Exhaustive qualitative LC-DAD-MSⁿ analysis of Arabica green coffee beans: cinnamoyl-glycosides and cinnamoylshikimic acids as new polyphenols in green coffee.

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Abstract

Coffee is one of the most consumed beverages in the world, due to its unique aroma and stimulant properties. Although its health effects are controversial, moderate intake seems to be beneficial. The present work deals with the characterization and quantification of polyphenols and methylxanthines in four Arabica green coffee beans from different geographical origin. The antioxidant activity was also evaluated. 43 polyphenols (cinnamic acid, cinnamoyl-amide, 5 cinammoyl-glycosides and 36 cinnamate esters) were identified using LC/MSⁿ. Among these, cinnamate esters of 6 different chemical groups (including 2 dimethoxycinnamoylquinic acid isomers, 3 caffeoyl-feruloylquinic acid isomers, caffeoyl-sinapoylquinic acid, *p*-coumaroyl-feruloylquinic acid, 2 of caffeoylshikimic acid isomers, and trimethoxycinnamoylshikimic acid) in addition to 5 isomers of cinnamoyl-glycosides called caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosic acid (CDOA), are described for the first time in Arabica green coffee beans. Moreover, 38 polyphenols (6-7% w/w) and 2 methylxanthines (1.3% w/w) were quantified by HPLC-DAD. Caffeoylquinic was the most abundant group of compounds (up to 85.5%) followed by dicaffeoylquinic and feruloylquinic acids (up to 8% and 7%, respectively), and the newly identified cinnamoyl-glycosides (CDOA) (up to 2.5%). Caffeine was the main methylxanthine (99.8%), with minimal amounts of theobromine (0.2%). African coffees (from Kenya and Ethiopia) showed higher polyphenolic content than American beans (from Brazil and Colombia), whereas methylxanthine contents varied randomly. Both phenols and methylxanthines contributed to the antioxidant capacity associated to green coffee, with a higher contribution of polyphenols. We conclude that green coffee represents an important source of polyphenols and methylxanthines, with high antioxidant capacity.

Keywords: Arabica green coffee beans, geographic origin, phenolic composition, chlorogenic acids, cinnamoyl conjugates, methylxanthines, LC-MSⁿ, antioxidant activity.

Abbreviations: AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid; CDOA, caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical scavenging capacity; TE, Trolox equivalents; TPTZ, 2,4,6-tripyridyl-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

1. Introduction

Cinnamic acids are *trans*-phenyl-3-propenoic acids widely found in plants. The different substituents in their ring give rise to different compounds such as *p*-coumaric, caffeic, ferulic, sinapic, dimethoxycinnamic and trimethoxycinnamic acids, among others. These compounds are usually conjugated with amino acids, polysaccharides, glycosides or different acids by their carboxylic group. The most common is the *trans*-esterification with quinic acid, (1*S*,3*R*,4*S*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid forming cinnamate esters, officially called cinnamoylquinic acids and collectively known as chlorogenic acids. In addition, cinnamic acids conjugated with shikimic acid,¹ a derivative from quinic acid defined as (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexanecarboxylic acid,² also belong to this group of cinnamate esters. The quinic and shikimic acids allow bonding of one or more cinnamic acids at positions 3, 4 and/or 5, forming a huge number of different cinnamate esters, such as monoacyl-, diacyl- or even triacyl- ester derivatives.

Cinnamate esters are widely found in fruits and vegetables¹ and, in particular, green coffee beans represent a rich source of this kind of polyphenols, containing up to 14% (w/w).³ Up to 74 different compounds have been identified in green *Coffea canephora* L. (Robusta) beans.⁴ 5-caffeoylquinic acid, caffeic acid esterified with quinic acid at position 5, is the most abundant cinnamate ester in green coffee beans, accounting for 50 to 60% of the total polyphenols.^{5,6} Other important cinnamate esters present in green coffee beans are dicaffeoylquinic and feruloylquinic acids, whose content amounts up to 20 and 10% of the total, respectively.^{5,7} Moreover, different cinnamoyl-amide as well as cinnamoyl-glycosides have been described in green coffee beans.⁸⁻¹⁰

Methylxanthines are also an important group of phytochemicals in coffee. This group of natural purine alkaloids has a xanthine base in common and they are mainly constituted of caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine), caffeine being the most abundant compound in green coffee beans.⁸

Identification of polyphenols has been done using HPLC-LC/MSⁿ, as this is the most appropriate technique to discriminate and carry out unambiguous structure elucidation among individual regioisomers of cinnamates and other compounds,¹¹ based on the fragmentation patterns observed in their tandem mass spectra.

Coffee is one of the most consumed beverages in the world due to its pleasant flavour, aroma and stimulatory effects, with nearly 76 million 60 kg-bags consumed in 2014 (<http://www.ico.org>). Green coffee beans are obtained from the cherries of *Coffea* plant. Although there are several species of the genus *Coffea*, only two have remarkable commercial value, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). Arabica coffee beans provide a high-quality brew with intense aroma and finer taste than Robusta coffee, which in contrast produces a bitterer brew, with a musty flavour and less body. Traditionally, the intake of coffee had been associated with negative health effects, although recently many studies have shown that the moderate intake of coffee reduces body fat and decreases oxidative damage related diseases, such as type 2 diabetes, cardiovascular complications, Alzheimer or Parkinson disease, among others.¹²⁻¹⁵ Moreover, several epidemiological studies have shown that the consumption of green coffee has important, beneficial health effects, such as anti-hypertensive, anti-diabetic, anti-obesity or hypotriglyceridemic properties.¹⁶⁻¹⁸ Cinnamic acid derivatives have been related with different biological effects, including anti-inflammatory, antioxidant, anti-carcinogenic or neuroprotective activities, among others,¹⁹⁻²² and consequently they contribute to the beneficial effects associated to coffee intake. However, other bioactive components in coffee like

methylxanthines can also play an important role. Many studies have confirmed that methylxanthines have neuroprotective, hypoglycemic, anti-inflammatory and cardiovascular protective properties.²³⁻²⁵

The chemical composition of green coffee depends on genetic factors and/or degree of maturation.^{3,26} Other factors related to the geographical area of production, such as environment or agricultural practices, may also affect the biosynthetic pathways of cinnamic acid derivatives or methylxanthines, contributing to both the variability and the final content of phenols and methylxanthines in green coffee beans^{8,5,27,28} and therefore to the beneficial health effects derived from its consumption. The aim of this study was to characterize the phenolic and methylxanthine composition in Arabica green coffee beans of four different geographical origins and to evaluate their antioxidant activity by complementary assays.

2. Material and methods

2.1. Chemical reagents and materials

Green coffee beans (*Coffea arabica* L.) from four different origins (Colombia, Brazil, Ethiopia and Kenya) were purchased in a local supermarket in Madrid (Spain). 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 5-caffeoylquinic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), caffeic acid, fluorescein sodium salt, gallic acid and theobromine were acquired from Sigma-Aldrich (Madrid, Spain). Folin-Ciocalteu reagent was from Panreac (Madrid, Spain). Caffeine, 2,4,6-tripyridyl-s-triazine (TPTZ) and potassium persulfate were obtained from Fluka (Madrid, Spain). 3,5-dicaffeoylquinic acid was from PhytoLab (Vestenbergsgreuth, Germany). All other reagents were of analytical or chromatographic grade.

2.2. Extraction of phenolic compounds and methylxanthines from green coffee beans

The method developed by Bravo & Saura-Calixto²⁹ was used to extract both phenolic compounds and methylxanthines in Arabica green coffee beans from different geographical origins. Briefly, the green coffee phenolic extracts were obtained from 1 g of *Coffea arabica* beans, previously grinded with a sieve of 5 µm. Each sample (n = 3) was extracted with 2 N hydrochloric acid in aqueous methanol (50:50, v/v) for 1 h at room temperature by constant shaking and centrifuged for 10 min at 3000 g. Supernatants were separated and the pellets washed with acetone:water (70:30, v/v) for 1 h at room temperature by constant shaking and centrifuged for 10 min at 3000 g. The supernatants were combined with the former and made up to 100 mL. An aliquot was concentrated under reduced pressure using a vacuum concentrator system (Speed-Vac, Thermo Fisher Scientific Inc., Waltham, MA, EEUU), resuspended in 1% formic acid in deionized water (v/v), filtered (0.45 µm pore size) and stored at -20 °C until chromatographic analysis.

2.3. Quantitative analysis of polyphenols and methylxanthines

Total phenolic content of green coffee phenolic extracts was determined by the Folin-Ciocalteu spectrophotometric assay.³⁰ The test samples were mixed with Folin-Ciocalteu reagent, 75 g/L sodium carbonate solution and distilled water (1:1:2:28, v/v) for 1 h and the absorbance was measured at 750 nm (Beckman DU-640, UV-Visible Spectrophotometer, Fullerton, CA, USA). Gallic acid was used as standard.

The polyphenolic content was additionally quantified by high performance liquid chromatography–diode array (HPLC-DAD) (1200 series, Agilent Technologies, Waldrom, Germany). The analysis also allowed determining the methylxanthine content in green coffee. Separation was achieved on a Superspher RP18 column (4 x 250 mm, 4 μ m particle size, Agilent Technologies) protected with an ODS RP18 guard column. Elution was performed with a gradient elution using a ternary system consisting of 1% formic acid in deionized water (solvent A), acetonitrile (solvent B) and methanol (solvent C) at a constant flow rate of 1 mL/min and 30 °C. The solvent gradient changed according to the following conditions: from 90% A – 5% B – 5% C to 80% A – 10% B – 10% C in 30 min, to 75% A – 13% B – 12% C in 10 min, to 65% A – 20% B – 15% C in 10 min, to 65% A – 17% B – 18% C in 5 min, and returning to initial conditions in 10 min (90% A – 5% B – 5% C) followed by 5 min of maintenance. Phenolic compounds previously identified through LC/MS analysis (see section 2.4) were acquired at 320 nm; whereas methylxanthines were detected at 272 nm and identified by comparing with the retention times and UV spectra of commercial standards. Phenolic compounds and methylxanthines were quantified using standard calibration curves: 5-caffeoylquinic and 3,5-dicaffeoylquinic acids were used to calculate the mono- and di-acylcinnamate esters content, respectively, and caffeic acid to quantify caffeic acid and caffeoyl-glycosides. Methylxanthines (theobromine and caffeine) were quantified using their respective standards. Limit of detection and quantification were calculated in order to determine the content of each component with the Chemstation Software (Agilent Technologies).

2.4. Qualitative analysis of polyphenols

A 1200 LC-DAD system coupled to an Accurate-Mass Quadrupole Time-of-Flight (Q-ToF) detector with electrospray ionization (ESI)-Jet Stream Technology (Agilent Technologies) was used to characterize the phenolic composition of the green coffee bean extracts. The chromatographic conditions and gradient elution were identical to those described above. Sample (1 μ L) was injected and compounds were separated on a Superspher RP18 column (4 x 250 mm, 4 μ m particle size, Agilent Technologies) and detected at 280 and 320 nm. The MS was fitted with an atmospheric pressure ESI source which operated in negative ion mode. The Q-ToF operating conditions were as follows: 12 L/min dry gas flow at 350 °C, 7 L/min sheath gas volume at 325 °C, nebulizing pressure at 45 psig, capillary voltage at 3500 V, fragmentor and nozzle voltages at 100 and 0 V, respectively. Mass spectrometry data were acquired in the 100-1000 m/z range. Mass Hunter Workstation Software was used to process data.

2.5. Antioxidant activity

Ferric reducing antioxidant power (FRAP) assay: the method modified by Pulido et al.³¹ was used to evaluate the reducing activity of green coffee beans. The formation of a colored TPTZ-Fe²⁺ complex after mixing test samples with FRAP reagent (0.3 M acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃ -3:1:1, v/v-), 0.3 M acetate buffer and dissolvent (1:6:20:3, v/v) was monitored at 595 nm for 30 min at 37°C nm in an automatized plate reader (Bio-Tek, Winooski, VT, USA). Trolox was used as a standard and results were expressed as μ mol Trolox equivalent (TE) per gram of dry matter.

ABTS assay: free radical scavenging capacity was assessed using the free radical cation ABTS^{•+}, whose absorbance decreases at 730 nm in the presence of an antioxidant.³² The radical cation was performed by reaction of ABTS with 2.45 mM potassium persulfate during 12-16 h at room temperature in the dark. Diluted

radical in methanol was incubated with the extracts of green coffee (6:23:1, v/v) to monitor the change of absorbance for 30 min at 37°C in an automatized plate reader (Bio-Tek). Trolox was used as standard and results expressed as $\mu\text{mol TE}$ per gram of dry matter.

Oxygen radical scavenging capacity (ORAC) assay: the capacity of green coffee extracts to prevent the fluorescence decay of fluorescein in the presence of a peroxy radical (AAPH) was evaluated following the method developed by Huang et al.³³, in order to provide a complementary measurement of free radical scavenging capacity. The fluorescence of the samples ($\lambda_{\text{excitation}}$ 485 nm, $\lambda_{\text{emission}}$ 528 nm) was measured for 90 min at 37 °C after mixing the green coffee bean extracts with 8.5×10^{-5} mM fluorescein in 75 mM phosphate buffer pH 7.4 and 153 mM AAPH (1:6:1.2, v/v) in an automatized plate reader (Bio-Tek). Trolox was used as standard, results were expressed as $\mu\text{mol TE}$ per gram of dry matter.

2.6. Statistical analysis

Statistical analyses were carried out using the program SPSS (version 19.0, SPSS, Inc., IMB Company). Firstly, homogeneity of variance of the data was tested using the Levene test. When variances were homogeneous, for multiple comparisons the one-way ANOVA test was used followed by a Bonferroni test; when variances were not homogeneous, the Games-Howell test was used. The significance level was set at $p < 0.05$. Results were expressed as mean $\pm$ standard deviation (SD).

3. Results and discussion

3.1. Identification and characterization of polyphenols in green coffee

The phenolic constituents present in green coffee beans from four different geographical origins were monitored by HPLC-DAD, analyzing UV spectra for their tentative identification. Afterwards, the samples were analyzed by high-resolution mass spectrometry using an LC-ESI-QTOF instrument in negative ion mode. Selected ion monitoring (SIM) located a total of 42 polyphenols grouped in four families: one cinnamic acid, one cinnamoyl-amide, five cinnamoyl-glycosides and 35 cinnamate esters. The green coffee beans from Colombia, Brazil and Kenya showed an additional compound (number **43** in Table 1), absent in Ethiopian green coffee. There were no differences in the chromatographic profiles of the analyzed coffees, except for the aforementioned compound. Figure 1 (b,c) shows a representative chromatogram at 320 nm. Lastly, samples were subjected to LC-MSⁿ by collision-induced dissociation mass spectrometer to complete the identification of polyphenols, especially isomers. MS fragmentation patterns observed after analysis by tandem MS spectra, chromatographic retention times, relative hydrophobicity and bonding strength to quinic acid have been used to develop structure-diagnostic hierarchical key for polyphenol identification. In this sense, the relative hydrophobicity of cinnamoyl derivatives depends on the substitution position of quinic and shikimic acids and the number and identity of the cinnamoyl residues. Accordingly, compounds with a greater number of free hydroxyl groups at position 4 or 5 on the quinic or shikimic acids are more hydrophilic than compounds possessing free hydroxyl groups at position 1 or 3.³⁴ Moreover, the cinnamoyl residue at position 5 is easily hydrolyzed, whereas links at position 4 are the strongest of all.³⁵ Figure 2b-c represents the structures of cinnamic acids (*p*-coumaric, caffeic, ferulic, dimethoxycinnamic, sinapic and trimethoxycinnamic

acids) as well as quinic and shikimic acids and tryptophan, which are involved in the chemical structures of the identified polyphenols of green coffee, Table 2 shows the cinnamate esters identified and the substituent and substituted position of quinic acid for each isomer. Table 1 includes the chemical characterization of all identified polyphenols by peak elution order: retention time, UV absorption maxima from DAD, molecular formula, quasimolecular ion $[M-H]^-$, MS/MS fragment ions with relative abundance and tentative name.

Caffeic acid (peak 8): In the present study, free caffeic acid (peak 8) was identified based on its $[M-H]^-$ at m/z 179 and its ion fragment at m/z 135, originated from the decarboxylation of caffeic acid. Its identity was confirmed by comparison with the corresponding commercial standard. Previous studies have identified up to three free cinnamic acids (caffeic, ferulic and dimethoxycinnamic acids) in green coffee beans.^{8,26}

Cinnamoylshikimic acids (peaks 15, 19 and 33): MS analysis showed two peaks (15 and 19) with parent ion at m/z 335.0737 and UV spectra similar to cinnamates (λ_{max} at 328-329 nm and shoulder at 296 nm). The accurate mass and molecular formula provided by Mass Hunter allowed the tentative identification as caffeoylshikimic acids, although the lack of fragmentation after MS² analysis did not allow to identify the substituted position in shikimic acid by caffeic acid. The esterification of shikimic acid with cinnamic acid has been previously described in yerba mate, sweet basil or roasted coffee.^{2,36,37} However to the best of authors' knowledge, this is the first time that caffeoylshikimic acids have been described in green coffee beans. Similarly, compound 33 showed a quasimolecular ion at m/z 393.1195 and UV spectra compatible with cinnamic acid derivatives. Based on its accurate mass, Mass Hunter software predicted it being trimethoxycinnamoylshikimic acid, which had not been previously reported in green coffee.

***p*-Coumaroylquinic acids (peaks 3, 13 and 14):** Three chromatographic peaks showed a MS spectrum with a quasimolecular ion at m/z 337 and a characteristic UV spectrum with a single maximum between 310-312 nm. The deprotonated product of the cinnamic moiety gave a base peak at m/z 163 for compound 3 after MS² analysis, being assigned as 3-*p*-coumaroylquinic acid based on previous studies.³⁵ The fragment ion of peak 13 at m/z 173 in MS² spectrum resulting from dehydration of quinic acid clearly indicated the substitution of quinic acid at position 4,³⁵ allowing the identification of peak 13 as 4-*p*-coumaroylquinic acid. The last isomer (14) showed the characteristic fragmentation of 5-acyl-quinic acid (MS² at m/z 191),³⁵ suggesting that this compound was 5-*p*-coumaroylquinic acid.

Caffeoylquinic acids (peaks 1, 2, 5, 7 and 11): A target MSⁿ experiment at m/z 353 applied to the extracts showed five caffeoylquinic acid isomers. Compound 5 was identified as 5-caffeoylquinic acid after comparison with the commercial standard, facilitating the distinction of compound 2 with a common base peak at m/z 191. Moreover, the fragment ions at m/z 179 and 135 observed in MS² spectrum of compound 2 allowed its identification as 3-caffeoylquinic acid based on previous studies^{8,38}. The MS² spectrum of peak 7 provided a fragment ion at m/z 173, indicative of the isomer 4-caffeoylquinic acid.³⁵ Lastly, the fragmentation pattern of isomers 1 and 11 was identical to 3- and 5-caffeoylquinic acids, suggesting that these compounds were the corresponding *cis*-isomers: *cis*-3-caffeoylquinic and *cis*-5-caffeoylquinic acid, respectively.³⁹

Caffeoyl-*N*-tryptophan (peak 38): The chromatographic peak labelled as 38 had a UV spectrum with two maxima at 290 and 324 nm, characteristic of caffeic or ferulic acids conjugated with tryptophan.^{8,26} The MS

analysis allowed to confirm that compound **38** was caffeoyl-*N*-tryptophan with a quasimolecular parent ion at m/z 365. While caffeoyl-*N*-tryptophan, present in all samples, did not provide additional information about the geographical origin, some cinnamoyl-amino acids can play an important role in the characterization of both the coffee species and the geographical origin. In fact, *p*-coumaroyl-*N*-tryptophan has been characterized in some cultivars of Arabica coffee^{8,26} and caffeoyl-*N*-phenylalanine and *p*-coumaroyl-*N*-tyrosine are indicators of Robusta coffee from Angola and Uganda.^{8,10,26}

Feruloylquinic acids (peaks 6, 10, 16, 18, 20): Five feruloylquinic acid isomers, with UV spectra similar to caffeoylquinic acid (λ_{max} at 325–326 nm and shoulder at 296 nm), were identified by their parent ion at m/z 367 in negative mode. In MS² of compound **6**, a base peak at m/z 193 derived from the deprotonated cinnamoyl moiety was observed, which allowed its identification as 3-feruloylquinic acid,³⁵ in agreement with the fragmentation pattern of the 3-*p*-coumaroylquinic acid with a base peak at m/z 163 (deprotonated *p*-coumaroyl moiety) or the 3-caffeoylquinic acid with the ion at m/z 179 (deprotonated caffeoyl moiety) as the most abundant secondary ion in MS² spectra. Compounds **16** and **18** were easily distinguished from the other isomers due to their characteristic base peak at m/z 173 (dehydration of quinic acid moiety) and 191 (deprotonated quinic acid moiety), respectively, which allowed their identification as 4-feruloylquinic (**16**) and 5-feruloylquinic acids (**18**). Lastly, the lack of fragmentation for compound **10** and the different MS² spectrum for compound **20** compared to the aforementioned isomers (**6**, **16** and **18**), hindered their specific identification of these feruloylquinic acid isomers.

Caffeoyl-glycosides (peaks 4, 9, 12, 17 and 21): Exhaustive analysis of the extracts allowed observing five compounds with a parent ion at m/z 381.0820, never before reported in green coffee beans, to the best of our knowledge. After searching in SciFinder (March 2014) and on the basis of their molecular formula (C₁₇H₁₈O₁₀) and fragment ions (only present in MS² spectra for the compounds **9** and **12**), compounds **4**, **9**, **12**, **17** and **21** were tentatively identified as caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosic acid (CDOA), a caffeic acid esterified to a monosaccharide of eight carbon atoms. This caffeoyl-glycoside had been identified in *Erigeron breviscapus*⁴⁰ and, recently, in yerba mate.⁴¹ Further work is required to define the position of the substituents, which will allow establishing the chemical structure of each isomer. Up to date, the only cinnamoyl-glycosides described in green coffee beans are formed with hexoses,^{8,9} being this the first report of this type of glycoside in green coffee.

Dimethoxycinnamoylquinic acid (peaks 22 and 23): MSⁿ analysis of compounds **22** and **23** showed two peaks with parent ion at m/z 381.1186 in negative mode and UV spectra characteristic of [cinnamate esters](#) (λ_{max} at 328 nm and shoulder at 296 nm). In spite of the similitude in m/z value with caffeoyl-glycosides, Mass Hunter software predicted a different molecular formula (C₁₈H₂₂O₉) compatible with dimethoxycinnamoylquinic acid, in agreement with the literature.⁴² In addition, MS² spectrum of peak **22** showed an intense fragment ion at m/z 173, which allowed its identification as 4-dimethoxycinnamoylquinic acid. The absence of fragmentation for compound **23** ruled out the possibility to identify the isomer. For the first time, dimethoxycinnamoylquinic acids have been described in Arabica green coffee beans, although previous studies reported the presence of these compounds in Robusta green coffee beans.^{9,4,42}

***p*-Coumaroyl-caffeoylquinic acids (peaks 26, 29 and 35):** Three chromatographic peaks with an UV spectrum maximum at 311–316 nm and quasimolecular ion at m/z 499 were assigned to *p*-coumaroyl-

caffeylquinic acids. The absence of the secondary ion at m/z 173 for compound **29** indicated no substituent at position 4, while the ion at m/z 163 corresponding to the dehydrated *p*-coumaric characteristic at position 3, suggested that compound **29** was 3-*p*-coumaroyl-5-caffeylquinic acid. In this sense, the ion at m/z 173 in MS² spectra of isomers **26** and **35** confirmed the existence of a residue at position 4. Regarding peak **26**, the MS² spectrum showed an intense fragment at m/z 353, originated from the loss of one dehydrated molecule of *p*-coumaric acid; while the base peak for compound **35** was at m/z 337 due to the loss of a dehydrated caffeyl residue. Considering all the above and the elution order of dicinnamoylquinic acids (3,4-, 3,5- and 4,5) as well as previous studies,⁴³ compounds were assigned as 3-*p*-coumaroyl-4-caffeylquinic acid (**26**) and 4-*p*-coumaroyl-5-caffeylquinic acid (**35**), respectively.

Dicafeoylquinic acids (peaks 24, 25, 27 and 39): A targeted MSⁿ experiment at m/z 515 allowed locating four dicafeoylquinic acid isomers with a typical UV-spectrum of cinnamate esters. The loss of one caffeyl residue gave a common base peak at m/z 353 for all compounds. The specific identification of each isomer was possible thanks to the secondary ions and the well-established elution order for di-acyl compounds (3,4-, 3,5- and 4,5-di-acyl).^{34,43} The use of a pure standard allowed to confirm the identity of compound **25** as 3,5-dicafeoylquinic acid. Moreover, the secondary ion at m/z 173 in MS² spectrum of peaks **24** and **27** was indicative of the presence of one caffeyl moiety at position 4, the fragment ion at m/z 179 in compound **24** of a deprotonated caffeic acid, and the well-known elution order of these isomers, allowed their unequivocal identification as 3,4-dicafeoylquinic acid (**24**) and 4,5-dicafeoylquinic acid (**27**).³⁴ Compound **39** was identified as *cis*-4,5-dicafeoylquinic based on the identical fragmentation pattern to peak **27**.³⁹

Caffeoyl-feruloylquinic acids (peaks 28, 30-32, 34, 37, 40 and 41): Eight compounds with specific UV spectra of cinnamate esters were identified as caffeoyl-feruloylquinic acids attending to their quasimolecular parent ion at m/z 529. Based on the fragmentation patterns of MS² analysis, elution order of these compounds previously established by Clifford et al.⁴³ in Robusta green coffee, and the strength of the bonds³⁵ seven peaks were identified. The absence of a secondary ion at m/z 173 in MS² spectrum of compounds **31** and **32** coupled to their base peak at m/z 367 and 353, respectively, allowed assigning them as 3-feruloyl-5-caffeylquinic (**31**) and 3-caffeyl-5-feruloylquinic (**32**) acid. The earlier elution of peaks **28** and **30**, and their intense ion at m/z 353 and 367 originated from the loss a feruloyl and caffeyl residue linkage through a labile bond, respectively, made possible their identification as 3-feruloyl-4-caffeylquinic acid (**28**) and 3-caffeyl-4-feruloylquinic acid (**30**). The similar fragmentation of compound **34** to **31** allowed its identification as the *cis*-isomer (*cis*-3-feruloyl-5-caffeylquinic acid). Base peak at m/z 367 and 353 was observed in MS² for peaks **37** and **40**, respectively, together with a secondary ion at m/z 173 in both compounds, which allowed their identification as 4-feruloyl-5-caffeylquinic acid (**37**) and 4-caffeyl-5-feruloylquinic acid (**40**).^{39,43} Lastly, the absence of secondary ions in MS² spectrum of compound **41** impeded to determine the substituted positions of quinic acid. Although some of these compounds had been positively identified in yerba mate and Robusta coffee,^{2,41,43} only four (**30**, **32**, **37** and **40**) had been previously described in Arabica green coffee beans.^{8,26}

Caffeoyl-sinapoylquinic acid (peak 36): One isomer of caffeoyl-sinapoylquinic acid was detected in the green coffee phenolic extracts when the m/z value for the extracted MS chromatograms was set at 559. The UV spectrum showed a single maximum at 325 nm compatible with sinapic acid. The MS² base peak at m/z 397 after losing a dehydrated caffeyl moiety along with the fragment ion at m/z 173 (dehydrated quinic acid),

which is indicative of the presence of a substituent in position 4 of quinic acid, together with the fragmentation pattern previously characterized in Robusta green coffee by Jaiswall et al.⁴ allowed its identification as 4-sinapoyl-5-caffeoylquinic acid. To our knowledge, this is the first time that this compound has been described in Arabica green coffee; in fact, sinapoylquinic acid derivatives have been considered as markers to distinguish Arabica and Robusta coffee varieties, as these compounds had been considered exclusive to Robusta coffee.⁴

p-Coumaroyl-feruloylquinic acid (**peak 43**): MSⁿ analysis showed an additional compound in Colombian, Brazilian and Kenyan green coffee extracts with a quasimolecular ion at *m/z* 513 and UV spectra that well-matched with cinnamate esters. Characterization by Clifford et al.,⁴³ facilitated the identification as *p*-coumaroyl-feruloylquinic acid. However, the lack of the secondary ions in MS² analysis complicated the identification of the specific isomer. As for compound **36**, *p*-coumaroyl-feruloylquinic is now reported in Arabica green coffee for the first time to the best of authors' knowledge.

Compound 42: Based on its UV spectra, with λ_{max} at 324 nm and shoulder at 296 nm, chromatographic peak **42** was assigned as a cinnamic acid derivative. The quasimolecular ion at *m/z* 543 in negative mode, was compatible with the chemical structure of three different compounds: diferuloylquinic, dimethoxycinnamoyl-caffeoylquinic and *p*-coumaroyl-sinapoylquinic acid.^{4,42} The lack of fragmentation after MS² analysis hindered its identification although attending to the abundance of compounds constituted by ferulic acid, it was tentatively assigned as diferuloylquinic acid.

Although in the present work many polyphenols have been identified in Arabica green coffee for the first time, it is worth mentioning that the authors failed to observe other polyphenols previously described in Arabica green coffee such as ferulic acid, mono- or diacyl-hexones or *p*-coumaroyl-*N*-tryptophan.^{8,9,26}

3.2. Quantitative determination of green coffee phenolic compounds

The content of phenolic compounds present in green coffee was chromatographically and spectrophotometrically determined using HPLC-DAD and Folin-Ciocalteu assay, respectively. In spite of the accepted un-specificity of Folin-Ciocalteu assay to determine phenolic compounds,⁴⁴ it is the most commonly used method to estimate phenolic contents. In this sense, the green coffee bean phenolic content ranged from 5.1 to 5.7% w/w of dry matter (Table 3), values comparable with those described by other authors for Arabica green beans (3.5-5.2 % w/w).⁴⁵⁻⁴⁷ Statistically significant differences in the phenolic content of green coffee from different geographical origins (Colombia, Brazil, Ethiopia and Kenya) were observed, being Ethiopian and Brazilian beans the richest and poorest in phenolic compounds, respectively, among the evaluated coffees.

Simultaneously, a chromatographic method was developed to deepen in the chemical structures of the phenolic compounds (section 3.1.) and, in addition, accurately determine their content. Thus, a triphasic gradient was used to analyze the green coffee extracts by HPLC-DAD allowing the quantification of 38 of the 43 identified polyphenols at 320 nm. For unresolved chromatographic peaks, quantification was performed for the most abundant compound: peaks **5-6** as 5-caffeoylquinic acid (**5**), **9-10** as CDOA (**9**), **11-12** as *cis*-5-caffeoylquinic acid (**11**), **16-17** as 4-feruloylquinic acid (**16**) and **39** and **43** as *cis*-4,5-dicafeoylquinic acid (**39**).

Table 4 summarizes the phenolic contents of the four coffee extracts grouped according to their chemical structure (Table 1 in supporting information shows the individual content of all quantified compounds).

These results confirm the effectiveness of this chromatographic program which improved the procedure developed by Rodrigues et al.²⁶, who quantified 21 phenolic compounds in Arabica green coffee beans. Similarly, in Robusta green coffee up to 74 different compounds, were identified,^{4,34,35,39,42,43} whereas only 26 polyphenols had been quantified by Alonso-Salces et al.⁹ The significant improvement of the chromatographic method here reported is based on using a triphasic gradient versus the biphasic one that has been widely applied, resulting in enhanced resolution and symmetry of the chromatographic peaks.

Using HPLC-DAD analysis, a high phenolic content (6-7% w/w) in Arabica green coffee beans extracts was observed in agreement with values previously reported in the same type of coffee (5.5-8% w/w)⁵⁻⁷, while slightly lower contents had been reported in Robusta green beans (8.5-9%, w/w).^{6,9,48} The most abundant phenolic group was caffeoylquinic acids (up to 85.5% of the phenols total), with 5-caffeoylquinic acid as the main polyphenol (62.1-69.6%) followed by 4-caffeoylquinic acid (8.5-11.4%) and 3-caffeoylquinic acid (4.4-6.8%). Dicafeoylquinic and feruloylquinic acids were the second and third most common groups of compounds in green coffee beans, representing up to 8 and 7% of the total polyphenol content, respectively; 3,5-dicafeoylquinic (2.5-4.6%) and 5-feruloylquinic (4.1-5.7%) acids were the main compounds within each group, respectively. These results are in agreement with those reported by other authors (80, 12 and 4-7% of phenolic total for caffeoylquinic, dicafeoylquinic and feruloylquinic acids, respectively).^{7,26} It is worth noting the high content in CDOA (approximately 2.5% of the total polyphenol content), particularly considering that it is a new group of compounds that had not been identified before in green coffee. Caffeoyl-glycosides represent the fourth most abundant group of compounds in green coffee, being higher than other mono- or dicinnamoylquinic acids typically associated with this beverage, such as *p*-coumaroylquinic acids or caffeoyl-feruloylquinic acids.

The total phenolic content determined chromatographically varied slightly among the analysed coffees (Colombia, Brazil, Ethiopia and Kenya), partially in agreement with previous data.^{5,9,27,49} As indicated, Ethiopian coffee showed the highest phenolic content and Brazilian beans had the lowest. This is in agreement with the results observed using the Folin-Ciocalteu assay (Table 3, $R^2 = 0.88$), with the chromatographic values slightly lower than the colorimetric ones, probably due to the low specificity of the Folin-Ciocalteu reagent. However, the present results differed from those reported by Dziki et al.⁵⁰, who described a higher phenolic content in Brazilian coffee than in Ethiopian. Some polyphenols have been identified as markers of the growing region, such as 3,4-dicafeoylquinic acid discriminating between green coffee from Africa and America and also from Ethiopia.^{5,9} Accordingly, the results of this study showed a higher content of 3,4-dicafeoylquinic acid in Colombian and Brazilian green coffee than in African beans. Moreover, the biosynthetic pathways of *p*-coumaroyl-caffeoylquinic and caffeoyl-feruloylquinic acids, as well as *p*-coumaroylquinic acid are favoured in American regions compared to African regions, which could be due to genetic and/or environmental factors.^{26,28,51} In fact, Colombian and Brazilian coffees showed higher contents of *p*-coumaroyl-caffeoylquinic, caffeoyl-feruloylquinic and *p*-coumaroylquinic acids than Ethiopian and Kenyan coffee beans.

3.3. Characterization and quantification of green coffee methylxanthines

The chromatographic method used to analyse phenolic compounds in green coffee beans allowed to determine methylxanthines at 272 nm (Figure 1a). Theobromine and caffeine, but not theophylline, were identified by comparison with UV spectra and retention time of standards. These results were in agreement with those previously published.²⁶ Moreover, these authors also described the absence of theobromine in some varieties of Arabica coffee depending on the geographical origin, suggesting that this methylxanthine might be considered a chemical marker to distinguish the origin of Arabica green coffee. However, its low content in green coffee^{9,26} may complicate its identification, which might justify the different results observed among different studies, and thus suggesting that this methylxanthine would be a doubtful marker.

Quantitative analysis showed that caffeine is the most abundant methylxanthine in all the analyzed green coffee extracts, with values ranging from 1.17 to 1.31% w/w of dry matter, whereas theobromine contents were 0.0018-0.032% (Table 5). Both compounds were within the values previously reported for Arabica green coffee beans.^{52,53} Ethiopian green coffee showed the highest methylxanthine content, while Kenyan coffee had the lowest concentration out of the samples tested. These results were in line with those previously published (1.3 and 1% w/w caffeine in Ethiopian and Kenyan coffees, respectively).^{9,49,53}

3.4. Antioxidant activity determination of green coffee beans

All the green coffee extracts had a high radical scavenging as well as ferric reducing activities (Table 3), in agreement with the values reported by other authors.^{52,54} A good relationship was found between the phenolic composition determined with the Folin-Ciocalteu assay and the antioxidant activity determined by FRAP ($R^2 = 0.86$), ABTS ($R^2 = 0.56$) and ORAC ($R^2 = 0.82$) assays. Similarly, a direct relationship was found between the antioxidant capacity and the phenolic content determined chromatographically ($R^2 = 0.98$, $R^2 = 0.85$ and $R^2 = 0.82$ for FRAP, ABTS and ORAC, respectively), observing better R^2 values than those above, probably due to the higher specificity of the chromatographic method compared to the colorimetric one. With respect to the antioxidant capacity associated to the methylxanthine content,⁵⁵ R^2 values ($R^2 = 0.72$ MX vs. FRAP, $R^2 = 0.28$ MX vs. ABTS and $R^2 = 0.52$ MX vs. ORAC) were higher with the reducing activity determined using the FRAP assay than with the radical scavenging activity determined using ABTS and ORAC assays. These results evidenced limited contribution of methylxanthines to the antioxidant activity of green coffee in accordance with Pellegrini et al.,⁵⁶ who observed only a 25% lower total antioxidant capacity in decaffeinated coffee beverages as compared to caffeinated coffee. In this sense, evaluation of the correlation between methylxanthine plus polyphenol contents, determined by HPLC-DAD, versus antioxidant activity provided similar R^2 values ($R^2 = 0.98$, $R^2 = 0.79$ and $R^2 = 0.80$ for FRAP, ABTS and ORAC, respectively) than those aforementioned between polyphenols and antioxidant activity, which supports the higher contribution of polyphenols than methylxanthines to the antioxidant capacity of green coffee beans.

In summary, two methylxanthines and 43 polyphenols (1 cinnamic acid, 1 cinnamoyl-amide, 5 cinammoyl-glycosides and 36 cinnamate esters) have been identified in Arabica green coffee beans using LC/MSⁿ. New cinnamate esters and cinnamoyl-glycosides (caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosic acid -CDOA-) have been described for the first time. Moreover, 38 polyphenols (6-7% w/w) and 2 methylxanthines (1.3% w/w) were quantified by HPLC-DAD. Up to 85.5% of the phenolic compounds were caffeoylquinic acids, followed by dicaffeoylquinic acids (8%), feruloylquinic acids (7%) and cinnamoyl-glycosides (2.5%), among others. Caffeine was the main methylxanthine (99.8%) with minimal amounts of theobromine (0.2%). African

435 coffees (from Kenya and Ethiopia) showed higher polyphenol contents than American beans (from Brazil and
436 Colombia), while methylxanthine contents varied randomly. Both phenols and methylxanthines contributed to
437 the antioxidant capacity associated to green coffee, although the contribution of polyphenols was more
438 remarkable. Therefore, green coffee represents an important source of polyphenols and methylxanthines, with
439 high antioxidant capacity, mainly associated to its phenolic fraction.

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616

617 **Figure captions**

618 **Figure 1.** Representative HPLC-DAD chromatograms of green coffee beans (in blue) at 272 nm (a), at 320
619 nm (b) and at 320 nm enlarged (c). Chromatographic profiles of methylxanthine standards (in green; TB,
620 theobromine; TP, theophylline; CAF, caffeine) registered at 272 nm (a).

621 **Figure 2.** Chemical structures of methylxanthines (a); cinnamic (b); quinic and shikimic acids and tryptophan
622 (c) identified in green coffee beans.

623

624

Figure 1.

Figure 2.

a.

Caffeine

Theobromine

b.

p-Coumaric acid

Caffeic acid

Ferulic acid

Dimethoxycinnamic acid

Sinapic acid

Trimethoxycinnamic acid

c.

Quinic acid

Shikimic acid

Tryptophan

Table 1. HPLC-DAD characterization and negative ion MS/MS fragmentation of phenolic compounds in green coffee beans.

No.	Name	RT (min)	λ_{max}	Molecular formula	MS ¹ <i>m/z</i>	MS ²								
						Base peak <i>m/z</i>	Secondary peak							
							<i>m/z</i>	% RA	<i>m/z</i>	% RA	<i>m/z</i>	% RA	<i>m/z</i>	% RA
1	<i>cis</i> -3-Caffeoylquinic acid	7.06	324, 296sh	C ₁₆ H ₁₈ O ₉	353.0893	191	179	93.2						
2	3-Caffeoylquinic acid	7.97	325, 296sh	C ₁₆ H ₁₈ O ₉	353.0889	191	179	97.8	135	13.2				
3	3- <i>p</i> -Coumaroylquinic acid	11.61	310	C ₁₆ H ₁₈ O ₈	337.0915	163	191	33.0						
4	CDOA	12.54	327, 294sh	C ₁₇ H ₁₈ O ₁₀	381.0820									
5	5-Caffeoylquinic acid	14.61	326, 296sh	C ₁₆ H ₁₈ O ₉	353.0894	191								
6	3-Feruloylquinic acid	14.61	*	C ₁₇ H ₂₀ O ₉	367.1036	193	191	96.3	173	65.3	134	11.0		
7	4-Caffeoylquinic acid	15.13	326, 296sh	C ₁₆ H ₁₈ O ₉	353.0898	173	191	96.7	179	88.9	135	13.0		
8	Caffeic acid	16.70	324, 296sh	C ₉ H ₈ O ₄	179.0338	135								
9	CDOA	17.36	325, 296sh	C ₁₇ H ₁₈ O ₁₀	381.0820	219	191	31.9	173	21.0				
10	Feruloylquinic acid	17.36	*	C ₁₇ H ₂₀ O ₉	367.1042									
11	<i>cis</i> -5-Caffeoylquinic acid	19.97	322, 298sh	C ₁₆ H ₁₈ O ₉	353.0877	191								
12	CDOA	19.97	*	C ₁₇ H ₁₈ O ₁₀	381.0820	179	219	23.5	173	13.5				
13	4- <i>p</i> -Coumaroylquinic acid	21.71	312	C ₁₆ H ₁₈ O ₈	337.0924	173	191	42.4	163	42.4	145	8.7		
14	5- <i>p</i> -Coumaroylquinic acid	22.75	312	C ₁₆ H ₁₈ O ₈	337.0933	191	173	7.1	163	10.3				
15	Caffeoylshikimic acid	24.94	328, 296sh	C ₁₆ H ₁₆ O ₈	335.0737									
16	4-Feruloylquinic acid	25.81	326, 296sh	C ₁₇ H ₂₀ O ₉	367.1035	173	193	5.6						
17	CDOA	25.81	*	C ₁₇ H ₁₈ O ₁₀	381.0820	219								
18	5-Feruloylquinic acid	27.02	325, 296sh	C ₁₇ H ₂₀ O ₉	367.1048	191	173	9.2						
19	Caffeoylshikimic acid	28.16	329, 296sh	C ₁₆ H ₁₆ O ₈	335.0737									
20	Feruloylquinic acid	32.48	326, 296sh	C ₁₇ H ₂₀ O ₉	367.1038	179	191	36.7	135	42.7				
21	CDOA	35.48	326, 296sh	C ₁₇ H ₁₈ O ₁₀	381.0820									
22	4-Dimethoxycinnamoylquinic acid	41.08	328, 296sh	C ₁₈ H ₂₂ O ₉	381.1186	173	207	24.7	191	60.2				
23	Dimethoxycinnamoylquinic acid	43.73	328, 296sh	C ₁₈ H ₂₂ O ₉	381.1186									
24	3,4-Dicaffeoylquinic acid	44.50	325, 296sh	C ₂₅ H ₂₄ O ₁₂	515.1195	353	191	12.2	179	24.7	173	38.5	135	11.1
25	3,5-Dicaffeoylquinic acid	45.86	327, 296sh	C ₂₅ H ₂₄ O ₁₂	515.1201	353	191	24.8	179	20.3				
26	3- <i>p</i> -coumaroyl-4-caffeoylquinic acid	49.33	311	C ₂₅ H ₂₄ O ₁₁	499.1240	353	173	40.7						
27	4,5-Dicaffeoylquinic acid	49.73	327, 296sh	C ₂₅ H ₂₄ O ₁₂	515.1201	353	515	30.5	173	31.6				
28	3-Feruloyl-4-caffeoylquinic acid	49.73	*	C ₂₆ H ₂₆ O ₁₂	529.1326	353	173	61.8						
29	3- <i>p</i> -coumaroyl-5-caffeoylquinic acid	50.26	312	C ₂₅ H ₂₄ O ₁₁	499.1237	353	337	87.4	163	88.3				
30	3-Caffeoyl-4-feruloylquinic acid	50.73	324, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1342	367	529	74.7	173	73.9				

31	3-Feruloyl-5-caffeoylquinic acid	51.54	327, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1358	367	193	60.2				
32	3-Caffeoyl-5-feruloylquinic acid	52.07	327, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1352	353	367	47.4	191	36.8	179	43.6
33	Trimethoxycinnamoylshikimic acid	52.77	329, 296sh	C ₁₉ H ₂₂ O ₉	393.1195							
34	<i>cis</i> -3-Feruloyl-5-caffeoylquinic acid	52.77	328, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1339	529	367	57.1				
35	4- <i>p</i> -coumaroyl-5-caffeoylquinic acid	53.51	316	C ₂₅ H ₂₄ O ₁₁	499.1242	337	173	69.5				
36	4-Sinapoyl-5-caffeoylquinic acid	53.51	325	C ₂₇ H ₂₈ O ₁₃	559.1451	397	223	12.9	173	67.4		
37	4-Feruloyl-5-caffeoylquinic acid	54.26	327, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1353	367	173	70.8				
38	Caffeoyl- <i>N</i> -tryptophan	54.26	290, 324	C ₂₀ H ₁₈ N ₂ O ₅	365.1151							
39	<i>cis</i> -4,5-Dicaffeoylquinic acid	54.89	327, 296sh	C ₂₅ H ₂₄ O ₁₂	515.1185	353	173	25.9				
40	4-Caffeoyl-5-feruloylquinic acid	54.91	327, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1346	353	173	36.3				
41	Caffeoyl-feruloylquinic acid	57.00	329, 296sh	C ₂₆ H ₂₆ O ₁₂	529.1355							
42	Tentative identification as Diferuloylquinic acid	58.52	324, 296sh	C ₂₇ H ₂₈ O ₁₂	543.1503							
43**	<i>p</i> -Coumaroyl-feruloylquinic acid	54.89	328, 296sh	C ₂₆ H ₂₆ O ₁₁	513.1397							

Abbreviations: CDOA = caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosic acid; RT = retention time; % RA = percentage of relative abundance.

* λ_{max} not detected by overlapping with the previous chromatographic compound.

** Compound only detected in Colombian, Brazilian and Kenyan green coffee beans.

641 **Table 2.** Cinnamate esters identified in Arabica green coffee and substituted position in quinic acid (R3, R4
642 and R5).

No.	Name	R3	R4	R5
1	<i>cis</i> -3-Caffeoylquinic acid	C	H	H
2	3-Caffeoylquinic acid	C	H	H
3	3- <i>p</i> -Coumaroylquinic acid	<i>p</i> -Co	H	H
5	5-Caffeoylquinic acid	H	H	C
6	3-Feruloylquinic acid	F	H	H
7	4-Caffeoylquinic acid	H	C	H
11	<i>cis</i> -5-Caffeoylquinic acid	H	H	C
13	4- <i>p</i> -Coumaroylquinic acid	H	<i>p</i> -Co	H
14	5- <i>p</i> -Coumaroylquinic acid	H	H	<i>p</i> -Co
16	4-Feruloylquinic acid	H	F	H
18	5-Feruloylquinic acid	H	H	F
22	4-Dimethoxycinnaoylquinic acid	H	D	H
24	3,4-Dicaffeoylquinic acid	C	C	H
25	3,5-Dicaffeoylquinic acid	C	H	C
26	3- <i>p</i> -Coumaroyl-4-caffeoylquinic acid	<i>p</i> -Co	C	H
27	4,5-Dicaffeoylquinic acid	H	C	C
28	3-Feruloyl-4-caffeoylquinic acid	F	C	H
29	3- <i>p</i> -Coumaroyl-5-caffeoylquinic acid	<i>p</i> -Co	H	C
30	3-Caffeoyl-4-feruloylquinic acid	C	F	H
31	3-Feruloyl-5-caffeoylquinic acid	F	H	C
32	3-Caffeoyl-5-feruloylquinic acid	C	H	F
34	<i>cis</i> -3-Feruloyl-5-caffeoylquinic acid	F	H	C
35	4- <i>p</i> -Coumaroyl-5-caffeoylquinic acid	H	<i>p</i> -Co	C
36	4-Sinapoyl-5-caffeoylquinic acid	H	S	C
37	4-Feruloyl-5-caffeoylquinic acid	H	F	C
39	<i>cis</i> -4,5-Dicaffeoylquinic acid	H	C	C
40	4-Caffeoyl-5-feruloylquinic acid	H	C	F

643 Abbreviations: C = caffeic acid; D = dimethoxycinnamic acid; F = ferulic acid; *p*-Co = *p*-coumaric acid; S = sinapic acid

Table 3. Total phenolic content and antioxidant capacity of green coffee bean extracts from different geographical origins. Values are means $\pm$ SD expressed on a dry matter basis ($n = 3$). Different letters within a column indicate statistically differences ($p < 0.05$).

	Total phenolic content	Antioxidant capacity		
	Folin method (g/100 g d.m.)	FRAP ($\mu\text{mol TE/g d.m.}$)	ABTS ($\mu\text{mol TE/g d.m.}$)	ORAC ($\mu\text{mol TE/g d.m.}$)
Colombia	5.44 ± 0.07^a	342.76 ± 13.48^a	194.02 ± 9.67^a	1053.72 ± 103.13^a
Brazil	5.13 ± 0.05^b	335.05 ± 9.88^a	190.09 ± 10.61^a	968.74 ± 89.70^b
Ethiopia	5.68 ± 0.08^c	370.40 ± 13.22^b	227.13 ± 10.31^b	1108.24 ± 98.73^a
Kenya	5.27 ± 0.09^d	341.74 ± 10.11^a	211.10 ± 8.88^c	1057.93 ± 90.31^a

649 **Table 4.** Phenolic content **determined chromatographically and** grouped by related chemical
650 structures identified in green coffee beans from different geographical origins. Values are
651 means $\pm$ SD expressed in mg/g dry matter (n = 3).

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Polyphenolic Group		Family	Colombia	Brazil	Ethiopia	Kenya
Cinnamic acids		Caffeic acid	0.061 ± 0.003	0.036 ± 0.001	0.060 ± 0.003	0.031 ± 0.001
CINNAMATE ESTERS	Cinnamoyl-quinic acids (CQAs)	<i>p</i> -Coumaroylquinic acids	0.582 ± 0.005	0.608 ± 0.018	0.457 ± 0.008	0.495 ± 0.012
		Caffeoylquinic acids	51.240 ± 0.566	49.821 ± 1.404	61.001 ± 1.223	52.937 ± 1.060
		Feruloylquinic acids	4.624 ± 0.048	3.854 ± 0.129	3.834 ± 0.074	4.056 ± 0.089
		Dimethoxycinnamoylquinic acids	0.069 ± 0.003	0.051 ± 0.001	0.069 ± 0.002	0.068 ± 0.001
		<i>p</i> -Coumaroyl-caffeoylquinic acids	0.079 ± 0.003	0.091 ± 0.002	0.039 ± 0.002	0.065 ± 0.001
		Dicaffeoylquinic acids	5.317 ± 0.123	4.840 ± 0.098	3.741 ± 0.105	4.723 ± 0.103
		Caffeoyl-feruloylquinic acids	0.361 ± 0.006	0.312 ± 0.005	0.185 ± 0.005	0.255 ± 0.003
		Diferuloylquinic acids	0.024 ± 0.001	0.013 ± 0.000	0.013 ± 0.001	0.015 ± 0.001
		Caffeoyl-sinapoylquinic acids	0.011 ± 0.001	0.010 ± 0.001	0.006 ± 0.001	0.007 ± 0.000
		Total CQAs	62.306 ± 0.581	59.600 ± 1.414	69.345 ± 1.229	62.621 ± 1.069
	Cinnamoyl-shikimic acids (CSAs)	Caffeoylshikimic acids	0.038 ± 0.002	0.043 ± 0.002	0.053 ± 0.002	0.043 ± 0.002
		Trimethoxycinnamoylshikimic acids	0.032 ± 0.003	0.025 ± 0.002	0.020 ± 0.001	0.022 ± 0.001
Total CSAs		0.070 ± 0.004	0.069 ± 0.003	0.074 ± 0.003	0.065 ± 0.003	
TOTAL CINNAMATE ESTERS			62.376 ± 0.581	59.668 ± 1.414	69.419 ± 1.229	62.686 ± 1.069
Cinnamoyl-amino acids	Caffeoyl-N-tryptophan		0.135 ± 0.007	0.071 ± 0.005	0.136 ± 0.010	0.145 ± 0.006
Cinnamoyl-glycosides	Caffeoyl-glycosides		1.538 ± 0.031	1.293 ± 0.032	1.766 ± 0.042	1.543 ± 0.039
TOTAL POLYPHENOLS			64.110 ± 0.582	61.069 ± 1.414	71.380 ± 1.230	64.405 ± 1.070

654 Abbreviations: CS = cinnamate esters

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Table 5. Methylxanthines in green coffee beans from different geographical origins. Values are means $\pm$ SD expressed in mg/g dry matter (n = 3).

	Colombia	Brazil	Ethiopia	Kenya
Theobromine	0.028 $\pm$ 0.001	0.025 $\pm$ 0.001	0.032 $\pm$ 0.002	0.018 $\pm$ 0.001
Caffeine	12.569 $\pm$ 0.310	11.783 $\pm$ 0.464	13.066 $\pm$ 0.314	11.654 $\pm$ 0.165
TOTAL	12.597 $\pm$ 0.310	11.808 $\pm$ 0.464	13.098 $\pm$ 0.314	11.671 $\pm$ 0.165

681 TOC

New polyphenols in green coffee: cinnamoyl-glycosides and cinnamoylshikimic acids

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