Comprehensive characterization of hydroxycinnamoyl derivatives in green and roasted coffee beans: A new group of methyl hydroxycinnamoyl quinate

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ABSTRACT

The aim of this study was to quantitatively characterize 19 green and roasted coffee beans by ultra-performance liquid chromatography coupled with diode array detector and quadrupole time-of-flight mass spectrometry. A total of 57 phenolic acids including nine methyl ester of mono-, di-caffeeoylquinic acid, and feruloxyquinic acid were identified. The methyl hydroxycinnamoyl quinates are reported for the first time from Coffea arabica and Coffea robusta. The total phenolic content ranged from 5628 ± 227 to 8581 ± 109 mg/100 g dry weight (DW) in green, and from 791 ± 63 to 1891 ± 37 mg/100 g DW roasted beans. The methyl caffeoylquinates accounted for 2.1% of the total phenolic acids. The result suggested that the phenolic composition was affected by the type of species, cultivars, and roasting process. Hence, to retain the balance between health beneficial phenolics and sensory attributes, optimization of roasting condition specific to the cultivar type substantially required.

1. Introduction

The pleasant aroma and psychostimulant effect of coffee make it one of the most popular non-alcoholic natural beverage worldwide. Coffea arabica and Coffea canephora (commonly known as Coffea robusta) are the two economically important species under the family Rubiaceae, used for coffee bean production. South West Ethiopia is recognized as the birthplace and center of diversity for C. arabica while evidence indicated that C. robusta appears to grow in its natural habitat of West Africa tropical forests (Teketay, 1999).

Hydroxycinnamoyl esters of quinic acid and shikimic acid are phenolic compounds that are widely distributed in the plant species. Caffeic, p-coumaric, ferulic, sinapic, dimethoxylicinnamic and trimethoxylicinnamic acid are the most common hydroxycinnamic acid...
that conjugate with quinic acid, shikimic acid, amino acid, or sugar molecules to form hydroxycinnamoyl-esters, amides or glycosides (Clifford et al., 2003, 2006a, 2006b). Naturally, trans-cinnamic acid is the most favored biosynthetic precursor to form hydroxycinnamoyl derivatives, however, depending on the part of the plant where compounds are accumulated and extent of exposure to UV radiation, the cis-isomers can also be biosynthesized (Clifford et al., 2008; Karakose et al., 2015).

The coffee bean is one of the plant resources containing a high concentration of these compounds which are predominantly responsible for coffee flavor (Frank et al., 2006; Jaiswal et al., 2014). From our literature review, so far a total of 137 different hydroxycinnamoyl derivatives have been identified from green and roasted beans of C. arabica and C. robusta (Table 1S in the Supplementary material). Upon roasting of the bean, these phenolic acids undergo chemical transformation such as hydrolysis, transesterification (acyl migration), lactonization, and isomerization to form the corresponding lactone compounds which are primarily responsible for coffee aroma and cup attributes (bitterness, body, acidity, and astringency) (Clifford et al., 2008; Deshpande et al., 2014; Honda et al., 2014).

The phytochemical profile of coffee bean affected by genetic traits, cultivar, environment, growing condition, level of maturation, processing condition, pre-, and post-harvest handling practices. Previous reports revealed that from 5202 to 7138 mg/100 g, dry weight (DW) and from 7577 to 8600 mg/100 g, DW of total phenolic content have been determined from green bean of C. arabica and C. robusta, respectively. Caffeoylquinic acid derivatives were the most abundant (68.2–95.0%) phenolic groups and 5-cafeoylquinic acid was being the major compound (48.1–63.4%) in both species (Perrone et al., 2008; Baeza et al., 2016; Mehari et al., 2016). Other metabolites such as, volatile compounds (Liu et al., 2019), hydroxycinnamoyl amide conjugates (Clifford & Knight, 2004), hydroxycinnamoyl hexose and methyl xanthines (Alonsa-Salces, Serra, Reniero, & Heberger, 2009), and triacylglycerol composition (Cossignani et al., 2016) have also shown influence to discriminate between these two species. The effect of geographical origin and types of cultivar to affect the phenolic acid composition among coffee samples were also observed from principal component analysis (PCA) data (Kuhnet et al., 2011; Mehari et al., 2016). Roasting process results in the loss of these metabolites and the level of degradation depend on the roasting condition and temperature. In medium dark roasting condition, 63.1–78.4% and 55.5–78.2% of total phenolic acid degradation have been determined in C. arabica and C. robusta beans, respectively (Farah et al., 2005; Perrone et al., 2008).

Apart from psychostimulating effect, consumption of coffee brew has positive health benefits. Coffee phenolic acids have shown antioxidant (Iwai et al., 2004; Honda et al., 2014; Baeza et al., 2016), anticancer (Gaascht et al., 2015), antihypertensive (Rozuma et al., 2005), and antiobesity activities (Murase et al., 2012; Narita & Inouye, 2011). Moreover, the dicaffeoylquinic acids (3,4-, 3,5-, and 4,5-O-di-cafeoylquinic acid) followed by 3- and 5-O-cafeoylquinic acids have exhibited potent antiobesity effect in in vivo animal model by modulating post-prandial energy metabolism (Shimoda et al., 2006). The phenolic ring system with hydroxy- and methoxy- substituent of hydroxycinnamoyl moiety, the number, position of substitution, and the molecular stereo configuration are the responsible structural activity requirements for the reported biological activities (Narita & Inouye, 2011; Shimoda et al., 2006).

Previous literatures revealed that comprehensive characterization of phenolic acids in green and roasted coffee bean samples from different geographical origin is limited. Moreover, comparison data of phenolic acid among coffee samples collected from the top coffee growing countries remain unknown. The analytical method used to characterize the hydroxycinnamoyl derivatives in coffee was based on liquid chromatography with tandem mass spectrometry (LC-MS/MS), and quadrupole time-of-flight mass spectrometry (QToF/MS) technologies (Clifford et al., 2006a; Frank et al., 2008; Blumberg et al., 2010; Jaiswal & Kuhnet, 2010; Mullen et al., 2013; Baeza et al., 2016). Therefore, in the present study, we quantitatively characterized a total of 57 hydroxycinnamoyl derivatives from 19 commercially popular green and roasted coffee beans using ultra-performance liquid chromatography coupled with diode array detector and quadrupole time-of-flight mass spectrometry (UPLC-DAD-QToF/MS). Nine methyl hydroxycinnamoyl quinates were identified for the first time in C. arabica and C. robusta beans. Percent (%) loss of phenolic acids after roasting process was also determined.

Table 1
Coffee bean samples name (origin), species and roasting condition.

| No. | Sample origin | Sample Code | Species | T<sub>in</sub> (°C) | T<sub>out</sub> (°C) | Roasting time (min) |
|-----|---------------|-------------|---------|---------------------|-------------------|-------------------|
| 1   | Brazil NY2 Sc-17/18 Pulped Natural Cerrado | Brazil-NC | Arabica | 206 | 199 | 10.35 |
| 2   | Brazil NY2 SC16 FC Red Bourbon | Brazil-RB | Arabica | 206 | 198 | 10.40 |
| 3   | Brazil [2016 COE Program] #10 Natural Siio Mundoigara | Brazil-NSM | Arabica | 205 | 199 | 10.40 |
| 4   | Colombia Supremo Medellin | Colombia-SM | Arabica | 210 | 203 | 12.15 |
| 5   | Colombia Supremo SC 17/18 Tolima FNC | Colombia-ST | Arabica | 210 | 204 | 11.45 |
| 6   | Ethiopia G1 Sidamo Guji Eguabayeb | Ethiopia-SG | Arabica | 202 | 200 | 11.10 |
| 7   | Ethiopia G1 Yirgachelle | Ethiopia-YC | Arabica | 202 | 201 | 11.05 |
| 8   | Guatemala Red Pache k-72 | Guatemala-RL | Arabica | 212 | 204 | 10.50 |
| 9   | Guatemala Red Typica k-72 | Guatemala-RT | Arabica | 212 | 204 | 11.05 |
| 10  | Guatemala SHB Mataquesquintela Reserve | Guatemala-MR | Arabica | 212 | 204 | 11.15 |
| 11  | Hawaiian Kona Extra Fancy | Hawaiian-K | Arabica | 205 | 200 | 9.15 |
| 12  | Honduras SHG EP Copan RFA | Honduras-C | Arabica | 210 | 204 | 11.00 |
| 13  | Honduras SHG Fermin Hernandez Copan | Honduras-FMC | Arabica | 210 | 203 | 10.20 |
| 14  | India A Monsooned Malabar | India-MM | Arabica | 205 | 200 | 10.10 |
| 15  | India Robusta Kenii Royal | India-RKR | Robusta | 205 | 204 | 12.10 |
| 16  | Kenya AA Top Ngamara Kainamui | Kenya-NK | Arabica | 202 | 202 | 11.50 |
| 17  | Kenya AA TOP Rungeto Karimikui | Kenya-RK | Robusta | 200 | 200 | 12.25 |
| 18  | Vietnam Robusta G1 Saigon Hama | Vietnam-R | Robusta | 205 | 204 | 12.40 |
| 19  | Vietnam Robusta G1 Ser18 | Vietnam-RSH | Robusta | 208 | 208 | 10.40 |

T<sub>in</sub> (input temperature) is the temperature just prior to the bean chamber, and T<sub>out</sub> (output temperature) is the temperature just after the exit of the bean chamber in the fluidized bed coffee roaster machine.

2. Material and methods

2.1. Plant materials

A total of 19 coffee samples, belonging to C. arabica and C. robusta, representing major coffee growing regions (Africa, Asia, and South America) were purchased from GSC International Co. Ltd., South Korea (Table 2S in the Supplementary material). The samples were
lyophilized prior to maintaining constant moisture content and roasted in commercial bed fluid roaster (semi-hard air roaster) in Jeonju Coffee Roasting Factory, South Korea. As described in Table 1, the roasting condition was used to produce a medium roasting degree which targeted Korean coffee consumers taste preference (Fig. 1S in the Supplementary material).

2.2. Chemicals and reagents

Standards of caffeic acid, 4-O-cafeoylquinic acid, 5-O-cafeoylquinic acid, 3,4-di-O-cafeoylquinic acid, 3,5-di-O-cafeoylquinic acid, 4,5-di-O-cafeoylquinic acid, and 2,4,5-trimethoxyxanic acid were purchased from Sigma Aldrich Co. (St. Louis, MO, USA); 1-O-cafeoylquinic acid, 3-O-feruloylquinic acid, 4-O-feruloylquinic acid, 5-O-feruloylquinic acid, 3-O-cafeoylquinic acid methyl ester, 3,4-di-O-cafeoylquinic acid methyl ester, 3,5-di-O-cafeoylquinic acid methyl ester, and 4,5-di-O-cafeoylquinic acid methyl ester were purchased from Chem Face (Wuhan, China); p-coumaric acid, ferulic acid, 3-O-cafeoylquinic acid and 5-O-cafeoylquinic acid methyl ester, and 1,3-di-O-cafeoylquinic acid from Extrasynthese (Genay Cedex, France); and 1,5-di-O-cafeoylquinic acid were purchased from Avention (Incheon, Republic of Korea).

2.3. Extraction of hydroxycinnamoyl derivatives

Sample extraction was conducted according to the method described by Kim et al. (2012) with a slight modification. Briefly, 1 g powdered sample was extracted with 10 mL of solvent (methanol: water: formic acid (80:15:5, v/v/v)) containing 500 mg/L of 2,4,5-trimethoxyxamic acid as an internal standard. The mixture (1 g/10 mL) was vortexed, shaken on an orbital shaker for 5 min at 200 rpm, and centrifuged (LABOGENE 1580R, Korea) for 15 min at 3000 rpm, 10 °C. The supernatant was filtered using a PVDF syringe filter (0.45 μm, Whatman, Kent, England). 0.5 mL of the filtrate was diluted with water to 5 mL of the final volume. The crude phenolic acid extract was then isolated by solid-phase extraction method using Sep-Pak C-18 cartridge (Waters Co., Milford, MA, USA). Sep-Pak C-18 cartridge was activated by washing with 2 mL of methanol, followed by 2 mL of water for conditioning. Then the diluted phenolic extract was loaded on the cartridge, and impurities were removed by washing with 2 mL of water. Finally, the total phenolic acid mixture was eluted from the cartridge by 3 mL of methanol. The purified phenolic extract was concentrated using N2 gas and re-dissolved with 500 μL of the extraction solvent without internal standard prior to analysis. All analysis was performed in three replicates.

2.4. UPLC-DAD-QToF/MS analysis

Phenolic acid profiling was conducted by UPLC-DAD-QToF/MS (Waters Micromass, Manchester, UK). UV–visible spectra were recorded in the region from 210 to 400 nm. Separation was conducted using C30® UPLC® T3 C18 column (2.1 mm × 150 mm, 1.6 μm) (Waters Co., Milford, MA, USA). Chromatographic conditions were set as column temperature 30 °C; mobile phase consists of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Flow rate was 0.35 mL/min; injection volume, 1 μL. The gradient profile was as follows: 0–4 min, 2% B; 8 min, 4% B; 20 min, 7% B; 32 min, 11% B; 55 min, 15% B; 75–87 min, 25% B; 85–87 min, 50% B; 90–92 min, 90% B; 95–100 min, 2% B. The mass spectrometric settings used were: electrospray ionization source operating in positive mode (+ ESI), capillary voltage was set to 3.5 kV, desolvation gas 1020 L/h, sample cone voltage 40 V. The source and desolvation temperatures were 140 °C and 500 °C, respectively. Data were recorded in the mass range of 50–800 m/z in full scan mode.

2.5. Identification of hydroxycinnamoyl derivatives

A coffee chemical library was developed by compiling reports obtained from published literature where the data are confirmed by LC-MS and NMR analysis. This library (Table 1S in the Supplementary material) was used as a reference to determine the presence of the compounds in C. arabica and C. robusta beans, elution order of the isomers, mass fragmentation pattern, and UV-absorbance characteristic of identified compounds. Moreover, authentic standards were used for confirmation of results wherever available.

2.6. Statistical analysis

All experiment was conducted in triplicate and data expressed in mean ± standard deviation. Phenolic content of identified compounds in the samples were analyzed by multivariate statistical analysis using SIMCA-P version 11.0 (Umetrics, Umea, Sweden). Partial Least Square Discriminant Analysis (PLS-DA) was used to visualize discrimination among the sample set.

3. Results and discussion

A total of 57 hydroxycinnamoyl derivatives were identified in the green and roasted beans of 19 C. arabica and C. robusta samples by UPLC-DAD-QToF/MS. This instrumental technique provided an excellent resolution of chromatographic separation with accurate mass measurement of parent and fragment ions for the individual compounds. The DAD chromatogram at 320 nm of representative samples from both species is presented in Fig. 1. The UV–visible absorbance and MS spectral data are summarized in Table 2. The compound nomenclature in this study was according to the IUPAC numbering system (IUPAC, 1976). The mass fragmentation scheme of representative hydroxycinnamoylquinic acids under (+) ESI mode is presented in Fig. 25 (a–g) (Supplementary material). The potassium (K+, m/z 39) and sodium (Na+, m/z 23) adduct ions were observed in addition to the pseudo molecular ions ([M+H]+) which helped to identify the exact molecular mass of the peak. The dehydrated protonated ions ([M+H–2H2O]+) and hydroxycinnamoyl moieties (HC) ([HC+H2O]+) were observed as a base peak. These phenolic acids showed different fragmentation pattern compared to (–) ESI mode (Clifford et al., 2006a; Jaiswal & Kuhnert, 2010). Basically, under negative ion mode, dehydrated ion from quinic moiety ([Quinic-H2O]+, m/z 191) was observed and this ion is important discriminant among rosinformers of hydroxycinnamoylquinic acids (Clifford et al., 2003). However, this characteristic fragment ion was not detected on (+) ESI ionization mode.

3.1. Hydroxycinnamic acids (HCA)

Caffeic acid (peak 5) showed [M+H]+ at m/z 181, dehydrated fragment ions at m/z 163 ([M+H2O]+) with 100% relative abundance (RA), and undergoes decarboxylation to produce m/z 135 [M + H2O–CO]+. Additional H2O molecule was removed from the phenyl moiety to form [M+H2H2O]+ ion at m/z 145. Ferulic acid (peak 18) detected at m/z 195 ([M+H]+), yielded dehydrated fragment ion (m/z 177 [M+H–2H2O]+) with 100% RA, and demethoxylated ion [M+H2O–CH3OH]+ at m/z 145. Minor fragment ions were observed at m/z 149 and 134 corresponds to subsequent decarboxylation and demethylation from [M+H2O]+ ion, respectively. This is the first report of ferulic acid in green beans of C. arabica and C. robusta.

3.2. Hydroxycinnamoylquinic acids (HQA)

3.2.1. Caffeoylquinic acids (CQA)

Three major peaks (2, 6 and 8) were extracted from the total ion current (TIC) chromatogram of green and roasted bean at m/z 355.
These isomers showed identical mass fragmentation pattern among each other and with authentic standard compounds (3-, 5-, and 4-CQA, respectively). The protonated ions [M+H]+ yielded fragment ion at m/z 337 ([M+H-H2O]+) due to the loss of H2O from the carboxylic group of the quinic moiety. Similar to negative ionization mode, quinic acid moiety was removed from the parent ions and the characteristic ions of caffeoyl moiety at m/z 181, 163, 145, and 135 were detected (Fig. 2S-a in the Supplementary material). The % RA of m/z 163 was 100% in all three isomers while the other fragment ions were below 10%. This indicated that irrespective of the position of acylation, losing the hydroxycinnamoyl moiety is the easiest and stable fragmentation pattern under positive ionization mode. The elution order was confirmed by comparing with authentic standards and peaks 2, 6, and 8 were assigned as 3CQA, 5CQA, and 4CQA, respectively. As mentioned above in Section 1, the roasting process resulted for the formation of two additional isomers (peak 1 and 4) having same MS spectral characteristics (m/z 355) in the roasted coffee bean chromatogram at a retention time (RT) = 8.04 min and 14.75 min, respectively. According to Clifford et al. (2008) and Jaiswal et al. (2014), the position of acylation and structural orientation of HCA in the quinic moiety determines the hydrophobicity of these compounds hence, the elution order follows trans-1CQA < trans-3CQA < cis-3CQA < trans-5CQA < trans-4CQA < cis-5CQA < cis-4CQA on reversed phase chromatographic column.

By this empirical rule, peak 1 was assigned as 1CQA and peak 4 which eluted following 3CQA identified as cis-3CQA. 1CQA was also further confirmed by comparing with an authentic standard. The presence of 1CQA in C. arabica is reported for the first time in this study.

Three isomers (peaks 32, 34, 35) of dicafeoylquinic acid (diCQA) were extracted from the TIC chromatogram of green and roasted coffee beans at m/z 517. The potassium adducts at m/z 555, sodium adducts at m/z 539 and base peak of [M+H-H2O]+ with 100% RA was detected at m/z 499. The protonated ions of CQA (m/z 355) were observed due to the loss of one caffeoyl moiety ([M+H-Ca]+) from the parent ions; eventually, the subsequent fragment ions corresponding to CQA were detected (Table 2 and Fig. 2S-b in the Supplementary material). The % RA of m/z 163 for peak 32 (50%) and 35 (58%) were predominant compared to peak 34 (14%) which revealed that in diacylated compounds the position of acylation might affect the fragmentation intensity. According to Clifford et al. (2005), the sequence of elution for the six diCQA isomers have been determined as 1,3 < < < 1,4 < 3,4 < 5,3 ~ 1,5(colo-eluted) << 4,5-diCQA. Further, we compared the retention time with the authentic standards and peaks 32, 34, and 35 unambiguously assigned as 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, respectively.

Fig. 1. UPLC-DAD chromatograms of coffee bean at 320 nm. *Coffee robusta* (Vietnam Robusta G1 Saigon Hama: a- green, b- roasted bean), and *Coffee arabica* (Ethiopia G1 Sidamo Guji Eguabaye: c- green, d- roasted bean). Compound name of individual peaks is presented in Table 2.
| Peak No. | Compounds assignment | Abbreviation | RT (min) | DAD λ max (nm) Formula [M+H]+ | Exp. Mass [M+H]+ | Adducts and Fragment ions of [M+H]+ |
|---------|----------------------|--------------|---------|--------------------------------|-----------------|----------------------------------|
| 5'c     | caffeic acid         | Caf          | 15.69   | 240a,295a,323 C7H7O4          | 181.0727        | 163[M+H]+, 145[M+H2O]+, 135[M+H2O-CO]+ |
| 11      | 5'-p-coumaroylquinic acid | 3pGQA       | 14.62   | 229,310 C7H7O4               | 339.0527        | 377[M+K]+, 361[M+Na]+, 147[Goum+H2O]+ |
| 12      | 4'-p-coumaroylquinic acid | 4pGQA       | 25.82   | 211,310 C7H7O4               | 339.0527        | 377[M+K]+, 361[M+Na]+, 147[Goum+H2O]+ |
| 1''     | 1'-carrageenonic acid | 1CQA        | 10.76   | 240a,295a,323 C7H7O4          | 355.0504        | 393[M+K]+, 377[M+Na]+, 337[M+H2O]+, 135[M+H2O-CO]+ |
| 6''     | 5'-carrageenonic acid (chlorogenic acid) | 5CQA       | 15.19   | 242a,297a,325 C7H7O4          | 355.0504        | 393[M+K]+, 377[M+Na]+, 337[M+H2O]+, 181[M+H]+, 161[M+H-CO]+ |
| 8''     | 4'-carrageenonic acid (cryptochlorogenic acid) | 4CQA       | 20.31   | 241a,297a,324 C7H7O4          | 355.0504        | 393[M+K]+, 377[M+Na]+, 337[M+H2O]+, 181[M+H]+, 161[M+H-CO]+ |
| 7''     | 3'-carrageenonic acid (chlorogenic acid) | 3CQA       | 18.87   | 234,295,323 C7H7O4            | 369.0629        | 407[M+K]+, 391[M+Na]+, 351[M+H2O]+, 195[Fr+H]+, 177[Fr+H2O]+ |
| 3''     | 3,4-di-carrageenonic acid (isochlorogenic acid B) | 3,4DCQA     | 52.44   | 241a,297a,324 C7H7O4          | 517.0549        | 555[M+K]+, 539[M+Na]+, 499[M+H2O]+, 355[M+H-Ca]+, 337[M+H-CaH2O]+, 181[M+H]+, 161[M+H-Ca]+, 145[M+Ca]+ |
| 34''    | 3,5-di-carrageenonic acid (isochlorogenic acid A) | 3,5DCQA     | 53.87   | 241a,297a,326 C7H7O4          | 517.0549        | 555[M+K]+, 539[M+Na]+, 499[M+H2O]+, 355[M+H-Ca]+, 337[M+H-CaH2O]+, 181[M+H]+, 161[M+H-Ca]+, 145[M+Ca]+ |
| 35''    | 4,5-di-carrageenonic acid | 4,5DCQA     | 60.58   | 242a,297a,326 C7H7O4          | 517.0549        | 555[M+K]+, 539[M+Na]+, 499[M+H2O]+, 355[M+H-Ca]+, 337[M+H-CaH2O]+, 181[M+H]+, 161[M+H-Ca]+, 145[M+Ca]+ |
| 36''    | 3-carrageenonic acid | 3CpGQA mix Trace | 399,313 C7H7O4 | 501.0670 | 539[M+K]+, 521[M+Na]+, 483[M+H2O]+, 147[M+H-Ca]+ |
| 37      | 3'-p-coumaryl-5'-p-carrageenonic acid | 3pCo, 5CQA | 61.77   | 299a,313 C7H7O4              | 501.0670        | 539[M+K]+, 521[M+Na]+, 483[M+H2O]+, 147[M+H-Ca]+ |
| 39      | 3'-carrageenonic acid | 3GpGQA       | 62.45   | 234,317 C7H7O4               | 501.0670        | 539[M+K]+, 521[M+Na]+, 483[M+H2O]+, 147[M+H-Ca]+ |
| 44      | 4'-p-coumaryl-5'-p-carrageenonic acid | 4pCo, 5CQA | 66.57   | 299a,314 C7H7O4              | 501.0670        | 539[M+K]+, 521[M+Na]+, 483[M+H2O]+, 147[M+H-Ca]+ |
| 46      | 4'-carrageenonic acid | 4GpGQA       | 67.33   | 232,308 C7H7O4               | 501.0670        | 539[M+K]+, 521[M+Na]+, 483[M+H2O]+, 147[M+H-Ca]+ |
| 38      | 3'-feruloyl-4'-carrageenonic acid | 3,4F,4CQA | 62.23   | 299a,323 C7H7O4              | 531.0665        | 569[M+K]+, 553[M+Na]+, 513[M+H2O]+, 351[M+H-Ca]+ |

Table 2
Characterization of 57 hydroxycinnamoyl derivatives in green and roasted beans of *Coffee arabica* and *Coffea robusta*.

| Peak No. | Individual phenolic acids | Abbreviation | RT (min) | DAD λ max (nm) Formula [M+H]+ | Exp. Mass [M+H]+ | Adducts and Fragment ions of [M+H]+ |
|---------|--------------------------|--------------|---------|--------------------------------|-----------------|----------------------------------|
| 40      | 3-carrageenyloyl-4-O-feruloylquinic acid | 3,4-C,4FQA | 63.48   | 242a,299h,324 C9H12O12       | 531.0665        | 569[M+K]+, 553[M+Na]+, 513[M+H2O]+, 195[Fr+H]+, 177[Fr+H2O]+, 163[Ca+F]+, 149[Fr+H2O]+, 145[Fr+H2O+CH3OH] |

(continued on next page)
| Peak No. | Individual phenolic acids | Abbreviation | RT (min) | DAD λ (nm) | Formula |
|---------|---------------------------|--------------|---------|------------|---------|
| 41      | 3,4-dicaffeoylquinic acid  | 3,4-dCAQM    | 66.28   | 242,298,235 | C_{17}H_{22}O_{12} |
| 49      | 4,5-dicaffeoylquinic acid  | 4,5-dCAQM    | 71.10   |            | C_{17}H_{22}O_{12} |
| 50      | 5-dicaffeoylquinic acid    | 5-dCAQM      | 72.10   |            | C_{17}H_{22}O_{12} |
| 55      | 5-hydroxycinnamoylquinic acid methyl ester | 5HCAQM | 71.81   | 242,298,235 | C_{17}H_{22}O_{12} |

(continued on next page)
| Peak No. | Individual phenolic acids | Abbreviation | RT (min) | DAD \( \lambda_{\text{max}} \) (nm) | Formula \[M+H\]^+ | ESI(+) QToF/MS (experimental ions, m/z) | Exp. Mass \[M+H\]^+ | Adducts and Fragment ions of \[M+H\]^+ |
|---------|--------------------------|--------------|---------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|
| 19      | 4-O-cafeoyl-1,3-quinide   | 4C-muco-γ-Q  | 30.59   | 244,294,327                     | C_16H_{17}O_8 | 337.1326                        | 375\[M+K\]^+, 359\[M+Na\]^+, 163\[Ca+H_2O\]^+  |
| 21      | 5-O-cafeoyl-1,4-quinide   | 5C-epi-δ-Q   | 32.95   | 245,296,327                     | C_16H_{17}O_8 | 337.1326                        | 375\[M+K\]^+, 359\[M+Na\]^+, 181\[Ca+H+2\]^+, 163\[Ca+H_2O\]^+  |
| 25      | 4-O-cafeoyl-1,5-quinide   | 4C-γ-Q       | 36.46   | 238,297,322                     | C_16H_{17}O_8 | 337.1326                        | 375\[M+K\]^+, 359\[M+Na\]^+, 181\[Ca+H+2\]^+, 163\[Ca+H_2O\]^+  |
| 23      | 3-O-feruloyl-epi-1,5-quinide | 3F-epi-γ-Q | mix     | Trace                           | C_16H_{17}O_8 | 351.1519                        | 389\[M+K\]^+, 373\[M+Na\]^+, 195\[Fr+H\]^+, 177\[Fr+H_2O\]^+, 145\[Fr+H_2O-CH_2OH\]^+  |
| 26      | 5-O-cafeoyl-1,3-quinide   | 5F-muco-γ-Q  | 40.35   | 238,295,324                     | C_16H_{17}O_8 | 351.1519                        | 389\[M+K\]^+, 373\[M+Na\]^+, 195\[Fr+H\]^+, 177\[Fr+H_2O\]^+, 149\[Fr+H_2O-CO\]^+  |
| 28      | 5-O-cafeoyl-1,5-quinide   | 5F-γ-Q       | 42.42   | 236,297,325                     | C_16H_{17}O_8 | 351.1519                        | 389\[M+K\]^+, 373\[M+Na\]^+, 195\[Fr+H\]^+, 177\[Fr+H_2O\]^+, 149\[Fr+H_2O-CO\]^+  |
| 30      | 3-O-feruloyl-epi-1,4-quinide | 5F-epi-δ-Q | 45.31   | 339,297,326                     | C_16H_{17}O_8 | 351.1519                        | 389\[M+K\]^+, 373\[M+Na\]^+, 195\[Fr+H\]^+, 177\[Fr+H_2O\]^+, 149\[Fr+H_2O-CO\]^+  |
| 33      | 4-O-feruloyl-1,3-quinide   | 4F-γ-Q       | 52.39   | Trace                           | C_16H_{17}O_8 | 351.1519                        | 389\[M+K\]^+, 373\[M+Na\]^+, 195\[Fr+H\]^+, 177\[Fr+H_2O\]^+, 149\[Fr+H_2O-CO\]^+  |
| 52      | 4,5-di-O-cafeoyl-1,3-quinide | 4,5diC-γ-Q   | 71.85   | 243,297,326                     | C_16H_{17}O_11 | 499.1866                        | 537\[M+K\]^+, 521\[M+Na\]^+, 337\[Ca+H\]^+, 181\[Ca+H+2\]^+, 163\[Ca+H_2O\]^+, 145\[Ca+2H_2O\]^+  |
| 57      | 4,5-di-O-cafeoyl-1,5-quinide | 4,5diC-muco-γ-Q | 76.11   | 241,299,332                     | C_16H_{17}O_11 | 499.1866                        | 537\[M+K\]^+, 521\[M+Na\]^+, 337\[Ca+H\]^+, 181\[Ca+H+2\]^+, 163\[Ca+H_2O\]^+, 135\[Ca+H_2O-CO\]^+  |

All samples analyzed in positive ESI-ionization mode \((m/z, [M+H]^+)\) of QToF/MS; \([M+K]^+, [M+Na]^+, \) and adducts presented; Caf: caffeic acid \((180 \text{ Da})\) or caffeyl \((162 \text{ Da})\); Coum: p-coumaric acid \((164 \text{ Da})\) or p-coumaroyl \((146 \text{ Da})\); Fr: ferulic acid \((194 \text{ Da})\) or feruloyl \((176 \text{ Da})\); Try: tryptophan \((204 \text{ Da})\); peak assignment was done by comparing UV-visible, MS fragmentation spectra, and elution order with the constructed library (Table 1S in the Supplementary material) and authentic standards, wherever available; (*) new compound identified; (**) tentatively identified compounds; (†) further confirmed in comparison with authentic standards, (mix); unresolved peak.
3.2.2. p-Coumarylquinic acids (pCQQA)

Peaks 3, 11, and 12 were assigned as pCQQA isomers (m/z 339) on the basis of UV-visible and MS spectral data. Minor intensity dehydrated protonated ions at m/z 321 ([M + H-H2O]+) was formed due to the loss of H2O from protonated ion (Fig. 25-c in the Supplementary material). The intense dehydrated ions of the p-coumaryl moiety ([Coum + H-H2O]+ 100% RA) detected at m/z 147 was the base peak in all three isomers. Additionally, minor fragment ion at m/z 119 resulted from decarboxylation (([Coum + H-H2O-CO]+) was observed. In our analytical condition, the authentic standards for CQA followed the elution order of 1 < 3 < 5 < 4. Hence, by considering this fact and naturally abundant isomers which have been previously reported, these isomers were assigned as 3pCQA, 5pCQA, and 4pCQA, respectively (Jaiswal et al., 2010; Ortiz et al., 2019).

3.2.3. Feruloylquinic acids (FQA)

Peaks 7, 14, and 15 were detected in the extracted TIC chromatogram of green and roasted beans at m/z 369. These peaks yielded a base peak at m/z 177 results from [Fr+H+H2O]+ (100% RA) fragment ion. This observation suggested that these isomers have feruloyl moiety. The sodium adduct at m/z 391 was moderate intense ion (65% RA). The dehydrated protonated ion at m/z 351 ([M + H-H2O]+) was formed due to the loss of H2O from protonated ion. The subsequent fragment ions corresponding to the feruloyl moiety were detected at lower abundance (Table 2 and Fig. 25-d in the Supplementary material). Assigning the elution order was done by comparing their retention time with authentic standards and identified as 3FQA, 5FQA, and 4FFQA, respectively. Moreover, the TIC analysis of roasted bean chromatogram showed one additional peak (13) at m/z 369 (Rt = 25.97 min). Similar to cis-CQA isomer, this isomer was formed during the roasting process; hence by considering the elution pattern, it was tentatively assigned as cis-3FFQA (Deshpande et al., 2014).

Selected ion monitoring (SIM) of the TIC chromatogram of green bean at m/z 545 showed five peaks having dehydrated protonated ions at m/z 527 ([M + H-H2O]+, 35–85% RA) as a base peak. Further examining the MS spectra, three peaks (50, 53, and 55) showed fragment ions corresponding to feruloyl moiety, suggesting that these isomers are diferuloylquinic acid (diFQA) (Fig. 3S-e in the Supplementary material). The retention time interval among three isomers and their elution pattern on DAD chromatogram were similar to that of diCQAs. The protonated ions of FQA after the loss of one feruloyl moiety ([M + H-Fr]+) was not detected, instead of moderate intense [Fr+H-H2O]+ (35–85% RA), fragment ion was observed (Table 2, Figs. 2S-e and 2S-a in the Supplementary material). Previous reports revealed that only three isomers of diFFQA have been detected in coffee, hence peaks 50, 53, 55 were tentatively assigned as 3,4-diFFQA, 3,5-diFFQA, and 4,5-diFFQA, respectively (Clifford et al., 2006a; Jaiswal & Kuhnert, 2010). This is the first time to report the presence of the latter two isomers in C. arabica beans.

3.2.4. Caffeoylferuloylquinic acids (CFFQA)

Peak 38, 40, 41, 43, 48, and 49 showed the same [M+H]+ at m/z 531 (5–20% RA), and dehydrated protonated ions at m/z 513 (55–100% RA). The corresponding caffeoyl and feruloyl moiety fragment ions also observed in the MS spectra data revealed that these isomers are CFFQAs (Table 2 and Fig. 2S-f in the Supplementary material). In the literature, a total of eight CFFQAs have been reported from both green and roasted coffee beans (Clifford et al., 2003; Jaiswal et al., 2014). Due to the limitation of (+) ESI ionization mode fragmentation scheme to discriminate isomers on the basis of their fragment ions formation and the absence of authentic standards, assignment of peaks was done based on the previous reports. In concordance with the description of Clifford et al. (2003), these isomers eluted in three pairs, and within the pair, the second isomer has a higher concentration than the first eluted compound (Fig. 3S-b in the Supplementary material). Apart from this, by considering the factors that affect the hydrophobicity of these isomers such as, the position of acyl substitute (3,4 < 3,5 < 4,5-diCQA) and the type of hydroxycinnamoyl moiety acylated with (caffeoyl < p-coumaroyl < feruloyl), the first pair of peaks (38, 40) assigned as 3F,4CQA and 3C,4FFQA, the second pairs (41, 43) 3F,5CQA and 3C,5FQA, and the last and the most hydrophobic pairs (peak 48, 49) as 4F,5CQA and 4C,5FQA, respectively.

3.2.5. p-Coumarylcaffeoylquinic acids (pCQAFF)

Five peaks (36, 37, 39, 44, and 46) were extracted from TIC chromatogram of green bean at m/z 501. The moderate intense peaks at m/z 163 and 147 suggested that these isomers are pCQAFF (Clifford et al., 2006b). Peak 39 did not show the dehydrated protonated ions of caffeoyl moiety. All isomers produce the base peak at m/z 483 (70–100% RA) resulted from the deprotonation of protonated ion ([M + H-H2O]+) (Table 2 and Fig. 2S-g in the Supplementary material). Basically, it is expected to observe fragment ions at m/z 355 and 339 correspond to the loss of p-coumaroyl (146 Da) and caffeoyl moiety (162 Da) from the parent ions, respectively. However, these fragment ions were not detected. Peak 36 was co-eluted with 4,5-diCQA (Fig. 1) and the other two pairs of peaks were detected in latter retention time (61–64 min and 66–68 min, respectively). To assign these peaks we used similar approach as CQA isomers since they showed similar elution pattern (Fig. 3S-c in the Supplementary material). Hence, the first isomer (peak 36) was tentatively identified as 3C,4pCQA assuming that 3pCo,4CQA eluted ∼ 1.2 min ahead of 3C,4pCoCQA. The second set (peak 37, 39) were assigned as 3pCo,5CQA and 3C,5pCoCQA, respectively, and the last two peaks (44, 46) identified as 4pCo,5CQA and 4C,5pCoCQA, respectively. Peak 36, 39 and 46 were reported from C. arabica bean for the first time in this study.

3.3. New hydroxycinnamoylquinic acid methyl ester (HCQM)

3.3.1. Caffeoylquinic acid methyl ester (CQM)

The SIM analysis of green bean TIC chromatogram at m/z 369 showed six peaks (Fig. 2a-1). The fragmentation pattern of peak 10, 20 and 24 was compared with that of FQAs and they all yielded a base peak at m/z 163 ([Caf+H-H2O]+, 100% RA). The sodium adduct [M + Na]+ at m/z 391 was the second most intense ion (38–90% RA) in all isomers. The [M+H]+, and [M+H-H2O]+ ions showed 14 Da increment than CQA (m/z 355), and minor intensity fragment ions corresponding to caffeoyl moiety were observed due to the loss of methyl quinate moiety (m/z 189) from protonated ions (Table 2 and Fig. 2b). Peak 20 and 24 were further compared with authentic standards and confirmed as 3-O-caffeoylquinic acid methyl ester (3CQM) and 5CQM, respectively. Jaiswal & Kuhnert, (2011) reported that methyl esters of caffeoylquinic have shown different elution order (3 < 1 < 4 < 5) from CQA isomers (1 > 3 > 5 > 4). However, from our analytical condition, there was no peak observed between 3- (Rt = 31.79 min) and 5-CQM (Rt = 35.79 min) and peak 10 was eluted earlier at Rt = 23.71 min (Figs. 1 and 2a). This brings an argument that the position of the acyl substituent or structural orientation (three-dimen- sional molecular structure) has an effect on the overall hydrophobicity of the compound than methylation of the quinic moiety to determine the elution order of these isomers. Hence, the first isomer (peak 10) was tentatively assigned as 1CQM.

3.3.2. Dicaffeoylquinic acid methyl ester (DiCQM)

The methyl ester derivatives of diCQA were also detected from the green and roasted bean of C. arabica and C. robusta extract. Nine peaks at m/z 531 ([M+H]+) were observed from SIM analysis (Fig. 2a-2). Three isomers at Rt = 64.73 min, 67.45 min, and 71.66 min unlike the other six isomers extracted at m/z 531, did not show feruloyl moiety related fragment ions (Table 2 and Fig. 2c). The protonated ions (4–12% RA), and the base peak [M + H-H2O]+ (100% RA) of these isomers showed 14 Da increment from dicaffeoylquinic acids. As shown in Fig. 2c, after losing caffeoyl moiety minor intensity fragment ions at...
3.3.3. Feruloylquinic acid methyl ester (FQM)

Three isomers (peaks 22, 27 and 31) were detected at m/z 383 (Fig. 2a-3) having similar fragmentation pattern with FQA derivatives but showed 14 Da increment on their [M+H]+, and [M+H-H2O]+ ions. They yielded a base peak at m/z 177, 100% RA and feruloyl moiety related fragment ions due to loss of methyl quinate moiety (m/z 189) from the parent ions (Table 2 and Fig. 2d). The sodium adduct [M+Na]+ at m/z 405 was the second most intense ion (40–92% RA) in all isomers. Due to the very low abundance of peak 22, fragment ions at m/z 145 and 134 was not detected. Similar to CQM, Jaiswal and Kuhnert (2011) have described the elution order of feruloylquinic acid methyl

m/z 369 ([M+H-Caf]+) and m/z 351([M+H-Caf-H2O]+) which correspond to CQM were observed. Moreover, the fragment ions of caffeoyl moiety were detected in the mass spectra. Thus, taking into the account of CQM elution order and Jaiswal and Kuhnert (2011) report, peaks 42, 47 and 51 were identified as 3,4-diCQM, 3,5-diCQM, and 4,5-diCQM, respectively.
ester (FQM) as $3 < 1 < 4 < 5$, however, based on the argument discussed for CQM in Section 3.3.1., peaks 22, 27 and 31 were tentatively assigned as 1FQM, 3FQM, and 5FQM, respectively.

Methyl ester of hydroxycinnamoyl quinate have been reported in plants such as Prunus species and Ilex paraguariensis (mate leaves) as a natural coexisting compound with other phenolic acids (Fujimoto et al., 2013; Jaiswal & Kuhnert, 2011; Jaiswal et al., 2013). Jaiswal and Kuhnert, (2011) have made an attempt to characterize HCQM after artificially synthesizing from crude extract of green coffee bean. As a result, the presence of these derivatives and contents as a naturally existing compound in coffee bean was not depicted. In our study, we reported nine methyl esters of hydroxycinnamoyl quinate in 19 samples of C. arabica and C. robusta green beans for the first time. Due to their low abundance only 5CQM, 5FQM, and 3,5-diCQM were detected in most of roasted coffee bean samples.

3.4. Hydroxycinnamoyl amides (HCAM)

The amino acid conjugates of HCA have been previously reported as a marker compound to discriminate among coffee cultivars (Clifford & Knight, 2004). Three peaks (45, 54 and 56) of hydroxycinnamoyl moiety conjugated with amino acid were detected from SIM extract of green coffee bean TIC chromatogram at $m/z$ 367, 351, and 381, respectively (Table 2 and Fig. 1). Peak 45 showed a moderate peak at $m/z$ 163 (15% RA) and 135. Fragment ion at $m/z$ 205 ($[\text{Try+H}]^+$) and 188 ($[\text{Try+H-NH}_3]^+$) corresponds to tryptophan amino acid were detected in this peak MS spectra. Thus, it was assigned as caffeoyl-N-tryptophan. Similarly, peak 54, and 56 yielded a moderate peak corresponding to the dehydrated fragment of $p$-coumaroyl ($m/z$ 147, 20% RA) and feruloyl moieties ($m/z$ 177, 20% RA), respectively. The mass unit difference between the parent ions and the dehydrated hydroxycinnamoyl

Fig. 2. (continued)
moieties ([HC+H2O]+) fragment ion was the same (204 Da) as observed for peak 45; hence, peak 54 and 56 identified as p-coumaroyl-N-tryptophan and feruloyl-N-tryptophan, respectively. All three peaks protonated ions showed 100% RA while the corresponding ([HC+H2O]+) fragment ions were minor, revealed that the amide bond is stronger than ester bond to remove the acyl substitute.

3.5. Hydroxycinnamoylquinic acid lactones (HCQL)

Roasting process is the crucial step to generate flavor in a coffee brew (Deshpande et al., 2014). The lactone derivatives of HCQ which are responsible for the characteristic coffee flavor were detected in 19 samples of roasted C. arabica and C. robusta beans. SIM extract of roasted bean TIC chromatogram at m/z 337 showed six peaks (Fig. 3S-d in the Supplementary material). The protonated ions of all isomers showed 75–100% RA. The base peak at m/z 163 ([Caf+H2O]+) of peak 15, 17 and 25 were detected at 100% RA while peak 9 and 19 showed less than 10% RA. The corresponding fragment ions related to caffeoyl moiety were also detected at the minor intensity (Table 2). Peak 9 has the most hydrophilic structural conformation as a result eluted earlier than the other isomers. The elution pattern was compared with the report by Frank et al. (2006, 2008) and found to be consistent. Hence, peaks tentatively assigned as 3C-epi-Q (9), 5C-muco-Q (15), 3C-γ-Q (17), 4C-muco-γ-Q (19), 3C-epi-δ-Q (21), and 4C-γ-Q (25). Similarly, the TIC chromatogram of roasted bean was evaluated for feruloylquinides at m/z 351 and six peaks having same fragmentation characteristics was observed. Feruloyl moiety related fragments ions at m/z 177, 149, 145 and 134 were detected at different % RA (Table 2). The protonated ions of all isomers showed 80–100% RA. The base peak [Fr+H2O]+ of peak 26 (100% RA) and peak 33 (80% RA) were intense while peak 23, 26 and 29 showed minor intensity fragment ions (2–8% RA). These peaks showed similar elution pattern with CQLs (Fig. 3S-e in the Supplementary material) hence, they were tentatively assigned as 3F-epi-γ-Q (23), 3F-muco-γ-Q (26), 3F-γ-Q (28), 4F-muco-γ-Q (29), 5F-epi-δ-Q (30), and 4F-γ-Q (33) (Frank et al., 2006, 2008). From our library (Table 15 in the Supplementary material), 3F-epi-γ-Q was not previously reported. Thus, we tentatively identified this compound for the first time in C. arabica and C. robusta beans.

Lastly, two peaks (52, 57) were extracted from TIC chromatogram of the roasted bean at m/z 499 (Rt = 71.85 min and 76.11 min). The sodium and potassium adducts at m/z 537 and 527, respectively, were used to confirm these peaks are diCQA lactone. The % RA of protonated ions (m/z 499) and the base peak (m/z 163) was 58% and 100% RA, respectively. Both peaks yielded product ions at m/z 337 due to the loss of one caffeoyl moiety, and the corresponding fragment ions of CQA were observed. Previous report revealed that three isomers have been detected in the roasted coffee bean as a bitter compound. Comparing the elution time difference among the isomers and the peak intensity with Frank et al. (2006) report, peak 52 was tentatively identified as 3,4-diC-γ-Q, and 57 as 4,5-diC-muco-γ-Q.

3.6. Quantification of hydroxycinnamoyl derivatives in green and roasted coffee beans

The contents of individual and group of hydroxycinnamoyl derivatives are presented in Table 3S (Supplementary material) and Table 3, respectively. The total phenolic acid content ranged from 5628 ± 227 to 8582 ± 109 mg/100 g dry weight (DW) in green and 791 ± 63 to 1891 ± 37 mg/100 g DW in the roasted coffee beans. The result revealed that the total phenolic acid level was affected by coffee species, geographical origin and roasting process. C. robusta was rich in diCQA and diFQA, and C. arabica showed a medium level of roasting condition (average temperature 206 °C and 233 °C). Hence, during sample preparation, it was aimed for a medium level of roasting condition (average temperature 206 °C and time 10.5 min) to develop the required flavor in the coffee brew. The most abundant lactone group was caffeoylquinides (11.7–19.7% of the total phenolic acids), with 3C-γ-Q (76.2 ± 1.7–128 ± 1.3 mg/100 g DW) as the major lactone followed by 4C-muco-γ-Q (46.6 ± 1.7–85.7 ± 4.2 mg/100 g DW). During roasting, 5CQA undergoes dehydration followed by acyl migration from 5-position to 3- or 4-position within each group of compounds and this result is in agreement with previous reports (Perrone et al., 2008; Mullenn et al., 2013; Baeza et al., 2016; Mehari et al., 2016).

The pCoQQA group represents the lowest portion of the total phenolic content in green coffee bean, being abundant in the Brazilian coffee and least in Colombian samples (up to 33.2 and 10.0 mg/100 g DW, respectively). Interestingly, the amide group of phenolic compounds showed significant variation between C. robusta (3.2–4.5%) and C. arabica (0.2–0.7%) bean. This has been well explained by Clifford and Knight (2004) that the amide conjugate of coffee phenolic used as a marker to discriminate between coffee species and cultivation regions.

Controlling critical processing points such as roasting temperature and time specific to the individual coffee samples is a crucial step to meet customer desirable coffee taste. The HCQL formation reaches to the maximum level at a medium roasting condition (230 °C for 7 min) (Farah et al., 2006). Hence, during sample preparation, it was aimed for a medium level of roasting condition (average temperature 206 °C and time 10.5 min) to develop the required flavor in the coffee brew. The most abundant lactone group was caffeoylquinides (11.7–19.7% of the total phenolic acids), with 3C-γ-Q (76.2 ± 1.7–128 ± 1.3 mg/100 g DW) as the major lactone followed by 4C-muco-γ-Q (46.6 ± 1.7–85.7 ± 4.2 mg/100 g DW). During roasting, 5CQA undergoes dehydration followed by acyl migration from 5-position to 3- and 4-position resulted in the formation of higher concentration of these two major caffeoylquinide isomers (Deshpande et al., 2014). In this study, substantial degradation of 5CQA (85.4–93.4%) was observed however, the levels of 3- and 4CQA were also reduced by 36.4–74.4% and 13.8–72.4%, respectively. Due to heat-induced isomerization 5CQA further utilized as a precursor to produce the cis-isomer, 1CQA, and free HCA (Farah et al., 2005; Fan et al., 2006). Similarly, 3F-γ-Q was determined as the main feruloylquinide and the content was higher in C. robusta samples (Table 3S in the Supplementary material). This result is also in agreement with previous report (Perrone et al., 2008).
Table 3
Content of hydroxycinnamoyl derivatives identified from green and roasted beans of 19 coffee samples and grouped by related chemical structure.

| Hydroxycinnamoyl groups (green\(^1\) and roasted\(^2\) beans) | Brazil-NC | Brazil-RB | Brazil-NSM | Colombia-SM | Colombia-ST | Ethiopia-SG | Ethiopia-YC | Guatemala-RP | Guatemala-RF | Guatemala-MR |
|-------------------------------------------------------------|-----------|-----------|-----------|-------------|-------------|------------|-------------|-------------|-------------|-------------|
| Hydroxycinnamic acid                                        | 115 ± 0.8 | 96.0 ± 0.1 | 10.8 ± 0.9 | 7.4 ± 0.0   | 15.2 ± 2.6  | 9.0 ± 0.5  | 16.9 ± 0.2  | 11.8 ± 0.6  | 10.1 ± 0.2  | 12.2 ± 4.4  |
| Caffeoylquinic acids                                        | 124 ± 0.9 | 11.0 ± 0.3 | 14.3 ± 0.2 | 21.1 ± 16   | 15.9 ± 1.5  | 21.2 ± 1.5 | 24.3 ± 1.3  | 22.6 ± 2.0  | 24.8 ± 6.1  | 197 ± 1.0   |
| Hydroxycinnamic acid                                        | 4467 ± 93.3 | 5035 ± 23.0 | 5235 ± 9.9 | 5769 ± 51.5 | 4036 ± 860 | 5480 ± 154 | 6172 ± 160 | 5034 ± 204 | 5613 ± 177 | 4654 ± 186 |
| Caffeoylquinic acids                                        | 649 ± 9.0  | 665 ± 41.5 | 806 ± 41.1 | 651 ± 15.9  | 490 ± 37.8  | 1080 ± 411 | 1087 ± 126 | 835 ± 56    | 990 ± 207   | 777 ± 42.6  |
| Methyl caffeoylquinic acids                                 | 140 ± 17.0 | 33.0 ± 2.7 | 165 ± 9.6  | 29.8 ± 18   | 126 ± 16.6  | 184 ± 6.0  | 109 ± 10.9  | 147 ± 14.0  | 293 ± 8.2   | 153 ± 27.5  |
| Dicaffeoylquinic acids                                      | 174 ± 4.0  | 184 ± 3.7 | 20.3 ± 7.2 | 20.7 ± 25   | 14.0 ± 1.4  | 28.0 ± 4.1 | 25.9 ± 7.7  | 26.7 ± 6.9  | 31.6 ± 9.8  | 226 ± 3.9   |
| L-Diferuloylquinic acids                                    | 919 ± 44.6 | 1158 ± 11.2 | 1025 ± 8.9 | 1212 ± 10.8 | 856 ± 68.8  | 867 ± 356  | 1018 ± 1.8  | 1061 ± 45.0 | 1298 ± 16.9 | 1043 ± 83.7 |
| Total caffeoyl derivatives                                  | 5536 ± 157 | 6243 ± 36.9 | 6426 ± 29.7 | 7010 ± 66.1 | 5019 ± 171  | 6494 ± 198 | 7218 ± 164  | 6242 ± 263  | 6941 ± 196  | 5849 ± 297  |
| Feruloylquinic acids                                       | 777 ± 15.2 | 761 ± 50.1 | 917 ± 53.0 | 733 ± 20.6  | 561 ± 42.6  | 1207 ± 287 | 1217 ± 289 | 966 ± 66.3  | 1132 ± 65.5 | 882 ± 51.2  |
| Hydroxycinnamic acid                                        | 455 ± 14.3 | 493 ± 5.2  | 54.4 ± 7.0 | 60.5 ± 7.1  | 438 ± 32.4  | 393 ± 109  | 474 ± 5.1   | 488 ± 14.7  | 504 ± 3.4   | 489 ± 42.4  |
| Caffeoylferuloylquinic acid                                 | 802 ± 0.6  | 780 ± 4.9  | 78.3 ± 3.9 | 101 ± 4.7   | 67.0 ± 5.1  | 110 ± 5.8  | 125 ± 8.1   | 86.1 ± 8.2  | 91.5 ± 10.0 | 968 ± 9.3   |
| Methyl feruloylquinic acids                                 | 9.3 ± 0.8  | 10.7 ± 0.5 | 2.9 ± 0.3  | 9.6 ± 1.2   | 11.7 ± 1.2  | 2.7 ± 0.2  | 10.1 ± 0.8  | 6.9 ± 12.2  | 8.3 ± 12.5  | 8.3 ± 0.5   |
| Diferuloylquinic acids                                      | ND         | ND         | ND         | ND          | ND          | ND         | ND          | ND          | ND          | ND          |
| Total feruloyl derivatives                                  | 464 ± 15.1 | 497 ± 5.2  | 451 ± 7.9  | 655 ± 8.1   | 448 ± 33.6  | 404 ± 121  | 476 ± 5.3   | 498 ± 15.5  | 507 ± 3.5   | 501 ± 45.1  |
| P-O-methylaroylquinic acids                                 | 380 ± 5.0  | 78.0 ± 49  | 78.3 ± 3.9 | 101 ± 4.7   | 67.0 ± 5.1  | 110 ± 5.8  | 125 ± 8.1   | 86.1 ± 8.2  | 91.5 ± 10.0 | 968 ± 9.3   |
| Caffeoylferuloylquinic acid                                 | 27.0 ± 0.8 | 28.2 ± 0.2 | 3.4 ± 0.1  | ND          | 3.0 ± 1.3   | 4.9 ± 1.7  | 6.0 ± 0.9   | 4.7 ± 1.0   | 17.0 ± 1.5  | 70.2 ± 6.1  |
| Total feruloylquinic acids                                  | 206 ± 26.2 | 33.2 ± 1.7 | 39.7 ± 2.2 | 26.6 ± 0.4  | 10.0 ± 2.2  | 14.8 ± 1.0 | 28.7 ± 1.4  | 32.1 ± 0.8  | 20.1 ± 3.6  | 25.5 ± 16.0 |
| Hydroxycinnamoyl amides                                     | 134 ± 1.9  | 16.6 ± 2.9 | 17.2 ± 1.4 | 38.5 ± 1.7  | 417 ± 5.0   | 172 ± 1.2  | 175 ± 0.8   | 19.4 ± 3.7  | 23.6 ± 1.2  | 130 ± 0.1   |
| Caffeoylferuloylquinic acid                                 | 170 ± 1.9  | 158 ± 5.7  | 164.1 ± 71 | 189.9 ± 140 | 272.2 ± 145 | 201.0 ± 232 | 225.6 ± 88  | 235.0 ± 220 | 220.5 ± 123 | 185 ± 9.6   |
| Feruloylquinic acids lactones                               | 198 ± 0.4  | 174 ± 0.5  | 15.6 ± 0.3 | 15.1 ± 16.9 | 18.7 ± 0.9  | 15.4 ± 0.5 | 24.1 ± 1.3  | 24.0 ± 0.3  | 23.1 ± 2.1  | 23.6 ± 0.9  |
| Total phenolic content                                      | 6165 ± 186 | 6938 ± 48.3 | 7059 ± 48.8 | 7885 ± 773 | 5628 ± 227 | 7039 ± 223 | 7868 ± 173 | 6922 ± 291 | 7635 ± 204 | 6521 ± 360 |
| % Loss of phenolic content                                  | 1024 ± 31.6 | 1032 ± 62.3 | 1195 ± 64.0 | 1069 ± 39.9 | 791 ± 63.4 | 1583 ± 70.5 | 1647 ± 527 | 1327 ± 189 | 1502 ± 145 | 1230 ± 71  |

Footnotes:
1. (Continued on next page)
Upon roasting, loss of 71.0–86.4% total phenolic acids was determined. A higher level of degradation was observed for dCiQA (88.8–96.8%) followed by CQA (72.2–89.6%) and FQL (71.8–85.4%) groups. The partial hydrolysis of the dCiQA may also contribute to the total content of mono-acylated compounds and free HCA. Up to 87.6% loss was determined for CQm group, and from FQM group only 5FQM was quantified in Ethiopia-YC, Guatemala, Honduras, India-MM, and Kenya coffee (Table 3). Moreover, 80.7–100% loss of hydroxycinnamoyl amide, pCoCQA, and dFQA groups were observed among the samples.

3.7. Partial list square discriminant analysis (PLS-DA)

PLS-DA analysis was conducted to assess possible variation among the coffee samples. Normalized data set (38 samples × 53 compounds quantified for phenolic acid content) was classified base on influencing factors such as type of compounds, processing condition (green or roasted), variety (Arabica or Robusta), geographical origin, and bean size which contributed for variation. The supervised PLS-DA analysis base on processing condition clearly distinguished green and roasted beans (Fig. 4S-A in the Supplementary material). The predictive strength was $Q^2_{Cum}(0.985)$, while the goodness of the fit was quantified as $R^2_X (0.735)$ and $R^2_Y (0.988)$. The variable importance in the prediction (VIP) value showed greater than 1 for hydroxycinnamoyl quinic acid lactones, 5CQA, 1CQA, 5FQA, cis-3CQA, and diCQAs (Fig. 4S-B in the Supplementary material). Moreover, Asia origin coffee beans (Vietnam-R, Vietnam-RSH, and India-KRK) having higher total phenolic acid content (8219 ± 106–8582 ± 109 mg/100 g, DW) were distinctly separated from the remaining samples (5628 ± 227–7885 ± 77.3 mg/100 g, DW) in green beans group. Discrimination of samples by species and geographical location were also observed (Fig 4S-C in the Supplementary material). The score plot of PLS-DA analysis separated the samples into two major groups corresponding to C. arabica and C. robusta. Previous studies revealed that some unique compounds which are specific to C. robusta such as, sinapoylquinic acid, trimethoxycinnamoylquinic acid and tricacyl quinic acid have shown discriminant ability on principal component analysis (Jaiswal et al., 2010; Kuhnert et al., 2011). However, on the present study these compounds were not detected in any of C. robusta beans hence, this clustering can be explained due to the difference in phenolic acid contents. The concentration of 4C,5FQA, 3C,5FQA, diFQAs, diCQAs, and HCAms in green bean were higher and their influence on VIP value was greater than 1 (Fig 4S-D in the Supplementary material). The C. arabica group further divided into two distinct groups (C. arabica 1 and 2) (Fig 4S-C in the Supplementary material), and this is in agreement with Kuhnert et al. (2011) finding. To understand the contributing factor for this variation among C. arabica samples green bean processing methods, bean size, altitude and latitude of cultivation areas were taken in to account and data matrix was analyzed by PLS-DA however, none of these factors were contributed to explain the discrimination among these C. arabica samples. From this result, we can hypothesis that coffee beans from Colombia-SM, Guatemala-RT, Brazil-RB, Ethiopia-YC, and India MM might have genetic similarity however, we suggest further studies on large number of representative samples collected from different geographical origin.

In conclusion, moderate variation in composition and concentration of hydroxycinnamoyl derivatives attributed to sample origin, cultivar, and species were observed among the coffee bean samples. The analytical method used demonstrated excellent chromatographic resolution to characterize and quantify different groups of compounds from a small amount (1 g) of the sample simultaneously. A total of 57 phenolic acids were identified, and of which 39 compounds in green and 42 compounds in roasted coffee bean quantitatively determined. Discrimination and characterization of regioisomers among a group of hydroxycinnamoyl derivative based on the fragment ion and its intensity they produced were the limitations observed in (+) ESI.
ionization mode. Higher level of total phenolic acid degradation was determined in roasted samples. Processing condition and species variation was influencing factor for the discrimination in PLS-DA analysis. This finding suggested that roasting condition should not only target the level of bean color during roasting (Fig. 1S in the Supplementary material), it further requires optimizing of the maximum condition to retain health benefitting phenolic compounds.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100033.

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