d	0.59 ± 0.00 ^c	39.42 ± 0.99 ^a
Peru	Green	192.70 ± 1.92 ^a	32.43 ± 0.95 ^a	4060.05 ± 11.82 ^a	0.23 ± 0.00 ^a	ND
	Light	104.44 ± 0.78 ^b	31.52 ± 0.84 ^a	1535.59 ± 16.37 ^b	0.55 ± 0.00 ^b	39.76 ± 1.93 ^a
	Medium	61.37 ± 0.26 ^c	30.22 ± 1.00 ^a	910.24 ± 18.42 ^c	0.61 ± 0.00 ^c	35.42 ± 2.24 ^b
	Dark	27.40 ± 0.40 ^d	30.17 ± 1.29 ^a	469.46 ± 16.47 ^d	0.61 ± 0.01 ^c	39.49 ± 1.70 ^a
Sumatra	Green	183.95 ± 1.06 ^a	33.01 ± 0.50 ^a	4013.08 ± 20.46 ^a	0.24 ± 0.00 ^a	ND
	Light	85.42 ± 0.65 ^b	32.44 ± 0.89 ^a	1311.06 ± 19.72 ^b	0.53 ± 0.00 ^b	36.41 ± 1.72 ^a
	Medium	56.20 ± 1.15 ^c	32.42 ± 0.81 ^a	814.50 ± 18.37 ^c	0.58 ± 0.08 ^c	35.42 ± 1.56 ^a
	Dark	30.36 ± 0.46 ^d	31.51 ± 0.79 ^a	533.04 ± 16.38 ^d	0.58 ± 0.00 ^d	37.16 ± 1.34 ^a
PNG	Green	166.33 ± 1.84 ^a	33.63 ± 1.03 ^a	4081.51 ± 15.27 ^a	0.23 ± 0.01 ^a	ND
	Light	102.53 ± 0.77 ^b	33.67 ± 0.96 ^a	1387.26 ± 14.33 ^b	0.54 ± 0.00 ^b	33.60 ± 2.25 ^{ab}
	Medium	67.55 ± 0.49 ^c	32.50 ± 1.05 ^a	898.46 ± 14.73 ^c	0.58 ± 0.00 ^c	30.72 ± 2.57 ^a
	Dark	33.91 ± 0.37 ^d	33.66 ± 0.96 ^a	464.35 ± 16.93 ^d	0.59 ± 0.00 ^c	35.52 ± 2.08 ^b
Ethiopia	Green	186.99 ± 1.74 ^a	32.67 ± 0.56 ^a	4083.31 ± 15.38 ^a	0.22 ± 0.01 ^a	ND
	Light	96.2 ± 0.78 ^b	32.34 ± 1.01 ^a	1155.25 ± 13.25 ^b	0.57 ± 0.00 ^b	39.26 ± 2.27 ^a
	Medium	50.38 ± 0.60 ^c	32.70 ± 0.46 ^a	568.70 ± 14.62 ^c	0.58 ± 0.00 ^c	37.39 ± 2.09 ^a
	Dark	28.15 ± 0.56 ^d	33.50 ± 1.07 ^a	415.65 ± 11.35 ^d	0.59 ± 0.00 ^c	38.38 ± 1.29 ^a

¹ Color characteristic, $E = L^2 + (a)^2 + (b)^2$, browning = absorbance at 420 nm; HMW MRPs = MW >10 kDa.

^{abcd} Represent means in columns that are significantly different among different roasting degree within the same geographic region by using Bonferroni post-tests. Level of confidence set at $p < 0.05$. ND = not detected in coffee brew extracts.

Table 2
Antioxidant activity of coffee brew extract evaluated by ABTS, ORAC, NO, Frémy's salt, and TEMPO assays.¹

Region	Roasting degree	Antioxidant activity (mmol Trolox Equivalent/g)				
		ABTS	ORAC	NO	Frémy's salt	TEMPO
Dominican	Green	0.41 ± 0.01 ^a	1.51 ± 0.04 ^a	0.62 ± 0.03 ^a	1.16 ± 0.03 ^a	0.00 ± 0.00 ^a
	Light	0.60 ± 0.03 ^b	2.04 ± 0.13 ^b	0.62 ± 0.03 ^a	0.94 ± 0.03 ^b	0.27 ± 0.01 ^b
	Medium	0.55 ± 0.02 ^c	1.87 ± 0.10 ^c	0.33 ± 0.02 ^b	0.66 ± 0.04 ^c	0.37 ± 0.01 ^c
	Dark	0.54 ± 0.02 ^c	1.43 ± 0.07 ^d	0.32 ± 0.03 ^b	0.67 ± 0.04 ^d	0.40 ± 0.01 ^d
Peru	Green	0.45 ± 0.02 ^a	1.69 ± 0.05 ^a	0.62 ± 0.04 ^a	1.28 ± 0.05 ^a	0.00 ± 0.00 ^a
	Light	0.61 ± 0.03 ^b	2.09 ± 0.07 ^b	0.61 ± 0.04 ^a	1.20 ± 0.03 ^b	0.30 ± 0.01 ^b
	Medium	0.53 ± 0.03 ^c	1.73 ± 0.06 ^a	0.35 ± 0.04 ^b	1.03 ± 0.04 ^c	0.34 ± 0.01 ^c
	Dark	0.53 ± 0.02 ^c	1.54 ± 0.06 ^c	0.34 ± 0.04 ^b	0.82 ± 0.04 ^d	0.38 ± 0.02 ^d
Sumatra	Green	0.42 ± 0.03 ^a	1.55 ± 0.06 ^a	0.67 ± 0.03 ^a	1.11 ± 0.03 ^{ac}	0.00 ± 0.00 ^a
	Light	0.53 ± 0.02 ^b	2.14 ± 0.10 ^b	0.69 ± 0.02 ^a	1.15 ± 0.06 ^{ab}	0.36 ± 0.01 ^b
	Medium	0.52 ± 0.03 ^b	1.93 ± 0.09 ^c	0.45 ± 0.03 ^b	0.91 ± 0.04 ^c	0.39 ± 0.02 ^c
	Dark	0.51 ± 0.03 ^b	1.74 ± 0.05 ^d	0.45 ± 0.03 ^b	0.80 ± 0.04 ^c	0.42 ± 0.02 ^d
PNG	Green	0.38 ± 0.02 ^a	1.65 ± 0.09 ^a	0.58 ± 0.04 ^a	1.17 ± 0.04 ^a	0.00 ± 0.00 ^a
	Light	0.51 ± 0.03 ^b	2.25 ± 0.11 ^b	0.60 ± 0.03 ^a	1.15 ± 0.04 ^a	0.34 ± 0.01 ^b
	Medium	0.51 ± 0.02 ^b	1.97 ± 0.10 ^c	0.38 ± 0.04 ^b	0.93 ± 0.04 ^b	0.35 ± 0.01 ^b
	Dark	0.49 ± 0.02 ^b	1.94 ± 0.07 ^c	0.38 ± 0.03 ^b	0.84 ± 0.03 ^c	0.36 ± 0.01 ^c
Ethiopia	Green	0.41 ± 0.02 ^a	1.73 ± 0.08 ^a	0.62 ± 0.05 ^a	1.13 ± 0.03 ^a	0.00 ± 0.00 ^a
	Light	0.53 ± 0.02 ^{bc}	2.16 ± 0.11 ^b	0.64 ± 0.05 ^a	1.04 ± 0.04 ^b	0.27 ± 0.02 ^b
	Medium	0.55 ± 0.03 ^b	2.16 ± 0.08 ^b	0.37 ± 0.03 ^b	0.92 ± 0.04 ^c	0.34 ± 0.01 ^c
	Dark	0.51 ± 0.02 ^c	1.66 ± 0.07 ^a	0.35 ± 0.04 ^b	0.81 ± 0.04 ^d	0.38 ± 0.01 ^d

¹ Values represent mean ± SD.

^{abcd} Represent means in columns that are significantly different among different roasting degree within the same geographic region by using Bonferroni post-tests. Level of confidence set at $p < 0.05$.

Our results showed that CGA isomers in green coffee beans will react to Frémy's salt, an inorganic stable radical, to a degree that is specific to the isomer. On the contrary, MRP generated partly from CGA transformation during roasting had stronger reactivity with the TEMPO radical, compared with Frémy's salt. Thus, the complex chemical composition of coffee brew being the basis for different atoms each possessing distinct electron densities, will ultimately govern the absolute response in different antioxidant assay measurements used to evaluate activity. Factors that include the variety or source of the coffee bean, exact roasting conditions and brewing times and procedures collectively influence the final composition of the coffee brew. These factors in turn might react differently with specific radicals used in various assays (Sacchetti, Di Mattia, Pittia, & Mastrocola, 2009) (Vignoli, Bassoli, & Benassi, 2011).

Despite notable decreases in total CGA in coffee brews derived from a light roast thermal process, the similar increase in scavenging activities observed with ABTS and peroxy radicals, respectively in all coffee varieties tested indicated that compounds derived from the browning reactions at a relatively earlier stage of roasting are more effective than the presence of total CGA in terms of interacting with these specific radical species. Maillard reactions that specifically occur in thermally processed coffee have been attributed to non-covalent interactions between CGA isomers and melanoidins to produce complexes with varying degrees of antioxidant activity (Wolfe & Liu, 2007). Delgado-Andrade et al. separated melanoidins from coffee brews prepared with different roasts and found that pure melanoidins contributed 10–15% to the total antioxidant activity of coffee brew when evaluated using ABTS assays (Delgado-Andrade et al., 2005). Others who have studied the contribution of CGA to the antioxidant activity of coffee brew concluded that the loss of CGA during the roasting process does not yield a decrease of antioxidant activity due to the simultaneous generation of browning products that also exhibit antioxidant activity (Perrone et al., 2012). The present study found similar NO reducing activities between light-roasted and unroasted coffee, but more activity ($p < 0.05$) compared with medium or dark roasted brews, respectively. Our results using Frémy's

salt and EPR measurements were similar but less striking. Table 2 also shows that green coffee did not have the same TEMPO radical scavenging capacity as roasted coffee brew extracts. In particular, TEMPO scavenging was highest among the dark roasted coffees, regardless of the geographic location where the coffee beans were sourced. Taken together, these results indicate that phenolic compounds in green coffee beans that are more reactive in both the NO reducing test and Frémy's salt were lost during roasting and likely transformed to compounds that were more responsive in the TEMPO radical assay. It is plausible that melanoidins generated during roasting that parallel the loss of phenolics, possess relatively lower hydrogen dissociation enthalpy or reducing capacity, which explains the typically lower antioxidant activity in both the NO and Frémy's salt assays. Hence, browning components generated during extended roasting processes do not possess enough antioxidant capacity to compensate for the loss of phenolic compounds that is attributed to higher antioxidant capacity in both light roasted and non-roasted brews. A shift to lower activity in the Frémy's salt assay with roasting corresponded to losses of CGA isomers, whereas the increased radical scavenging activity of TEMPO in dark roasts more effectively reflected the contribution of MRP to total antioxidant capacity.

3.3. Coffee brew extracts protect Caco-2 cells against AAPH-induced intracellular oxidative stress

Although chemical-based antioxidant assays are useful for analysing the antioxidant activity of different coffee brews, the results cannot be fully extrapolated to biological systems. Cellular antioxidant activity assays provide biologically relevant methods to measure the activity of antioxidants since they account for important factors such as cellular uptake, distribution and to some extent, metabolism in the final assessment of activity (Wolfe & Liu, 2007). We assessed cellular antioxidant activity in Caco-2 cells after their differentiation into intestinal-like cells (Takenaka et al., 2015). Coffee brews in a concentration range of 0.125–1.0 mg/mL did not adversely affect Caco-2 cell viability, but significantly inhibited fluorescence endpoint measures in

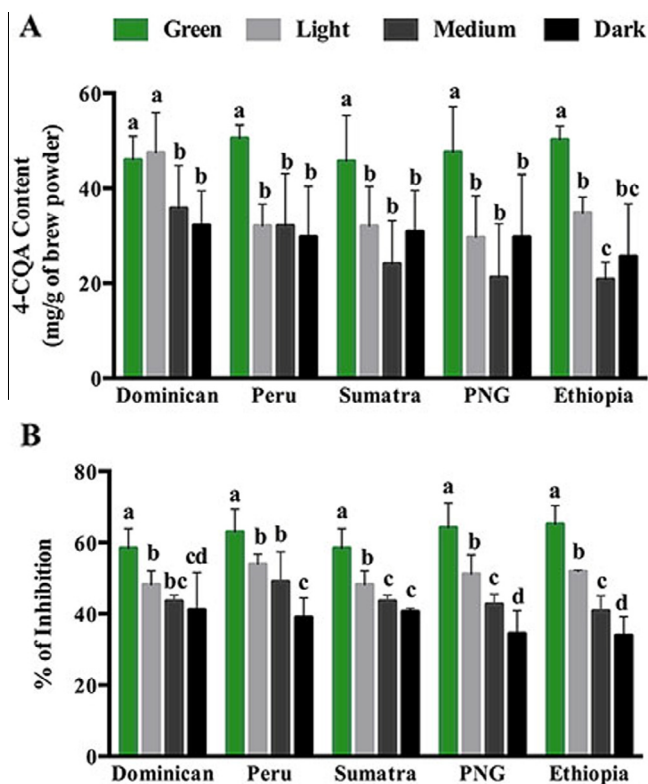


Fig. 2. Capacity of coffee brew extracts at concentration of (A) 0.5 mg/ml and (B) 1 mg/ml to prevent AAPH-induced oxidative stress determined by DCFH-DA probe in differentiated Caco-2 cells. Data were expressed as % of Inhibition on fluorescence signal. Different letters indicate significant differences ($p < 0.05$) among different roasted coffee brew extracts from each geographic region.

Caco-2 cells incubated with AAPH. All coffee brews, regardless of the source of the beans dose-dependently (0.5–1.0 mg/mL) reduced intracellular oxidative stress induced by AAPH, which generates peroxy radicals (Fig. 2a and b). At concentration of 1 mg/mL, brew extract generated intracellular inhibition effects in the order of brews from green coffee > light > medium > dark roasts. Intestinal cells uptake and metabolize CGA isomers that are relatively small molecules with known peroxy radical scavenging capacity (Gómez-Ruiz, Leake, & Ames, 2007). The decrease in the ratio (%) of intracellular oxidation in Caco-2 cells incubated with roasted coffee brew extracts, compared with green coffee brew extracts can be explained as the proportional losses of CGA isomers during roasting and the relatively reduced capacity of browning products to be absorbed into intracellular compartment to quench peroxy radicals. Since, MRP have scavenging capacity for peroxy radicals

in chemical assays, this finding could reflect limited bioavailability of HMW MRPs in Caco-2 cells.

3.4. The relationships between physicochemical characteristics and antioxidant properties

The results of a Pearson correlation analysis confirmed the relevance of physicochemical characteristics of coffee brews that influence antioxidant capacity estimated using different types of antioxidant assays (Table 3). The strong positive correlations ($r = 0.748$ – 0.783) between specific CGA isomers (5-CQA, 3,4-diCQA, 4,5-diCQA, and 3,5-diCQA) and intracellular oxidative capacity contrasted with the negative correlations ($r = -0.984$ to -0.928) between specific CGA isomers and the finding of assays of radical scavenging capacity using TEMPO. We also observed a negative correlation ($r = -0.705$) between 5-CQA and ABTS radical scavenging capacity. This result agrees with recent findings that showed 5-CQA concentrations decreased gradually during roasting, but the ABTS radical scavenging capacity of coffee brew increased with increased roasting (Kocadağlı & Gökmen, 2016).

We also used PCA to help interpret relationships between the chemical composition of coffee brews and estimated antioxidant activity. Since CGA isomers and browning compounds are major contributors to the antioxidant activity of coffee, their concentrations were included in the PCA along with antioxidant activities determined from different types of assays. Two principle components, PC1 and PC2, with eigenvalue greater than one, explained 65.01% and 18.00% respectively, of the total variance in the data set.

Fig. 3 shows a PCA bi-plot that allows the display of information about both samples (dots) and variables (vectors) as a data matrix in a single figure. The blue scale represents the loading plot in which the projection of variables is reflected in PC1 and PC2 planes. This result indicated that the content of particular CGA isomers (5-CQA, 3,4-diCQA, 4,5-diCQA, and 3,5-diCQA) have strong positive correlation to the intracellular peroxy radical scavenging capacity and strong negative correlation to TEMPO radical scavenging capacity and browning. The black scale on the scatter plot provides the location of each sample point where levels of a given component tend to cluster with a particular characteristic. Fig. 3 shows that clustered sample points together have the same roasting degree. Green coffee samples had a high content of 5-CQA, dicaffeoylquinic acids and more intracellular antioxidant activity. Light-roasted coffee samples had a relatively high 3-CQA and 4-CQA content accompanied by powerful peroxy radical observed in ORAC assay. Medium- and dark-roasted coffee samples with melanoidin content clustered with TEMPO radical scavenging capacity. In summary, we analyzed physicochemical changes in green and roasted coffee beans that ultimately affect antioxidant

Table 3

Pearson correlation coefficients between physicochemical parameters and antioxidant activities in coffee brews.

Parameters	ABTS	ORAC	NO	Frémy's salt	TEMPO	Intracellular oxidative assay
Caffeine	-0.001	-0.149*	-0.006	-0.242**	-0.095	-0.026
3-CQA	0.196**	0.487***	0.678***	0.480***	-0.266***	0.402***
4-CQA	-0.129	0.285***	0.821***	0.655***	-0.595***	0.634***
5-CQA	-0.705***	-0.281***	0.696***	0.642***	-0.981***	0.783***
3,4-diCQA	-0.606***	-0.180*	0.748***	0.630***	-0.928***	0.754***
3,5-diCQA	-0.776***	-0.374***	0.613***	0.593***	-0.984***	0.748***
4,5-diCQA	-0.704***	-0.247**	0.704***	0.619***	-0.967***	0.771***
HMW MRPs	0.835***	0.444***	-0.513***	-0.522***	0.951***	-0.690***
420 nm absorbance	0.799***	0.406**	-0.611***	-0.564***	0.975***	-0.726***
E	0.499***	-0.040	-0.838***	-0.755***	0.867***	-0.796***

* Denoted significance set at $p < 0.05$.

** Denoted significance set at $p < 0.01$.

*** Denoted significance set at $p < 0.001$.

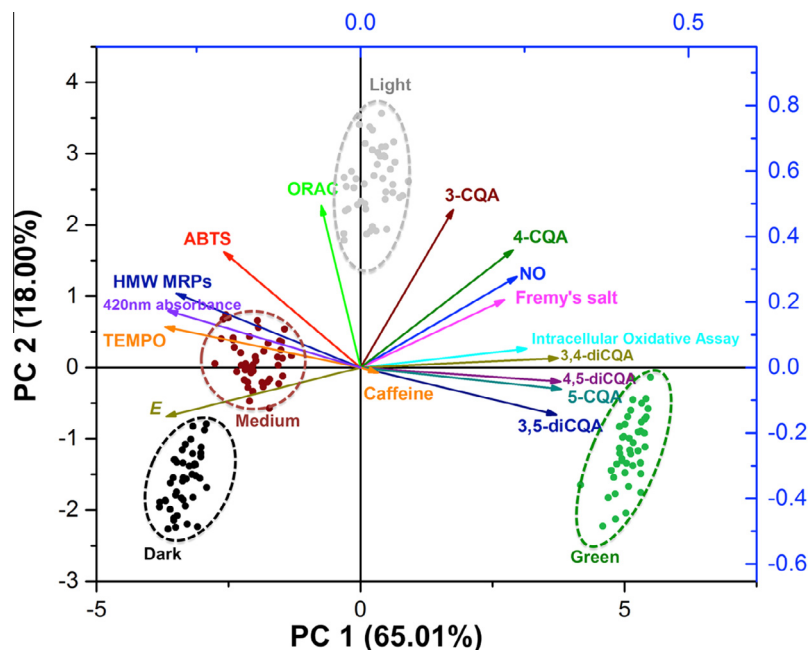


Fig. 3. Bi-plot of principal component analysis of antioxidant activity and coffee physicochemical characteristics of green, light, medium and dark roasted coffee brew extracts. The black scale denotes the coffee sample scatter plot and the blue scale denotes the scale for variable projection. Green, light, medium and dark refer the different roasting conditions used to produce the coffee brews. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

values among *Arabica* coffee brews. The findings, albeit specific to *Arabica* coffee, but sourced from five geographic locations, imply that the complex mixture of chemical components in coffee brews collectively influences the characteristic antioxidant activity, which in turn is relevant to the underlying mechanisms of action of various types of antioxidant assays.

4. Conclusion

We concluded that the complexity of coffee brews particularly that created by the influence of roasting, precludes using a single assay to adequately describe the antioxidant activity of coffee. Moreover, while chemical assays of free radical scavenging are useful to evaluate the quality attributes of a coffee source, cell-based antioxidant tests are more effective as predictors of bioactive potential that are relevant to human health. The use of PCA in the final interpretation of the data not only allowed for visual displays of the complex relationships among various physicochemical and functional coffee parameters including the CGA isomer content, but also provided an immediate view of the effects of processing and geographic region on each parameter.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (DDK). NL is a recipient of a 4 year UBC academic research scholarship. Authors want to thank Ingrid Elisia's technical help during this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.06.041>.

References

- Almeida, M. M. B., de Sousa, P. H. M., Arriaga, Â. M. C., do Prado, G. M., Magalhães, C. E. D. C., Maia, G. A., & de Lemos, T. L. G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International*, 44(7), 2155–2159.
- Anese, M., & Nicolini, M. C. (2003). Antioxidant properties of ready-to-drink coffee brews. *Journal of Agricultural and Food Chemistry*, 51(4), 942–946.
- Anthony, F., Clifford, M. N., & Noiro, M. (1993). Biochemical diversity in the genus *Coffea* L.: Chlorogenic acids, caffeine and moxambioside contents. *Genetic Resources and Crop Evolution*, 40, 61–70.
- Bekedam, E. K., Schols, H. A., Van Boekel, M. A., & Smit, G. (2008). Incorporation of chlorogenic acids in coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, 56(6), 2055–2063.
- Brezová, V., Šlebodová, A., & Staško, A. (2009). Coffee as a source of antioxidants: An EPR study. *Food Chemistry*, 114(3), 859–868.
- Campa, C., Doulbeau, S., Dussert, S., Hamon, S., & Noiro, M. (2005). Qualitative relationship between caffeine and chlorogenic acid contents among wild *Coffea* species. *Food Chemistry*, 93(1), 135–139.
- Crozier, T. W. M., Stalmach, A., Lean, M. E. J., & Crozier, A. (2012). Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. *Food & Function*, 3(1), 30–33.
- Delgado-Andrade, C., Rufian-Henares, J. A., & Morales, F. J. (2005). Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. *Journal of Agricultural and Food Chemistry*, 53(20), 7832–7836.
- Deshpande, S., Jaiswal, R., Matei, M. F., & Kuhnert, N. (2014). Investigation of acyl migration in mono- and dicaffeoylquinic acids under aqueous basic, aqueous acidic, and dry roasting conditions. *Journal of Agricultural and Food Chemistry*, 62, 9160–9170.
- Ding, M., Bhupathiraju, S. N., Chen, M., van Dam, R. M., & Hu, F. B. (2014). Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: A systematic review and a dose-response meta-analysis. *Diabetes Care*, 37(2), 569–586.
- Ding, M., Satija, A., Bhupathiraju, S. N., Hu, Y., Sun, Q., Han, J., ... Hu, F. B. (2015). Association of coffee consumption with total and cause-specific mortality in 3 large prospective cohorts. *Circulation*, 132(24), 2305–2315.
- Farah, A., Monteiro, M., Donangelo, C. M., & Lafay, S. (2008). Chlorogenic acids from green coffee extract are highly bioavailable in humans. *Journal of Nutrition*, 138(12), 2309–2315.
- Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chemistry*, 106(1), 217–221.
- Gómez-Ruiz, J. Á., Leake, D. S., & Ames, J. M. (2007). In vitro antioxidant activity of coffee compounds and their metabolites. *Journal of Agricultural and Food Chemistry*, 55(17), 6962–6969.
- Granato, D., de Araújo Calado, V. M., & Jarvis, B. (2014). Observations on the use of statistical methods in Food Science and Technology. *Food Research International*, 55, 137–149.
- Hossain, M. B., Patras, A., Barry-Ryan, C., Martin-Diana, A. B., & Brunton, N. P. (2011). Application of principal component and hierarchical cluster analysis to classify

- different spices based on in vitro antioxidant activity and individual polyphenolic antioxidant compounds. *Journal of Functional Foods*, 3(3), 179–189.
- Hu, C., Kwok, B. H. L., & Kitts, D. D. (2005). Saskatoon berries (*Amelanchier alnifolia* Nutt.) scavenge free radicals and inhibit intracellular oxidation. *Food Research International*, 38(8–9), 1079–1085.
- Kamiyama, M., Moon, J.-K., Jang, H. W., & Shibamoto, T. (2015). Role of degradation products of chlorogenic acid in the antioxidant activity of roasted coffee. *Journal of Agricultural and Food Chemistry*, 63(7), 1996.
- Kang, K. S., Yokozawa, T., Kim, H. Y., & Park, J. H. (2006). Study on the nitric oxide scavenging effects of ginseng and its compounds. *Journal of Agricultural and Food Chemistry*, 54(7), 2558–2562.
- Kitts, D. D., & Hu, C. (2005). Biological and chemical assessment of antioxidant activity of sugar-lysine model Maillard reaction products. *Annals of the New York Academy of Sciences*, 1043, 501–512.
- Kocadağh, T., & Gökmen, V. (2016). Effect of roasting and brewing on the antioxidant capacity of espresso brews determined by the QUENCHER procedure. *Food Research International*. 10.1016/j.foodres.2016.03.004.
- Ky, C. L., Louarn, J., Guyot, B., Charrier, A., Hamon, S., & Noirot, M. (1999). Relations between and inheritance of chlorogenic acid contents in an interspecific cross between *Coffea pseudozanguebariae* and *Coffea liberica* var 'dewevrei'. *Theoretical and Applied Genetics*, 98(3), 628–637.
- Li, G., Ma, D., Zhang, Y., Zheng, W., & Wang, P. (2012). Coffee consumption and risk of colorectal cancer: a meta-analysis of observational studies. *Public Health Nutrition*, 16(2), 1–12.
- Liang, N. J., & Kitts, D. D. (2014). Antioxidant property of coffee components: assessment of methods that define mechanisms of action. *Molecules*, 19(11), 19180–19208.
- Liu, Y., & Kitts, D. D. (2011). Confirmation that the Maillard reaction is the principle contributor to the antioxidant capacity of coffee brews. *Food Research International*, 44(8), 2418–2424.
- Liu, Y., & Kitts, D. D. (2012). Activation of antioxidant response element (ARE)-dependent genes by roasted coffee extracts. *Food & Function*, 3(9), 950–954.
- Moon, J.-K., Yoo, H. S., & Shibamoto, T. (2009). Role of roasting conditions in the level of chlorogenic acid content in coffee beans: correlation with coffee acidity. *Journal of Agricultural and Food Chemistry*, 57(12), 5365.
- Perrone, D., Farah, A., & Donangelo, C. M. (2012). Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *Journal of Agricultural and Food Chemistry*, 60(17), 4265–4275.
- Sacchetti, G., Di Mattia, C., Pittia, P., & Mastrocola, D. (2009). Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. *Journal of Food Engineering*, 90(1), 74–80.
- Smrke, S., Opitz, S. E. W., Vovk, I., & Yeretian, C. (2013). How does roasting affect the antioxidants of a coffee brew? Exploring the antioxidant capacity of coffee via on-line antioxidant assays coupled with size exclusion chromatography. *Food & Function*, 4(7), 182–192.
- Takenaka, T., Naomoto, H., Kuze, J., Chiba, M., Iwao, T., & Matsunaga, T. (2015). Human small intestinal epithelial cells differentiated from adult intestinal stem cells as a novel system for prediction oral drug absorption in humans. *Drug Metabolism Reviews*, 47, 161–162.
- Vignoli, J. A., Bassoli, D. G., & Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chemistry*, 124(3), 863–868.
- Wijewickreme, A. N., & Kitts, D. D. (1998). Modulation of metal induced cytotoxicity by Maillard reaction products isolated from coffee brew. *Journal of Toxicology and Environmental Health*, 55, 151–168.
- Wolfe, K. L., & Liu, R. H. (2007). Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *Journal of Agricultural and Food Chemistry*, 55(22), 8896–8907.