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Abstract

Green coffee beans of *Coffea arabica* and *Coffea canephora* accessions from different geographical origin (Brazil, Colombia, Ethiopia, Honduras, Kenya, Mexico, Peru, Uganda and Vietnam) were extracted and the extracts analysed by HPLC-ESI-MS/MS for the identification and quantification of chlorogenic acids and caffeine content. Principal component and cluster analyses were used to identify chemical patterns separating the different species and accessions based on their geographical origin. *C. canephora* showed always a higher caffeine content with respect to *C. arabica*, whereas the *C. arabica* accessions from Kenya showed a higher chlorogenic acids and a lower caffeine content. The antioxidant capacity of green coffee extracts was assayed by the reducing power and DPPH assays. The antioxidant capacity correlated with the chlorogenic acids content. The results show that the *C. arabica* from Kenya possesses the highest chlorogenic acids/caffeine ratio and, among the *C. arabica* accessions, the highest antioxidant capacity. Therefore, the *C. arabica* from Kenya is the most suitable green coffee source for nutraceutical applications because of its high antioxidant capacity and low caffeine content.

Keywords

Coffea arabica, *Coffea canephora*, Rubiaceae, Green coffee, Chlorogenic acids, Caffeine, Geographical origin, Antioxidant capacity

1. Introduction

Coffee is one of the major industrial products; currently grown in about 80 countries of four continents it is one of the most popular beverages in the world (Anderson and Smith, 2002; Bicho et al., 2013). The production of coffee is based on three main *Coffea* (Rubiaceae) species: *C. arabica* L. (also known as arabica coffee), *C. canephora* Pierre ex A. Froehner (also known as robusta coffee) and *C. liberica* Bull. ex Hiern (also knowns as Liberian or Liberica coffee, or excelsa coffee) (Davis et al., 2006). The most important commercial species is *C. arabica*, which provides more than 95% of the

world's coffee (Vega et al., 2003). The chemical composition of green coffee is characterized by the presence of caffeine that can reach about 1.45% and 2.38% in *C. arabica* and in *C. canephora*, respectively (Bicho et al., 2013). Caffeine is well known as a mild stimulant of the central nervous system and the impact of caffeine on the human organism is well understood (Herman and Herman, 2013). Besides caffeine, green coffee contains high levels of phenolic compounds, with chlorogenic acids being the most abundant molecules (Clifford, 1999). Chlorogenic acids are a group of compounds comprising hydroxycinnamates, such as caffeic acid, ferulic acid, and *p*-coumaric acid, linked to quinic acid to form a range of conjugated structures known as caffeoylquinic acids, feruloylquinic acids, and *p*-coumaroylquinic acids all of which exist in several isomeric forms (Baeza et al., 2014; Clifford, 1999; Clifford and Knight, 2004; Del Rio et al., 2010; Ky et al., 2013).

Despite the controversial effects of caffeine and other compounds present in the roasted coffee, green coffee beans utilization is gaining an increasing interest in the nutraceutical and pharmaceutical industry because they are accepted as a rich source of compounds possessing antioxidant and radical scavenging activities (Anissi et al., 2014; Bresciani et al., 2014; Brezova et al., 2009 and references cited therein). Chlorogenic acids are the major contributors to the antioxidant properties of green coffee (Abrahao et al., 2010; Farah et al., 2006; Lima et al., 2010). On the other hand, a high content of caffeine lowers the applicability of green coffee extracts to prepare dietary supplements to be used as antioxidants, owing to the its known effects on the nervous system. Therefore, the market requires the identification of *C. arabica* and *C. canephora* accession with a high chlorogenic acid/caffeine ratio. The aim of our this study was the chemical characterization of several *C. arabica* and *C. canephora* accessions of green coffee from different geographic origin in order to identify specific chemical patterns and antioxidant capacity that may be suitable for nutraceutical and pharmaceutical applications.

2. Results and discussion

2.1. Total hydroxycinnamic acid derivatives and caffeine determination in Coffea canephora *and* Coffea arabica

The chemical composition of green coffee from different geographical origins is characterized by the presence of several chlorogenic acids, including esters of *trans*-cinnamic acids and quinic acid (Table 1). Caffeic acid (**1**), *p*-coumaroyl quinic acid (**2**), *p*-coumaroyl-*N*-tryptophan (**3**), chlorogenic acid (3-*O*-caffeoylquinic acid (**4**), neochlorogenic acid (5-*O*-caffeoylquinic acid, **5**), cryptochlorogenic acid (4-*O*-caffeoylquinic acid, **6**), caffeoyl-*N*-tryptophan (**7**), 3-*O*-feruloylquinic acid (**8**), 5-*O*-feruloylquinic acid (**9**), 3,4-*O*-dicaffeoylquinic acid (**10**), 3,5-*O*-dicaffeoylquinic acid (**11**), 4,5-*O*-dicaffeoylquinic acid (**12**), 3-*O*-feruloyl-4-caffeoylquinic acid (**13**), 3-*O*-feruloyl-5-caffeoylquinic acid (**14**), 4-*O*-feruloyl-5-caffeoylquinic acid (**15**), and caffeine (**16**) were isolated (Figure 1), as is typical of the *C. arabica* and *C. canephora* species (Alonso-Salces et al., 2009; Clifford, 1999; Clifford and Knight, 2004; Del Rio et al., 2010). Among phenolic compounds, neochlorogenic acid (**5**) was the most abundant compound in all samples analyzed, followed by chlorogenic acid (**4**) (Table 1). Caffeine (**16**) was the second most abundant compound in all analyzed samples (Table 1).

The total amount of identified compounds shows a clear and significant distinction between *C. canephora* from Uganda and Vietnam and all other *C. arabica* accessions. Among the latter, *C. arabica* from Ethiopia showed the lowest amount (Figure 2). These data are in agreement with the literature data, confirming a higher content of chlorogenic acids and caffeine in the *C. canephora* accessions (Alonso-Salces et al., 2009; Correia et al., 1995; Guerrero and Suarez, 2001).

One of the crucial parameters for the use of green coffee in dietary supplements is the ratio between caffeine and total chlorogenic acids, the latter being responsible for most of the antioxidant capacity of green coffee extracts. Moreover, concentrations of phenolic compounds and methylxanthines are considered reliable geographical indicators, as well as chemotaxonomical markers (Alonso-Salces et al., 2009). The plot of caffeine against total chlorogenic acids shows a clear distinction between *C. canephora* accessions from Uganda and Vietnam and all the other *C. arabica* accessions (Figure 3).

In particular, *C. canephora* (robusta) shows high levels of both caffeine and total chlorogenic acids, which agrees with the highest total content of extracted compounds (Figure 2). With the sole exception for Honduras accessions, a clear separation was found among *C. arabica* accessions according to their geographical origin, with a narrow differentiation based on caffeine and a broad differentiation based on their content of total chlorogenic acids (Figure 3). In particular, the accessions from Ethiopia and Mexico show the lowest content of both caffeine and chlorogenic acids, whereas the accessions from Kenya have a relatively low caffeine content and the highest content of chlorogenic acids (Figure 3).

2.2. Principal component analysis and cluster analyses of C. arabica *and* C. canephora *accessions*

Different classes of compounds have been used for the authentication of different coffee varieties (Alonso-Salces et al., 2009 and references cited therein) and for distinguishing the species and origins of green coffee bean samples of *C. arabica* and *C. canephora* from different geographic regions (Rodrigues et al., 2009; Sberveglieri et al., 2011; Serra et al., 2005; Wei et al., 2012). We used the data set of Table 1 to calculate a Principal Component Analysis (PCA). Figure 4 shows the scatter plot of the two main PCA factor loadings, which explained the 96% and 3% of the total variance for Factor 1 and Factor 2, respectively. A discrimination between the *C. canephora* accessions and all other *C. arabica* species was observed, mainly because of Factor 2 scores (negative for *C. canephora* and positive for *C. arabica*). Among *C. arabica*, a separation was found for *C. arabica* from Kenya and all other accessions, which were separated by positive values of Factor 1. A separation was also observed for the "caracol" accession from Brasil (C-Brasil). The "caracol" (or "snail" in Spanish, also called peaberry) is a natural mutation of the *C. canephora* coffee bean inside its cherry that affects about 5% of the world coffee. The phenotype of these "caracol" green coffee produces a single, rather than a double bean that appears smaller, denser and with a more rounded shape with respect to

the wild type (Cilas and Bouharmont, 2005; Giomo et al., 2008). As expected, the chemical pattern of the C-Brasil accession places it closer to the *C. canephora* group (Figure 4).

A Cluster Analysis (CA) calculated on the data matrix of Table 1, by using Euclidean distance with median linkage method, shows a first cluster that isolates the A-Kenya accessions because of the higher content of neochlorogenic acid (**5**). A second cluster is made by all *C. canephora* accessions, with a close statistical linkage between R-Vietnam1 and R-Vietnam2. This cluster is generated because of the high amount of caffeine (**16**). A third cluster isolates the C-Brasil accession because of the presence of 4-O-feruloyl-5-caffeoylquinic acid (**15**), whereas a fourth cluster gathers the A-Peru1 accession containing a higher content of caffeic acid (**1**). All other *C. arabica* accessions are present in the last cluster because of the high amount of chlorogenic acids. Several subclusters compose this last cluster (see Supplementary Figure S1).

2.3. In Vitro Antioxidant capacity of C. arabica *and* C. canephora

In order to assess the antioxidant capacity of the green coffee extracts, we evaluated their reducing power by ferric thiocyanate assay and the free radical scavenging activities by DPPH radical assay. In general, the extracts were more active as antioxidants when tested by the reducing power assay (Figure 5), since the steric accessibility of DPPH nitrogen-centered radical strongly affects the reaction rate of antioxidant compounds (Prior et al., 2005). Figure 5 depicts the scatter plot of IC_{50} values from both tests. As expected, the four *C. canephora* accessions showed the highest antioxidant activity (lowest IC_{50} values for both assays) with respect to all other accessions. Although weaker than chlorogenic acid (Zhao et al., 2015), caffeine as well possesses antioxidant capacity, as recently demonstrated in *in vivo* experiments (Tsoi et al., 2015). Therefore, the higher content of both chlorogenic acids and caffeine correlates with a higher antioxidant capacity.

Among the *C. arabica* accessions, the samples from Kenya showed the highest antioxidant capacity, followed by one of the accessions from Peru (A-Peru2) and one from Honduras (A-Honduras2). The

lowest antioxidant capacity was observed in the Ethiopian (A-Ethiopia) and Guatemala (A-Guatemala) accessions, whereas intermediate antioxidant activities were observed for the remaining green coffee accessions.

3. Concluding remarks

Green coffee is a natural source of antioxidants, with chlorogenic acids being the most active compounds. We confirmed that analysis of chlorogenic acids, along with the caffeine content, is a powerful tool for the chemical partition between *C. arabica* (arabica) and *C. canephora* (robusta). Our preliminary study indicates the possibility to extend this analysis for the identification of different green coffee accessions also at the geographical level, which could be indicative for further works using the approach here presented. However, to better assess the geographical origin and not only the species effect, more samples from different countries and from the same species should be evaluated. Finally, the antioxidant capacity of green coffee was found to correlate with the content of chlorogenic acids and we identified in the *C. arabica* from Kenya the best source of high chlorogenic and low caffeine content. Therefore, the Kenya accessions of *C. arabica* can be considered an optimal source of green coffee for nutraceutical and pharmaceutical applications.

4. Experimental

4.1. Plant material

Coffea arabica L.and *C. canephora* Pierre ex A. Froehner (Rubiaceae) green coffee beans were kindly provided by Green Elite Coffee (Genova – Italy). Table 2 summarized the species, cultivars and the place of origin of the different green coffee accessions.

4.2. Extraction of green coffee compounds

Green coffee beans were ground with a blender and passed through a coarse sieve with 2 mm diameter holes. Ten grams of ground material were extracted with 50 ml of a 50% v/v ethanol:water solution by maceration in the dark for 7 days, by shaking samples twice a day. This period of maceration was selected after several trials from 3 to 14 days because of a better extraction and reproducibility. Kinetics of extraction showed no qualitative variations between 1 to 7 days of maceration (data not shown). The extract was then filtered and stored at 4 °C until chemical analysis.

4.3. Isolation and chemical characterization of green coffee phenolics and caffeine

The characterization of chlorogenic acids and caffeine was performed according to Campa et al. (2005) and Alonso-Salces et al. (2009) with some modifications. Green coffee extracts were analysed by high performance liquid chromatography (1200 HPLC, Agilent Technologies, USA) equipped with a C18 (2.6 µm, 100 x 3.0 mm) Kinetex (Phenomenex, USA) reverse phase column. The solvent system was: A) MilliQ H_2O (Millipore, U.S.A.) with 0.1% v/v of formic acid and B), acetonitrile (Panreac, Spain) with 0.1% v/v of formic acid. The separation was performed at a constant flow rate (200 µl min^{-1}) with the following conditions: linear gradient from 3% to 15% of B in 10 min, from 15% to 55% in 25 min, from 55% to 98% in 3 min, then isocratic elution for 5 min and a linear gradient from 98% to 3% in 4 min. Tandem mass spectrometry analyses were performed with a 6330 Series Ion Trap LC-MS System (Agilent Technologies, U.S.A.) equipped with an electrospray ionization source (ESI) operating in negative mode for the determination of phenolic compounds and in positive mode for caffeine analysis. Supplementary Table S1 lists the *m/z* and mass fragmentation data of all identified compounds. Qualitative analyses were performed in scan mode (50-800 m/z) while quantitative analyses were performed in Multiple Reaction Monitoring (MRM). For quantitative determinations, calibration curves were built with caffeic acid (R^2=0.996), chlorogenic acid (R^2=0.996) and caffeine (R^2=0.998) (Sigma-Aldrich, USA).

4.4. Antioxidant capacity

The reducing power assay was performed by mixing a proper dilution of samples (100 µl) with 250 µl of phosphate buffer (0.2 M, pH 6.6) and 250 µl of 1% w/v potassium ferricyanide (Capuzzo et al., 2014; Oyaizu, 1986). The mixture was incubated at 50°C for 20 min. At the end of incubation, 250 µl of 10% w/v of trichloracetic acid were added and the mixture and centrifuged at 1000 g for 2 min. The supernatant (500 µl) was mixed with distilled water (500 µl) and 0.1% w/v iron (III) chloride (100 µl). The absorbance was measured at 700 nm against a blank sample.

The free radical scavenging activity was measured using 1,1-diphenyl-2-picryl-hydrazil stable radical (DPPH•), based on the method described by Vladimir-Knezevic and co-workers (2011). Briefly, 500 µl DPPH working solution (0.1 mM in 95 % v/v ethanol) were added to different volumes (10-500 µl) of diluted samples (50% v/v ethanol). The reaction mixture was left to stand for 30 min in the dark at room temperature and periodically shaken. The absorbance was measured at 517 nm against a blank sample. For each extract, the antioxidant activity of samples was calculated using the following equation:

$$\%=[(A_{blank}-A_{sample})/A_{blank}]*100$$

where A_{blank} is the absorbance of blank, and A_{sample} the absorbance of sample at 517 nm.

IC_{50} values were determined from the plot of scavenging activity against the compounds concentrations, which were defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

4.5. Statistical analyses

The overall data sets is expressed as mean values of at least three biological replicates. Three technical replicates were run for each biological replicate (i.e., 3 sub-samples were taken from one sample for analysis as triplicate analysis, and each of sub-samples was then measured 3 times during instrumental analysis). ANOVA and Tukey–Kramer's HSD test ($P<0.05$) were used to determine significant differences among extractions and compounds. Principal component analysis was performed using covariant matrix of extraction and varimax rotation. Cluster analysis was calculated by using

Euclidean distance with median linkage method. All statistical analyses were performed by using the SYSTAT 10 software.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at:….. These data include mass spectrometry data of the identified compounds and the cluster analysis of the most important compounds described in this article.

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Table 1. Chemical composition of green coffee beans. Values are expressed as g kg^{-1} dry wt. (Standard Deviation).

Code name	Compounds															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
A-Brasil	0.02 (0.01)	0.16 (0.01)	0.01 (0.001)	0.37 (0.03)	1.73 (0.33)	tr	0.04 (0.002)	0.01 (0.002)	0.09 (0.03)	0.05 (0.01)	0.09 (0.03)	0.09 (0.02)	tr	tr	0.01 (0.001)	1.20 (0.10)
A-Colombia	0.01 (0.004)	0.14 (0.01)	0.01 (0.001)	0.37 (0.05)	1.68 (0.43)	tr	0.07 (0.004)	0.01 (0.003)	0.11 (0.05)	0.04 (0.01)	0.11 (0.02)	0.09 (0.02)	tr	0.01 (0.002)	0.01 (0.001)	1.03 (0.03)
A-Ethiopia	tr	0.11 (0.01)	tr	0.19 (0.04)	1.56 (0.13)	0.02 (0.03)	0.03 (0.003)	tr	0.07 (0.02)	0.02 (0.004)	0.13 (0.02)	0.04 (0.01)	tr	tr	tr	0.92 (0.11)
A-Guatemala	0.01 (0.003)	0.14 (0.02)	0.01 (0.001)	0.36 (0.12)	1.65 (0.33)	0.02 (0.03)	0.04 (0.004)	0.01 (0.003)	0.08 (0.02)	0.04 (0.01)	0.08 (0.02)	0.07 (0.01)	tr	tr	tr	1.00 (0.02)
A-Honduras1	0.02 (0.003)	0.11 (0.02)	tr	0.32 (0.08)	1.54 (0.09)	0.03 (0.02)	0.06 (0.01)	0.01 (0.001)	0.08 (0.03)	0.03 (0.01)	0.06 (0.001)	0.08 (0.01)	tr	tr	tr	1.02 (0.09)
A-Honduras2	0.03 (0.01)	0.14 (0.01)	0.01 (0.001)	0.45 (0.08)	1.79 (0.22)	0.02 (0.03)	0.07 (0.002)	0.01 (0.003)	0.09 (0.03)	0.05 (0.02)	0.08 (0.01)	0.09 (0.03)	tr	tr	0.01 (0.003)	1.08 (0.07)
A-Honduras3	0.01 (0.01)	0.13 (0.02)	0.01 (0.001)	0.27 (0.04)	1.56 (0.32)	0.04 (0.04)	0.05 (0.003)	0.01 (0.002)	0.10 (0.03)	0.03 (0.01)	0.11 (0.03)	0.08 (0.02)	tr	0.01 (0.001)	tr	1.09 (0.03)

Code name	Compounds															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
A-Honduras4	0.03 (0.01)	0.13 (0.01)	0.01 (0.001)	0.40 (0.04)	1.72 (0.23)	0.06 (0.002)	0.07 (0.02)	0.01 (0.003)	0.11 (0.06)	0.05 (0.01)	0.09 (0.01)	0.10 (0.03)	tr	tr	0.01 (0.001)	1.06 (0.06)
A-Honduras5	0.03 (0.004)	0.12 (0.01)	0.01 (0.002)	0.37 (0.04)	1.81 (0.16)	0.06 (0.001)	0.07 (0.06)	0.01 (0.003)	0.11 (0.05)	0.04 (0.002)	0.07 (0.01)	0.12 (0.03)	tr	tr	0.01 (0.001)	1.01 (0.03)
A-Kenia1	0.06 (0.003)	0.18 (0.017)	tr	0.45 (0.04)	1.96 (0.26)	0.06 (0.03)	0.03 (0.004)	0.01 (0.01)	0.13 (0.07)	0.05 (0.01)	0.10 (0.02)	0.13 (0.03)	tr	0.01 (0.001)	0.01 (0.003)	0.96 (0.05)
A-Kenia2	0.04 (0.01)	0.13 (0.01)	tr	0.32 (0.03)	1.15 (0.09)	0.61 (0.03)	0.03 (0.002)	0.02 (0.001)	0.60 (0.02)	0.04 (0.01)	0.10 (0.01)	0.07 (0.02)	tr	0.01 (0.001)	0.01 (0.001)	0.81 (0.04)
A-Kenia3	0.01 (0.01)	0.14 (0.01)	tr	0.33 (0.02)	1.18 (0.13)	0.66 (0.03)	0.04 (0.001)	0.02 (0.002)	0.62 (0.04)	0.03 (0.01)	0.11 (0.01)	0.06 (0.01)	tr	0.01 (0.002)	0.01 (0.001)	0.79 (0.03)
A-Mexico	0.02 (0.003)	0.12 (0.01)	tr	0.26 (0.03)	1.54 (0.27)	0.02 (0.001)	0.03 (0.01)	tr	0.08 (0.02)	0.03 (0.01)	0.10 (0.02)	0.07 (0.003)	tr	tr	tr	0.99 (0.07)
A-Peru1	0.21 (0.01)	0.14 (0.01)	tr	0.49 (0.01)	1.62 (0.10)	0.01 (0.01)	0.03 (0.002)	0.01 (0.001)	0.09 (0.02)	0.06 (0.01)	0.08 (0.01)	0.10 (0.02)	tr	tr	0.01 (0.001)	1.18 (0.06)
A-Peru2	0.10 (0.01)	0.16 (0.01)	tr	0.44 (0.06)	1.74 (0.20)	0.02 (0.017)	0.02 (0.004)	0.02 (0.004)	0.09 (0.043)	0.06 (0.004)	0.08 (0.03)	0.10 (0.03)	tr	0.01 (0.002)	0.01 (0.002)	1.01 (0.003)
C-Brasil	0.01 (0.004)	0.12 (0.01)	tr	0.28 (0.08)	1.54 (0.50)	0.02 (0.03)	0.03 (0.02)	0.02 (0.03)	0.06 (0.01)	0.02 (0.01)	0.05 (0.001)	0.05 (0.02)	tr	tr	tr	1.27 (0.24)

Code name	Compounds															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
R-Uganda1	0.02 (0.001)	0.07 (0.01)	0.11 (0.04)	0.36 (0.06)	1.50 (0.34)	0.04 (0.02)	0.33 (0.01)	0.02 (0.01)	0.12 (0.04)	0.06 (0.004)	0.07 (0.02)	0.08 (0.03)	0.02 (0.003)	0.02 (0.003)	0.03 (0.01)	1.78 (0.13)
R-Uganda2	0.02 (0.003)	0.07 (0.004)	0.11 (0.03)	0.35 (0.07)	1.39 (0.17)	0.09 (0.09)	0.42 (0.04)	0.02 (0.01)	0.12 (0.05)	0.07 (0.02)	0.08 (0.002)	0.10 (0.01)	0.02 (0.002)	0.01 (0.003)	0.03 (0.01)	1.84 (0.20)
R-Vietnam1	0.04 (0.001)	0.07 (0.002)	0.12 (0.02)	0.38 (0.07)	1.41 (0.22)	0.01 (0.01)	0.38 (0.004)	0.02 (0.01)	0.13 (0.05)	0.08 (0.02)	0.13 (0.03)	0.15 (0.04)	0.02 (0.01)	0.02 (0.004)	0.03 (0.01)	1.71 (0.09)
R-Vietnam2	0.05 (0.01)	0.08 (0.01)	0.12 (0.02)	0.40 (0.03)	1.52 (0.23)	0.03 (0.03)	0.39 (0.04)	0.02 (0.004)	0.14 (0.04)	0.09 (0.03)	0.10 (0.01)	0.12 (0.02)	0.02 (0.004)	0.02 (0.003)	0.04 (0.01)	1.72 (0.17)

1 = Caffeic acid; 2 = *p*-coumaroyl quinic acid; 3 = *p*-coumaroyl-*N*-tryptophan; 4 = chlorogenic acid (3-*O*-caffeoylquinic acid); 5 = neochlorogenic acid (5-*O*-caffeoylquinic acid); 6 = cryptochlorogenic acid (4-*O*-caffeoylquinic acid); 7 = caffeoyl-*N*-tryptophan; 8 = 3-*O*-feruloylquinic acid; 9 = 5-*O*-feruloylquinic acid; 10 = 3,4-*O*-dicaffeoylquinic acid; 11 = 3,5-*O*-dicaffeoylquinic acid; 12 = 4,5-*O*-dicaffeoylquinic acid; 13 = 3-*O*-feruloyl-4-caffeoylquinic acid; 14 = 3-*O*-feruloyl-5-caffeoylquinic acid; 15 = 4-*O*-feruloyl-5-caffeoylquinic acid; 16 = caffeine. Compouds below 0.01 g kg^{-1} are indicated as traces (tr).

Table 2. List of *Coffea arabica* (arabica) and *C. canephora* (robusta) code names and country of origin.

Code Name	Species	Commercial name	Cultivar	Country of Origin
A-Brasil	*C. arabica*	Arabica	Arabica Natural Terraforte	Brasil
A-Colombia	*C. arabica*	Arabica	Colombia Excelso Raphael Lavato	Colombia
A-Ethiopia	*C. arabica*	Arabica	Sidamo Grade 2	Ethiopia
A-Guatemala	*C. arabica*	Arabica	HB ep	Guatemala
A-Honduras1	*C. arabica*	Arabica	HG ep "Margay"	Honduras
A-Honduras2	*C. arabica*	Arabica	HG ep "Margay"	Honduras
A-Honduras3	*C. arabica*	Arabica	Catuahi,Caturra,Icatu	Honduras
A-Honduras4	*C. arabica*	Arabica	HG ep "Margay"	Honduras
A-Honduras5	*C. arabica*	Arabica	Catuahi,Caturra,Icatu	Honduras
A-Kenya1	*C. arabica*	Arabica	Arabica low grade	Kenya
A-Kenya2	*C. arabica*	Arabica	Arabica AK2	Kenya
A-Kenya3	*C. arabica*	Arabica	Arabica AK3	Kenya
A-Mexico	*C. arabica*	Arabica	PW ep	Mexico
A-Peru1	*C. arabica*	Arabica	Jacamar	Peru
A-Peru2	*C. arabica*	Arabica	Tinamous	Peru
C-Brasil	*C. canephora*	Caracol	Moka Fine Crop	Brasil
R-Uganda1	*C. canephora*	Robusta	Jolly Quartz	Uganda
R-Uganda2	*C. canephora*	Robusta	Jolly Quartz	Uganda
R-Vietnam1	*C. canephora*	Robusta	Unwahed Vietnam	Vietnam
R-Vietnam2	*C. canephora*	Robusta	Clean Vietnam	Vietnam

Legend for figures

Figure 1. Structure formulae of the identified compounds extracted from *Coffea arabica* and *Coffea canephora*. **1** Caffeic acid; **2** *p*-coumaroyl quinic acid; **3** *p*-coumaroyl-*N*-tryptophan; **4** chlorogenic acid (3-*O*-caffeoylquinic acid); **5** neochlorogenic acid (5-*O*-caffeoylquinic acid); **6** cryptochlorogenic acid (4-*O*-caffeoylquinic acid); **7** caffeoyl-*N*-tryptophan; **8** 3-*O*-feruloylquinic acid; **9** 5-*O*-feruloylquinic acid; **10** 3,4-*O*-dicaffeoylquinic acid; **11** 3,5-*O*-dicaffeoylquinic acid; **12** 4,5-*O*-dicaffeoylquinic acid; **13** 3-*O*-feruloyl-4-caffeoylquinic acid; **14** 3-*O*-feruloyl-5-caffeoylquinic acid; **15** 4-*O*-feruloyl-5-caffeoylquinic acid; **16** caffeine.

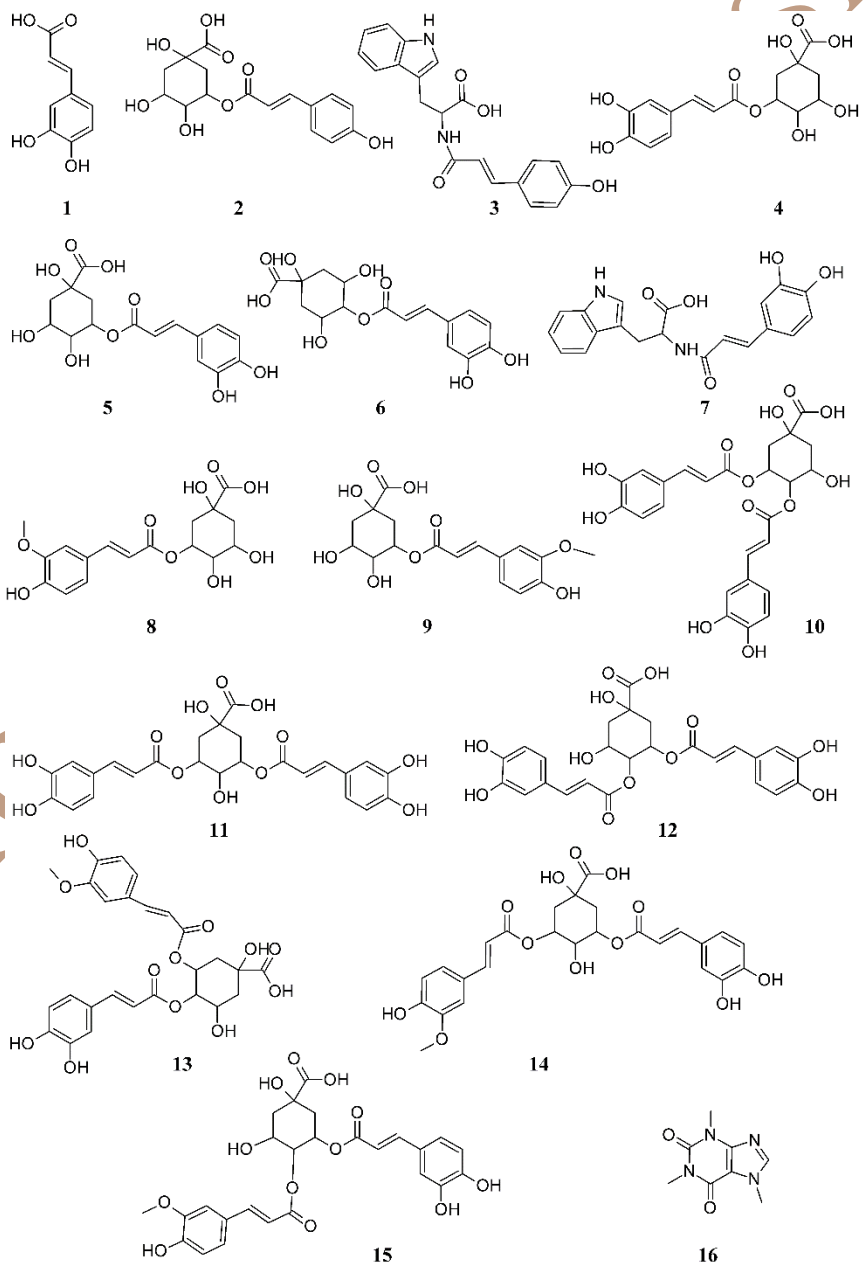

Figure 2. Distribution of total chlorogenic acids and caffeine (expressed as g kg^{-1} of dry weight g/kg) from *C. arabica* and *C. canephora* of different geographical origin. *C. canephora* accessions show the highest content with respect to all other *C. arabica* accessions. See Table 2 for code names.

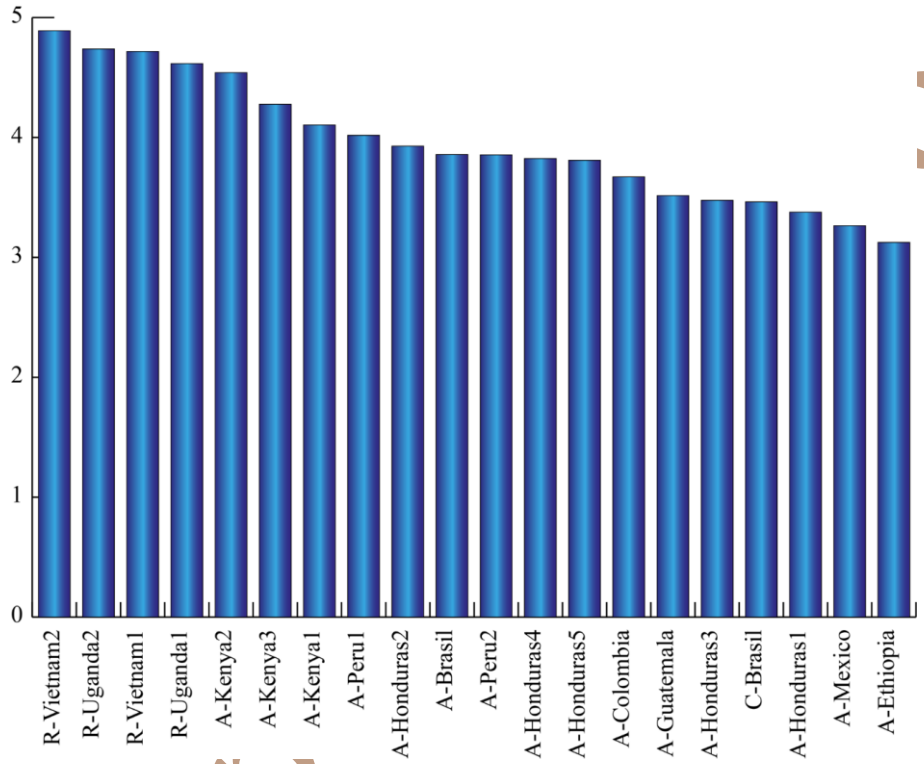

Figure 3. Scatter plot of total chlorogenic acids against caffeine content. A clear distinction is evident between *C. canephora* (R-Uganda and R-Vietnam) and *C. arabica* accessions. The *C. arabica* accessions from Kenya shows the highest content of chlorogenic acid and the lowest amount of caffeine. Metric bars indicate standard error; see Table 2 for code names.

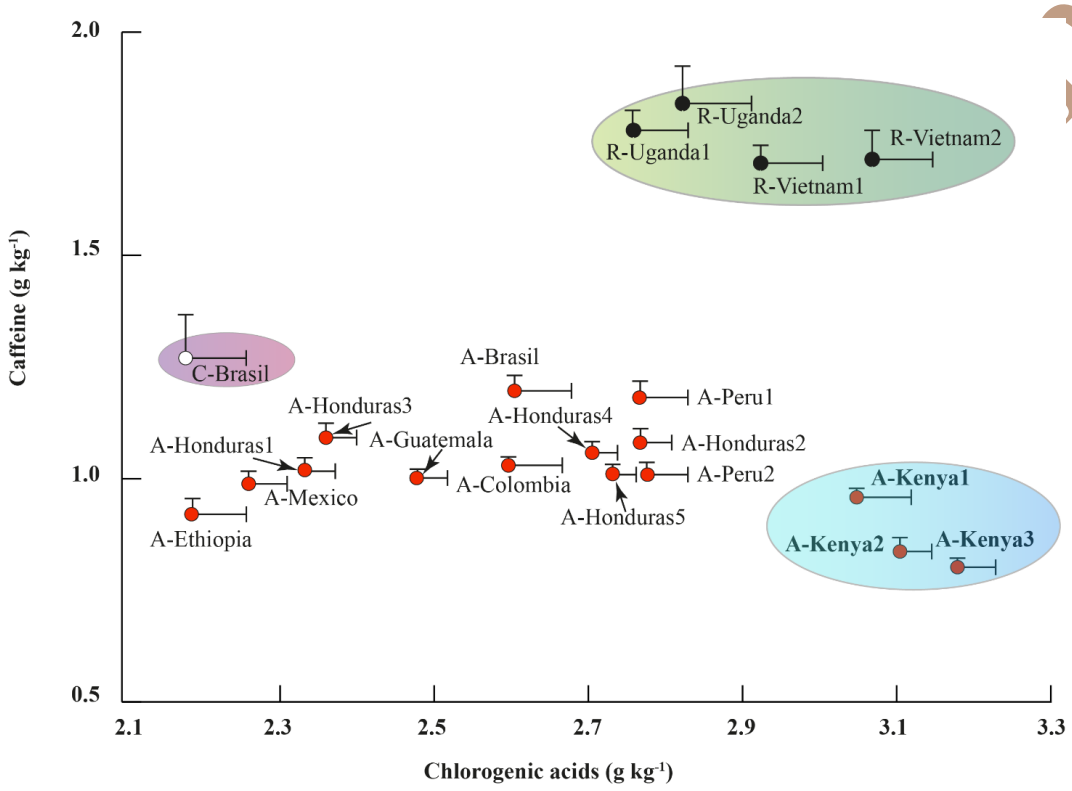

Figure 4. Scatter plot of the principal components analysis factor scores showing the separation of all *C. canephora* from *C. arabica* accessions. A clear separation is present inside the *C. canephora* group between Uganda, Vietnam and the "caracol" mutation from Brasil (C-Brasil). Among *C. arabica* accessions, accessions from Kenya show an opposite partition from all other varieties; see Table 2 for code names.

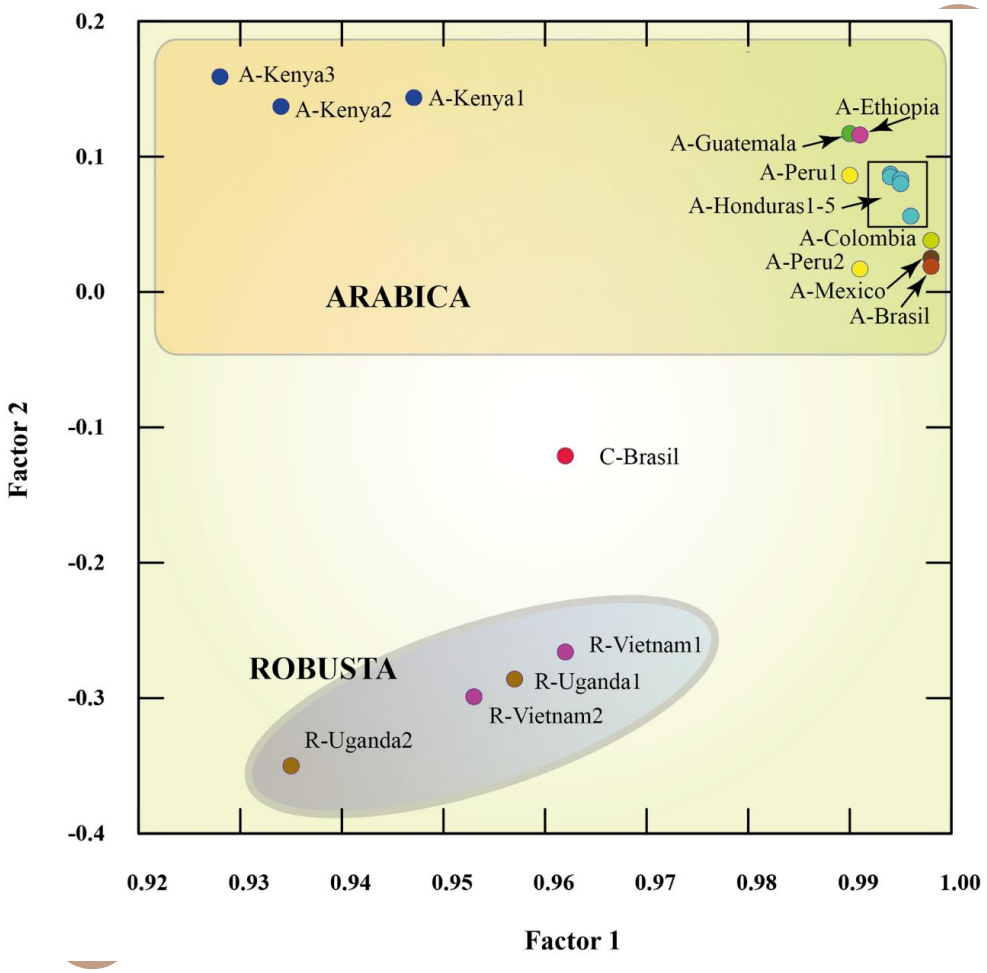

Figure 5. Scatter plot of the IC_{50} values from the reducing power and DPPH assays. The *C. canephora* accessions show the highest antioxidant capacity (lower IC_{50} values) than all other *C. arabica* accessions. Among *C. arabica*, the accessions from Kenya shows the highest antioxidant capacity. Metric bars indicate standard error; see Table 2 for code names.