Distribution of *p*-coumaroylquinic acids in commercial *Coffea spp*. of different geographical origin and in other wild coffee species

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ABSTRACT

Quantitative analyses of mono-*p*-coumaroylquinic acids (*p*CoQAs) and total chlorogenic acids (CGAs) in green coffee commercial lots of *C. arabica C. canephora* and *C. liberica* from different geographic origins and eight wild *Coffea* species were carried out. Among the commercial lots, *p*CoQAs average content of *C. arabica* (0.67 mg/g) is higher than that of *C. canephora* (0.40 mg/g) being *C. liberica* intermediate (0.58 mg/g). As far as the analyzed wild *Coffea* species is concerned, *C. pseudozanguebariae* is characterized by the lower *p*CoQAs content (0.12 mg/g) whereas *C. sessiliflora* is by far the richest source of *p*CoQAs (2.18 mg/g). Effect of the roasting process on the mono-*p*-coumaroylquinic acids profile was evaluated for the economically exploited species *C. arabica* and *C. canephora*. For the first time distribution of mono-*p*-coumaroylquinic acid isomers

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in wild coffee species by fast and accurate UHPLC-DAD analyses using authentic standards previously synthetized, is reported.

KEYWORDS

Coffee; UHPLC; chlorogenic acids; *p*-coumaroylquinic acids

Chemical compounds studied in this article:

5-*p*-coumaroylquinic acid (Pubchem CID: 6441280); 3-*p*-coumaroylquinic acid (Pubchem CID: 9945785); 4-*p*-coumaroylquinic acid (Pubchem CID: 5281766); 5-caffeoylquinic acid (Pubchem CID: 5280633).

ABBREVIATIONS

CGAs: chlorogenic acids; CQAs: caffeoylquinic acids; *p*CoQAs: *p*-coumaroylquinic acids; FQAs: feruloylquinic acids.

1. Introduction

Chlorogenic acids (CGAs) are naturally occurring esters formed between (-)-quinic acid and different *trans*-cinnamic acids (such as caffeic, ferulic and *p*-coumaric acid) and since quinic acid possesses four hydroxyl groups, a great variety of chlorogenic acid isomers can be formed. In facts, in addition to monoesters, a wide range of di- and tri-esters with the same or different cinnamic acids is known (Clifford, 1985). From a functional point of view, CGAs are plants secondary metabolites which have been suggested to be involved in the defense mechanism against environmental aggressions (Farah & Donangelo, 2006).

In the last few years, more than 80 chlorogenic acids have been isolated and identified in coffee. From a quantitative point of view, caffeoylquinic acids (CQAs) monoesters with the hydroxyl groups at C-3, C-4 and C-5 of the quinic ring esterified with caffeic acid are by far the most abundant,

reaching together more than 80% of total chlorogenic acids (Perrone, Farah, Donangelo, De Paulis, & Martin, 2008). So far, no esterification was observed at the C-1 hydroxyl group in coffee species (Narita & Inouye, 2015). In addition to CQAs isomers, green coffee beans are particularly rich in other classes of chlorogenic acid compounds including dicaffeoylquinic acids (diCQAs), mono-feruloylquinic acids (FQAs) and *p*CoQAs (Figure 1). (Farah & Donangelo, 2006) Since coffee beans represent an important dietary source (Tajik, Tajik, Mack & Enck, 2017) of these compounds several investigations on coffee composition regarding CQAs, diCQAs and FQAs have been reported in the literature. (Perrone, Farah, Donangelo, De Paulis, & Martin, 2008) (Farah & Donangelo, 2006) These polyphenols have recently attracted the attention of several Research groups also thanks to their biological activity including antioxidant, anti-inflammatory and antiviral properties (Sinisi et al., 2017).

Moreover, due to their potential beneficial effects, in the last years pharmaceutical and nutrition industries have paid special attention to determine the CGAs profile in green coffee extracts proposed as nutriceuticals (Onakpoya, Terry, & Ernst, 2011).

Although *p*CoQAs represent relevant intermediates in one of the proposed biochemical pathways of chlorogenic acid biosynthesis (Koshiro et al., 2007; Clifford et al. 2017a), they are the less studied class of CGAs. In 2008 Perrone et al. reported a quantitative analysis of *p*CoQAs in the green beans of the two most important commercial species *C. arabica* (commonly known as Arabica) and *C. canephora* (commonly known as Robusta), where they were present in the range from 1.0% to 0.60% respectively of the total chlorogenic acids content, leading this class of chlorogenic acids to be the fourth in order of quantitative importance. In addition to coffee, other vegetables and fruits including potatoes, apples and walnuts are relatively rich source of mono-*p*-coumaroylquinic acids, being sweet cherries the main dietary source (Aversano et al., 2017; Gutiérrez Ortiz et al., 2018).

However, even in these matrices, investigations focused on *p*CoQAs are rather scarce and with no information on the different isomers (Ballistreri et al., 2013; Goulas et al., 2015; Khanizadeh, Tsao,

Rekika, Yang, & DeEll, 2007; Serra, Duarte, Bronze, & Duarte, 2011), probably due to the lack of available *p*CoQAs standards which makes the need of a MS mandatory for their investigation (Regueiro et al., 2014). Recent advances in UHPLC methods made it possible to develop reliable, fast and accurate method for CGAs identification and quantification, without the need of an expensive MS detector but using a more cheap UV detector (Craig, Fields, Liang, Kitts, & Erickson, 2016).

In this study a reliable method for the identification of these minor coffee compounds via UHPLC-DAD was successfully optimized, thanks to the availability of previously synthetized mono-*p*-coumaroylquinic acid isomers and used as valuable standards (Gutiérrez Ortiz et al., 2017). Their quantification was performed by using high purity 5-CQA standard as recommended in the literature (Clifford & Madala, 2017b).

During coffee roasting process, CGAs content decreases due to chemical transformations leading to important volatile aroma compounds and to the formation of their corresponding lactones which contribute to the bitterness of the final beverage (Farah, de Paulis, Trugo, & Martin, 2005; Frank, Zehentbauer, & Hofmann, 2006). Depending on the intensity of the thermal treatment, and then on the roasting degree, chlorogenic acids may be lost up to 95% in dark roasted coffee (Farah & Donangelo, 2006). Although the detection of *p*CoQAs could be difficult due to the low amount in the raw material and the thermal treatment transformations part of the present work was dedicated to follow the fate of *p*CoQA isomers during roasting conditions simulation of the two commercially exploited species, Arabica and Robusta.

Moreover, the present investigation was enlarged to the analyses of other coffee species in order to quantify both the total *p*CoQAs content and their isomers composition in green coffee beans.

In spite of more than one hundred coffee species being present in the world (Davis, Govaerts, Bridson, & Stoffelen, 2006) both qualitative and quantitative determination of CGAs content have been focused almost exclusively on the two economically relevant species, Arabica and Robusta (Farah & Donangelo, 2006; Rodrigues & Bragagnolo, 2013). CGAs profiles of other wild species reported in the literature include primarily CQAs, diCQAs and only in few cases FQAs (C. Campa,

Doulbeau, Dussert, Hamon, & Noirot, 2005; Clifford, Williams, & Bridson, 1989; Narita & Inouye, 2015). As already mentioned, the three isomers of *p*CoQAs were quantified in Arabica and Robusta species only by Perrone et al. 2008. Although the CGAs content depends on agronomical practices, geographical origin and soil composition (Farah & Donangelo, 2006), genetic factors are also very important and the determination of CGAs in wild coffee species might provide useful data to establish a taxonomic classification based on the chemical patterns. In the present work, the content of *p*CoQA isomers has been compared with total CGAs content, calculated as sum of CQA, diCQA, FQA and *p*CoQA isomers in commercial lots of *C. arabica*, *C. canephora* and *C. liberica* from different geographical origins and in eight wild *Coffea* species: *C. liberica*, Arabusta coffee (*C. arabica* L. x *C. canephora* Pierre), *C. eugenioides*, *C. sessiliflora*, *C. congensis*, *C. pseudozanguebariae*, *C. racemosa* and *C. brevipes*. To our knowledge, this is the first time that all three isomers of this class of CGAs are quantified in wild coffee species.

The different stereocenters of the carbon atoms of the quinic acid ring make it necessary to detail the numbering of the carbon atoms to avoid possible confusion in the CGAs names already put in evidence in the literature (Abrankó & Clifford, 2017). In the present work the IUPAC numbering system is used (IUPAC, CBM, & IUPAC-IUB, 1976).

2. Materials and Methods

2.1 Chemicals

5-caffeoylquinic acid (5-CQA) was purchased from Phytolab (Vestenbergsgreuth, Germany). Acetonitrile and methanol (HPLC grade) were purchased from Sigma –Aldrich S.r.l. (Milano, Italy) while formic acid was obtained from CARLO ERBA Reagents S.r.l. (Cornaredo, Italy). Standards not commercially available (*p*-coumaroylquinic acids) were synthetized according to the literature (Gutiérrez Ortiz et al., 2017) and their identity was confirmed by ^{1}H and ^{13}C NMR spectroscopy. 3,4 dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), 4,5-dicaffeoylquinic acid

120 (4,5-diCQA) were purchased from Phytolab, feruoylquinic acids (FQAs) were obtained from the
121 division of organic chemistry and biochemistry at Ruđer Bošković Institute (Zagreb, Croatia) (Dokli,
122 Navarini, & Hameršak, 2013). Water was treated in a Milli-Q water purification system (Millipore
123 Academic).

124 *2.2 Samples*

125 A total of twenty seven fresh green coffee samples from different geographical origins and different
126 species were analyzed. Commercial wet processed lots of Arabica were from Brazil, Colombia,
127 Ethiopia (lots 1 and 2), Honduras, India, Yemen (lots 1 and 2) and Guatemala (cultivar *laurina*);
128 Commercial wet polished lots of Robusta were from Vietnam and India (parchment AB grade); *C.*
129 *liberica* lots (Liberica lots 1, 2 and 3) were from Indonesia. Non commercial samples are listed in
130 Table 1. Wild species from CATIE were cultivated and post-harvest treated in Turrialba – Costa Rica.

131 **Table 1** – Non commercial samples of green wild coffee species

Coffee species	**Identification code**	**Geographical origin of original material**	**Notes**
C. liberica - 4	CATIE T.03447	Honduras	in parchment
C. liberica - 5	CATIE T.03475	Ceylon	in parchment
C. liberica - 6	CATIE T.03476	Ceylon	in parchment
Coffea canephora x *Coffea arabica* Arabusta - 1	CIRAD 15	French Guiana	wet processed

Arabusta - 2	CIRAD 24	French Guiana	wet processed
C. eugenioides	CATIE T.21387	France	in parchment
C. eugenioides - 2	CATIE T.02725	East Africa	in parchment
C. sessiliflora	CATIE T.21348	France	in parchment
C. sessiliflora - 2	CATIE T.21345	France	in parchment
C. congensis	CATIE T.05241	Portugal	in parchment
C. pseudozanguebariae	CATIE T.21352	France	in parchment
C. racemosa		Mozambique	wet processed
C. brevipes	CATIE T.21372	France	in parchment

2.3 Extraction of chlorogenic acids and sample preparation

Green and roasted coffee beans were ground to a fine powder in a mixer ball mill MM400 (Retsch, Germany) and extraction was performed in duplicate by dynamic heat-assisted water extraction. For this purpose 1g of powdered green coffee for each species was added to 100 mL of boiling water (Gutiérrez Ortiz et al., 2018) and the mixture was stirred for 10 min at 200 rpm on a heated plate (Arex Velp Scientifica) and filtered through qualitative filter paper n.302 (VWR International Srl, Milano, Italy). The aqueous extract was frozen with liquid nitrogen and freeze dried for 3 days.

For quantification purposes, lyophilized crude material was redissolved in water to afford concentrations of 30 mg/mL. In order to analyze each compound accurately, every class of compounds was quantified on a specific diluted solution, because concentration of caffeoylquinic acids is appreciably higher than other minor compounds, so dilution of 1:2, 1:4 1:10 and 1:20 were prepared in water and filtered across a nylon filter (pore size 0.2 µm), transferred into a vial and immediately analyzed by UHPLC-DAD.

2.4 Roasting conditions

147 To simulate roasting conditions, green coffee beans of *C. arabica* and *C. canephora* were thermally
148 treated in a thermoblock Thermostatic Dry Bath G-Block (Fratelli Galli, Italy) at 211°C at different
149 times (0, 3, 5, 7, 10, 12, 15, 20, 25, 30, 35, 40 and 45 minutes). Roasting has been carried out in
150 duplicate to assess reproducibility. By monitoring the total weight loss, in our experimental set-up
151 the true roasting conditions were achieved starting from 15 minutes for Arabica and 20 minutes for
152 Robusta (corresponding to total dehydration) afterward. In this way, we obtained total weight losses
153 ranging from 11% to 18.5% in order to mimic roasting degree from very light to dark one. Total
154 weight loss has been calculated as follows: '(green bean weight – roasted bean weight)/ green bean
155 weight x 100'. Extraction and analysis of roasted samples were performed with the same protocol
156 used for green coffee.

157 *2.5 Analyses of Chlorogenic Acids (CGAs)*

158 Analyses of caffeoylquinic acids (CQAs), dicaffeoylquinic acids (diCQAs), feruloylquinic acids
159 (FQAs) and *p*-coumaroylquinic acids (*p*CoQAs) were performed using a 1290 UHPLC system
160 (Agilent, Germany), consisting of degasser, quaternary pump, column thermostat and diode array
161 detector (DAD) operating at 305 nm (specific for *p*CoQA) and 324 nm. A Kinetex XB-C18 column
162 2.6 μm 100 x 2.1 mm (Phenomenex, USA) was used at 25°C. Solvents were delivered at a total flow
163 rate of 0.5 mL/min and the volume of injection was 2.0 μl. Solvent A was water/formic acid
164 (1000:0.001 v/v) and solvent B acetonitrile. The gradient profile was from initial 97% of solvent A
165 to 85% of A in 8 min, then 60% of A until 11min, and a return to 97% A at 12 min to re-equilibrate.
166 Qualitatively identification of CGAs was achieved by comparison of specific retention times of
167 standard solutions as recently described for walnut leaves (Gutiérrez Ortiz et al., 2018), additionally
168 UHPLC/MS analyses were performed to unequivocally identify the investigated analytes comparing
169 the obtained results to those obtained by Clifford et al. as well as to those observed from diluted pure
170 standard solutions. (Clifford, Johnston, Knight, & Kuhnert, 2003) ABSciex Triple Quad 4500
171 detector was coupled to the UHPLC system; MS was operating in negative mode, ionization voltage

of 4500, desolvation temperature of 350°C and gas flows of GS1 30 and GS2 40. Fragmentation of pseudomolecular ion $[M-H]^-$ at m/z 337 were found for *p*CoQAs, yielding a base peak at m/z 163 for 3*p*CoQA, 174 for 4*p*CoQA and 191 for 5*p*CoQA.

Quantitative determination was performed by UHPLC based on the diode array value for peak areas, using calibration curve of *trans*-5CQA and converting the dichlorogenic acids quantification with an extinction relation (factor 0.77) according to the norm method ("DIN 10767 - Analysis of coffee and coffee products; determination of chlorogenic acids content; HPLC method," 2015). The total content of CGAs is expressed as the sum of all identified CGAs, i.e. the three isomers of CQAs, the three isomers of FQAs, the three isomers of *p*CoQAs and three diCQAs. Standard stock solutions were prepared in MeOH:H_2O (1:1) at appropriate concentrations and different diluted solutions were prepared from stock solutions.

Results obtained are given on dry weight basis (dwb) in order to establish clear comparison with data already reported in the literature and since some samples consisted of a few quantity of seeds a 10% moisture content was assumed as it has been done before by others authors. (Anthony et al., 1993)

3. Results and discussion

The lack of authentic polyphenols standards is a big problem in the identification of these compounds in complex food matrices. In the absence of standards, when liquid chromatography is used to study the polyphenol composition, identification by UV-vis spectra scan of individual components and comparison with published spectra is frequently performed. (Milinović et al., 2016; Mozetič & Trebše, 2004; Zhang & Hamauzu, 2004) Unfortunately, the spectral characteristics of polyphenols like chlorogenic acids, even if unique, are not selective. The synthesis of *p*CoQA isomers, recently performed by our group (Gutiérrez Ortiz et al., 2017), largely stimulated the present investigation, aimed at characterizing this class of chlorogenic acid in a high value food matrix such as coffee. In fact, synthetized and purified 3-, 4- and 5-*p*CoQA isomers have been used for their identification in

197 both green and roasted coffee samples of the present investigation. LC-MS analyses have also been
198 performed to fully confirm the presence of all isomers in every coffee sample analyzed. As far as
199 quantification is concerned, 5-caffeoylquinic acid standard has been used according to Clifford and
200 Madala (2017b).

201 Calibration curve of 5-CQA showed a good response linearity with a coefficient of correlation (r^2) of
202 0.999. Limit of quantification (LOQ) and limit of detection (LOD) were calculated as 3 times lower
203 concentration of analyte on signal to noise ratio (LOD) or 10 times lowest concentration of analyte
204 on signal to noise ratio (LOQ) resulting 0.88 μg/mL for LOQ and 0.26 μg/mL for LOD.
205 The distribution of *p*CoQAs and the total CGAs content, expressed in mg/g, are reported in table 2,
206 the mean of duplicate analyses are reported with standard deviation in brackets, highlighting a good
207 reproducibility of the method, being std always less than 10%.
208
209 **Table 2 –** Quantification of total *p*-coumaroylquinic acids and total chlorogenic acids (mg/g dwb)
210 in commercial samples and wild species of green coffee.

Samples	*p*CoQAs (std)	Mean	range	Total CGAs (std)	Mean	range
C. arabica Brazil	0.73 (0.01)			54.00 (0.28)		
C. arabica Colombia	0.63 (0.01)			65.62 (5.51)		
C. arabica Etiopia - lot 1	0.55 (0.01)			51.92 (3.79)		
C. arabica Etiopia - lot 2	0.56 (0.01)			56.86 (0.47)		
C. arabica Honduras	0.61 (0.01)			58.28 (1.61)		
C. arabica India	0.70 (0.02)			60.91 (3.80)		
C. arabica Yemen - lot 1	0.77 (0.01)			64.77 (1.32)		
C. arabica Yemen - lot 2	0.93 (0.01)			63.35 (1.84)		
C. arabica var. laurina Guatemala	0.51 (0.01)			61.82 (4.92)		

C. arabica		0.67	0.55 – 0.93	59.73	51.92-65.62
C. canephora Vietnam	0.37 (0.01)			57.97 (2.13)	
C. canephora India	0.43 (0.01)			83.95 (4.07)	
C. canephora		0.40	0.37 – 0.43	70.96	57.97-83.95
C. liberica – lot 1	0.26 (0.01)			52.02 (1.47)	
C. liberica – lot 2	0.80 (0.01)			59.78 (5.77)	
C. liberica – lot 3	0.68 (0.01)			46.64 (0.61)	
C. liberica		0.58	0.26 – 0.80	52.81	46.64 - 59.78
C. liberica – 4 (wild)	0.29 (0.01)			63.29 (2.49)	
C. liberica – 5 (wild)	0.64 (0.01)			47.09 (0.98)	
C. liberica – 6 (wild)	0.93 (0.01)			47.91 (2.24)	
C. liberica (wild)		0.62	0.29 - 0.93	52.76	47.09 - 63.29
Arabusta – 1 (wild)	1.03 (0.01)			56.41 (3.27)	
Arabusta – 2 (wild)	1.10 (0.01)			57.45 (1.19)	
Arabusta (wild)		1.07	1.03 - 1.10	56.93	56.41 – 57.45
C. eugenioides (wild)	0.19 (0.01)			29.54 (0.06)	
C. eugenioides-2 (wild)	0.29 (0.01)			32.56 (2.89)	
C. eugenioides (wild)		0.24	0.19 – 0.29	31.05	29.54 – 32.56
C. sessiliflora (wild)	2.12 (0.04)			45.38 (2.25)	
C. sessiliflora-2 (wild)	2.24 (0.04)			51.69 (1.52)	
C. sessiliflora (wild)		2.18	2.12 – 2.24	48.54	45.38 – 51.69
C. congensis (wild)	0.53 (0.01)			66.40 (1.81)	
C. pseudozanguebariae (wild)	0.12 (0.01)			2.13 (0.03)	
C. racemosa (wild)	0.37 (0.01)			62.14 (4.20)	
C. brevipes (wild)	0.25 (0.01)			70.50 (5.15)	

211

In all the analyzed green coffee samples, *p*CoQAs accounted for an amount lower than 1 mg/g for commercial samples, reaching a maximum of 2.24 mg/g for wild species, and all the three isomers were identified (table 4). 5*p*CoQA is the major compound of the three isomers, as expected (Koshiro et al., 2007), except in two commercial lots of *C. liberica* (lot 2 and lot 3) where the 3*p*CoQA was the major isomer detected. In the case of commercial lots, we don't have varietal details, except for *C. arabica var. laurina* from Guatemala, however, for Liberica, in view of its very marginal relevance in the coffee markets and low value, it cannot be excluded that the commercial lots may derived by blending in the farm different non homogeneous products in terms of varieties, degree of ripeness and defective beans which may affect the isomers distribution. Even the first commercial lot of Liberica (lot 1) shows some peculiarity. In particular, differently from the great majority of the analyzed samples, 5*p*CoQA is almost equal to 3*p*CoQA, as observed also in *C. canephora* from India. On the contrary, the three Liberica of the wild species showed the usual prevalence of the 5-isomer leading to a possible genetic origin of the peculiar distribution observed. Unfortunately, the lack of published data on *p*CoQAs isomers distribution cannot permit to draw any conclusion on this point.

The total CGAs content for *C. canephora* is higher (mean value 70.96 mg/g dwb) when compared to *C. Arabica* (mean value 59.73 mg/g dwb), as already reported in the literature. Our results are in accordance with the results obtained by Trugo (1984) where the extraction method was very similar to the one used in the present work. The same results were obtained by Perrone et al. although a different extraction procedure was used.

On the contrary the *p*CoQAs content is higher in Arabica (mean value 0.67 mg/g dwb) than Robusta (mean value 0.40 mg/g dwb). These findings are in full agreement with previous data reported by Perrone et al., the only work which reports the total content of *p*CoQAs (all three isomers are identified). Alonso-Salces in 2009 quantified the only 5-*p*CoQA but the results onìbtained are very lower than the one we obtained for the only 5-*p*COQA, probably due to a different extraction method. Anyway, the amount of 5-*p*CoQA in Arabica green coffee beans is confirmed to be higher with

237 respect to Robusta. Also Babova et al. reported results on the amount of 5-pCoQA although the
238 stereochemistry of all compounds analyzed in their work is not specified. Morover the old
239 nomenclature for chlorogenic acids is used and the *p*CoQA is not defined. We can assume that the
240 only stereoisomer they obtained was the 5-*p*CoQA and results were similar to the ones obtained by
241 Salces et al. It must be again underlined that different methods of extractions were used so results can
242 not be compared with our results. Babova et al. used a further different method of extraction (a
243 solution of 50% v/v ethanol:water by maceration in the dark for 7 days, by shaking samples twice a
244 day).
245 To our knowledge, no *p*CoQAs quantitative data have been reported in the literature for the other
246 wild coffee species. For the latter, *C. pseudozanguebariae* is characterized by the lowest *p*CoQAs
247 content (0.12 mg/g) whereas *C. sessiliflora* is by far the richest source of *p*CoQAs (2.18 mg/g).
248 However, considering the distribution of the different classes of chlorogenic acids with respect to the
249 total CGAs amount (2.13 ± 0.03 mg/g), *C. pseudozanguebariae* is the *Coffea* species showing the
250 most relevant contribution of the *p*CoQA class (0.12 mg/g), which is present in the relative percentage
251 of 5.6%. *C. sessiliflora* is the second *Coffea* specie with a relative content of *p*CoQAs of about 4.5%.
252 In general, it seems that the *p*CoQAs relative content with respect to the total amount of CGAs is
253 inversely proportional to the total CGAs amount, in agreement with the few data published so far
254 (Perrone et al., 2008).

255 It is noteworthy that investigations on 5-caffeoylquinic acid biosynthesis in coffee plant put in
256 evidence a route involving direct 3'-hydroxylation of 5-*p*-coumaroylquinic acid and the subsequent
257 role played by 5-*p*CoQA as a transient intermediate rapidly converted to downstream compound.
258 (Koshiro et al., 2007; Lepelley et al., 2007). This view is consistent with the low amount of *p*-
259 coumaroylquinic acids observed, up to now, in all coffee samples. In studies regarding chlorogenic
260 acid biosynthesis in globe artichoke (*Cynara cardunculus* L), the low concentration of *p*-
261 coumaroylquinic acid detected in all samples studied, has been ascribed to the transient intermediate

nature of this compound, within the pathway for the biosynthesis of 5-caffeoylquinic acid where the *p*-coumaroyl ester 3'-hydoxylase catalyzes the last step (Comino et al., 2007). In coffee, an alternative pathway has also been proposed, and in particular, the *p*-coumaric acid coming from phenylalanine through cinnamic acid, instead to lead to *p*CoQA, is converted to caffeic acid leading to 5-CQA by its trans-esterification with quinic acid through the formation of caffeoyl-CoA (Koshiro et al., 2007). In view of the different *p*CoQAs contribution to total CGAs observed in the wild coffee species, it cannot be excluded *a priori* that the chlorogenic acid biosynthesis in coffee could follow different routes depending on genotype. Similar hypothesis has been already formulated in discussing CGA evolution during grain development of *C. canephora* when compared to the wild species *C. pseudozanguebariae* (Lepelley et al., 2007).

The total CGAs contents of the cultivated species of *C. arabica* and *C. canephora* were between 52 – 66 mg/g on dry matter basis and 58 – 84 mg/g on dry matter basis respectively, in full agreement with values extensively reported in the literature (Farah et al., 2005; Monteiro & Farah, 2012), confirming a significant difference between these two main commercial species (table 2). The difference is even more pronounced for wild species. In fact, an average total CGA content of wild *C. arabica* and wild *C. canephora* equal to 41 and 113 mg/g, respectively, has been reported (Campa, Rakotomalala, Kochko, & Hamon, 2008; Farah & Donangelo, 2006).

Results obtained for *C. liberica*, with a range of determined total CGAs between 47 mg/g and 64 mg/g, put this specie similar to the Arabica one.

The variability inside the same species may depend on the geographical origin and then on the agronomical practices, as reported in the literature (Babova, Occhipinti, & Maffei, 2016; Narita & Inouye, 2015) but many other factors can affect the chemical composition such as edaphoclimatic circumstances, annual variations, ripening, storage and method of analysis. In the present work, we focused on the influence of the geographical origin only for the Arabica samples, being the most

valuable product in the coffee sector, but our data proved that the environmental factor was not relevant. Similar picture has been drawn by other authors (Babova et al., 2016; Kuhnert et al., 2011). In the case of wild coffee species, our values are in line with data reported by Anthony et al., (Anthony, Clifford, & Noirot, 1993) except for Arabusta, *C. eugeniodes* and *C. sessiliflora* which are lower than expected on the basis of the literature. However, it has to be taken in mind that the scarce amount of available data and the low number of samples analyzed so far (including differences in the source and degree of ripeness of seeds and/or analytical procedures), does not permit to draw any general conclusion. In all cases, the present investigation provide valuable quantitative data on a neglected class of coffee chlorogenic acid isomers.

Comparing the total CGAs content of all analyzed wild coffee species, *C. brevipes* and *C. congensis* are the two species with the higher content of total CGA while *C. liberica*, *C. racemosa* and Arabusta have a similar content of total CGA. *C. eugenioides* and *C. sessiliflora* show a lower content of CGAs with respect to the other wild species. In the case of *C. eugenioides*, even if our average total CGAs content is lower than expected, it has to be evidenced that it is considered a low accumulating chlorogenic acids coffee specie (Campa et al., 2008). *C. pseudozanguebariae* is confirmed to be the wild species with the lower total CGAs content (0.21%).

It is interesting to observe that, in almost all samples analyzed, the CGAs data of this study are within the range of the ones reported in the literature (Table 3), perhaps the only exceptions are those reported by Anthony et al. 1993 when they analyzed germplasm originating mainly from Africa (Côte-d'Ivoire, Madagascar, Tanzania) and Brazil.

Table 3 - Current data and literature values for total CGAs (% w/w dmb) in commercial and wild Coffea Spp.

	Total CGAs		
Coffea species	**Current data**	**Literature data**	**Ref**
	% dry matter	% dry matter	

C. arabica	5.20 – 6.70	4.05[a]–7.85[b]	[a](Narita & Inouye, 2015) [b](Campa et al., 2008)
C. canephora	5.80 – 8.40	5.19[a]-14.4[c]	[a](Narita & Inouye, 2015) [c](Campa et al., 2005)
C. liberica	4.66 – 6.41	3.29[d]-10.7[c]	[d](Martinez, Clemente, Lacerda, Neves, & Pedrosa, 2014) [c](Campa et al., 2005)
Arabusta	5.64 – 5.75	7.23-8.28[e]	[e](Clifford, 1985)
C. eugenioides	2.95 – 3.26	4.53-6.27[f]	[f](Anthony et al., 1993)
C. sessiliflora	4.54 – 5.17	5.61-9.93[f]	[f](Anthony et al., 1993)
C. congensis	6.64	4.86[d]-8.77[c]	[c](Campa et al., 2005) [d](Martinez et al., 2014)
C. pseudozanguebariae	0.21	0.87-1.75[f]	[f](Anthony et al., 1993)
C. racemosa	6.21	4.78[c]-6.03[e]	[c](Campa et al., 2005)e [e](Clifford, 1985)
C. brevipes	7.05	6.41[a]-12.3[c]	[a](Narita & Inouye, 2015) [c](Campa et al., 2005)

Table 4 – Distribution of *p*CoQAs isomers expressed as mg/100g dwb (standard deviation)

	3-*p*CoQA	**4-*p*CoQA**	**5-*p*CoQA**
C. arabica Brazil	7.8 (0.3)	11.8 (0.1)	53.5 (0.8)
C. arabica Colombia	6.8 (0.1)	6.4 (0.1)	49.7 (0.2)
C. arabica Etiopia – lot 1	3.2 (0.1)	4.4 (0.1)	47.4 (0.7)
C. arabica Etiopia – lot 2	3.6 (0.4)	4.7 (0.1)	47.9 (0.2)

C. arabica Honduras	7.5 (0.2)	8.7 (0.1)	45.1 (1.0)
C. arabica India	8.5 (0.3)	9.8 (0.3)	51.5 (1.0)
C. arabica Yemen – lot 1	8.8 (0.1)	9.4 (0.1)	58.4 (0.6)
C. arabica Yemen – lot 2	7.7 (0.7)	6.9 (0.1)	78.6 (0.7)
C. arabica var. laurina	4.9 (0.1)	6.8 (0.1)	39.0 (0.1)
C. canephora Vietnam	3.9 (0.4)	6.3 (0.1)	26.8 (0.1)
C. canephora India	19.7 (0.1)	6.1 (0.1	16.9 (0.2)
C. liberica – lot 1	9.5 (0.1)	3.1 (0.1)	13.7 (0.6)
C. liberica – lot 2	55.3 (0.4)	3.1 (0.1)	21.4 (0.2)
C. liberica – lot 3	48.0 (0.1)	2.6 (0.1)	17.1 (0.1)
C. liberica – 4	5.1 (0.1)	2.6 (0.1)	21.1 (0.4)
C. liberica – 5	15.3 (0.2)	17.8 (0.1)	30.8 (0.3)
C. liberica – 6	8.5 (0.1)	18.1 (0.1)	66.3 (0.1)
Arabusta – 1	18.1 (0.2)	17.7 (0.2)	67.2 (0.5)
Arabusta – 2	19.1 (0.1)	2.4 (0.1)	72.3 (0.1)
C. eugenioides	2.4 (0.1)	2.7 (0.1)	14.0 (0.1)
C. eugenioides – 2	7.1 (0.1)	1.6 (0.1)	20.0 (0.1)
C. sessiliflora	12.2 (0.1)	23.3 (0.2)	176.3 (4.1)
C. sessiliflora – 2	13.8 (0.1)	26.2 (0.1)	184.3 (4.3)
C. congensis	10.4 (0.1)	9.0 (0.1)	34.0 (1.2)
C. pseudozanguebariae	0.5 (0.1)	1.5 (0.1)	10.0 (0.1)
C. racemosa	8.6 (0.1)	9.6 (0.1)	19.0 (0.1)
C. brevipes	3.8 (0.1)	2.9 (0.1)	18.2 (0.1)

310

311 *Roasted coffee beans*

312 It is well known that during coffee roasting, chlorogenic acids are partially degraded because of
313 pyrolysis, generating other derivatives which are remarkably important in the development of coffee
314 aroma. At the same time, by a condensation process, phenolic lactones are formed, which contribute
315 to the bitter taste of the bevarage. The observed loss of chlorogenic acid during roasting (up to 95%
316 in dark roasted coffee), which has been extensively reported (Farah et al., 2005; Farah & Donangelo,
317 2006; Trugo & Macrae, 1984), is a consequence of the thermal degradation, which starts with
318 isomerization and epimerization processes in the initial roasting stages, followed by lactonization and
319 degradation reactions in the later stages. The effect of the roasting process on *p*CoQAs isomers
320 content in coffee has only been investigated on two different cultivars of *C. arabica* and one cultivar
321 of *C. canephora* from Brazil by Perrone et al. 2008. In addition to this study, no other data focused
322 on *p*CoQAs fate during coffee roasting have been published as far as we know.

323 In order to provide useful further data, and in view of the economic importance of Arabica and
324 Robusta coffee species, we selected two commercial samples (Arabica from Brazil and Robusta from
325 Vietnam) to carry out a preliminary exploration on the degradation of *p*CoQAs induced by thermal
326 treatments mimicking industrial roasting process. In the case of Arabica, the chosen roasting time
327 conditions led to the following total weight loss: 11.1%, 13.2%, 14.1%, 15.3%, 15.5%, 16.6% and
328 16.3% whereas for Robusta, 11.1%, 12.6%, 13.5%, 14.5%, 15.4%, and 18.5%. It has to be stressed
329 that total weight loss around 15% are typical of medium roasting degree, below and above this value,
330 roasting degree is known as light and dark, respectively. In figure 2, where the *p*CoQA isomers
331 content during roasting time evolution is reported, the overall and progressive decrease from light to
332 dark roasting degree is evident, as expected. Of the three isomers present, 5-*p*CoQA is clearly less
333 thermally stable than both 3-*p*CoQA and 4-*p*CoQA. However, the isomers' profile is somewhat
334 different depending on botanical species. In the case of Robusta sample, by considering the total
335 *p*CoQAs content it may be calculated that 1% loss of dry weight induced an average of 2% total
336 *p*CoQAs loss, whereas, in the case of Arabica, the average total *p*CoQAs loss, for 1% of dry weight

337 loss, was 4.2%. In spite of both the different roasting conditions and the experimental set up, these
338 findings are in good agreement with those of the previous study, particularly for Arabica. In fact, in
339 the range of roasting degree similar to that of the present investigation, Perrone et al., found 4.8%
340 and 5.3% of average *p*CoQAs losses for every 1% loss of dry weight for *C. arabica* cv. Mundo Novo
341 and *C. arabica* cv. Catuaì Vermelho, respectively. Regarding *C. canephora* cv. Conillon, 5.3% of
342 average *p*CoQAs losses for every 1% loss of dry weight was reported putting in evidence a very
343 similar behavior between Arabica and Robusta, which was not confirmed by the present data.

344 We also analyzed the degradation process of CQAs during roasting time in Arabica as reported in fig.
345 1S (see supplementary material) to confirm the data obtained by Perrone et al. Since they observed
346 an enhancement of the values of 3-CQA and 4-CQA at the beginning of roasting we decided to
347 analyze our roasting at shorter time. We also noticed a decrease of the amount of 5-CQA and a
348 simultaneous increase of the amount of both 3-CQA and 4-CQA. This evidence may suggest the
349 occurrence of an acyl transfer from 5-CQA to the other two isomers. The acyl migration was already
350 observed by Deshpande et al. (2014) but in completely different conditions. In the work of Deshpande
351 et al. acyl migration was studied in basic hydrolytic conditions and by thermal treatment without
352 solvent and different results were obtained: 5-CQA resulted stable after thermal treatment while acyl
353 migration was observed with basic hydrolytic treatment.

354 It has to be highlighted that we could detect and quantify *p*CoQA isomers in both botanical species,
355 even at the higher total weight loss, corresponding to a dark roasting degree while in previous works
356 reported in the literature this was not possible. This discrepancy may reflect both the different sample
357 preparation and the different analytical procedure adopted in the present work.

358 **4. Conclusions**

359 *p*CoQA isomers profile was determined in a variety of commercial lots of green coffee samples from
360 different geographical origins belonging to the three economically exploited botanical species *C.*
361 *arabica*, *C. canephora* and *C. liberica* and, for the first time, in several different wild species

362 belonging to the *Eucoffea* section. Total CGAs contents were also quantified to make possible the
363 estimation of the contribution of *p*CoQAs to the most important class of coffee polyphenols. In
364 addition, to confirm the higher amount of *p*CoQAs in Arabica than in Robusta commercial lots, the
365 characterization of the wild coffee species evidenced a more relevant contribution of *p*CoQAs to the
366 total CGAs content in botanical species accumulating low amount of chlorogenic acids such as *C.*
367 *pseudozanguebariae*. *C. sessiliflora* proved to be the richest source of *p*CoQAs among the different
368 analyzed wild coffee species. The low amount of *p*CoQAs in coffee beans may reflect the role played
369 by these isomers, particularly 5-*p*CoQA, as intermediate in one of the route proposed for the
370 biosynthesis of the most abundant coffee chlorogenic acid (5-CQA). In the case of Arabica
371 commercial lots, the observed intraspecific total CGAs variability is not related to the geographical
372 origin. Furthermore, the effect of roasting conditions on *p*CoQAs, evaluated in two selected samples
373 belonging to commercial species, was also preliminary explored. Mono-*p*-coumaroylquinic acids
374 considerably decreased with increasing of the roasting degree as already reported. However, the
375 thermal stability of this class of chlorogenic acids seems to be affected by the in-bean local
376 environment of the two investigated botanical species. In particular, when compared to Arabica,
377 *p*CoQAs in Robusta sample showed a smaller tendency to degrade with possible implications in the
378 aroma development. This finding, however, should be confirmed by enlarging the screening to a
379 wider number of samples. In perspective, we believe that it is necessary to further investigate the role
380 played by *p*CoQAs as aroma precursors, as bioactive coffee compounds or as chlorogenic acids
381 biosynthesis intermediates. The whole set of experimental data highlights the need to deepen the
382 knowledge on neglected classes of coffee chlorogenic acids.

383 **Funding**

384 This project has received funding from the European Union's Horizon 2020 research and innovation
385 programme under the Marie Sklodowska-Curie [grant agreement No 642014].

386 **Acknowledgment**

The authors would like to thank Mr. Gary Sjafwan and Mr. Sri Mulato for the kind gift of two *C. liberica* samples and Mr. Carlo Fornaciari for kindly gifted *C. racemosa* sample from Mozambique. Thank you to Mr. Benoit Bertrand from CIRAD (France) and to Mr. Stefano Scanferla, Mr. Luca Turello and Mr. Gianluca Malvicini from illycaffè for samples, supporting information and helpful discussion.

Declarations of interest: none

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530

531 **Figure 1.** Chemical structures of the hydroxycinnamic acid derivatives: 3-*p*-coumaroylquinic acid
532 (3-*p*CoQA) **1**; 4-*p*-coumaroylquinic acid (4-*p*CoQA) **2**; 5-*p*-coumaroylquinic acid (5-*p*CoQA) **3**; 3-
533 caffeoylquinic acid (3-CQA**) 4**; 4-caffeoylquinic acid (4-CQA**) 5**; 5-caffeoylquinic acid (5-CQA**) 6**;
534 3-feruloylquinic acid (3-FQA) **7**; 4-feruloylquinic acid (4-FQA) **8**; 5-feruloylquinic acid (5-FQA) **9**;
535 3,4-dicaffeoylquinic acid (3,4-diCQA) **10**; 3,5-dicaffeoylquinic acid (3,5-diCQA) **11**; 4,5-

536 dicaffeoylquinic acid (4,5-diCQA) **12**; *p*-coumaric acid (*p*-CoA) **13**; caffeic acid (CA) **14**; ferulic acid

537 (FA) **15**.

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539 **Figure 2** – Different isomers of *p*CoQAs content during roasting

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