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The polyphenolic and hydroxycinnamate contents of whole coffee fruits from China, India and Mexico

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1 **Abstract**

2 Air dried whole coffee fruits, beans and husks from China, India and Mexico were analysed
3 for their chlorogenic acids (CGA), caffeine, and polyphenolic content. Analysis was by
4 HPLC and Orbitrap exact mass spectrometry. Total phenol, total flavonol and antioxidant
5 capacity were measured.

6 The hydroxycinnamate profile consisted of caffeoylquinic acids, feruloyquinic acids,
7 dicaffeoylquinic acids and caffeoyl-feruloylquinic acids. A range of flavan-3-ols as well as
8 flavonol conjugates were detected. The CGA content was similar for both Mexico and India
9 coffee fruits but was much lower in China samples. Highest levels of flavan-3-ols were
10 found in the Indian samples whereas Mexico samples contained the highest flavonols.
11 Amounts of CGAs in the beans were similar to those in the whole fruits, but flavan-3-ols and
12 flavonols were not detected. The husks contained the same range of polyphenols as in the
13 whole fruits. Highest levels of caffeine were found in the Robusta samples.

14

15 **Keywords:** Chlorogenic acid, Caffeine, quercetin, procyanidins, total flavonol, total phenol
16 and antioxidant.

17

18 **INTRODUCTION**

19 Chlorogenic acids are a group of phytochemical compounds, the most common being caffeic
20 acid, ferulic acid and *p*-coumaric acid which form esters with quinic acid.[1-3]. They belong to
21 the hydroxycinnamic acids, a type of phenolic compounds having a C6-C3 skeleton. The
22 structures of some of these compounds are shown in Figure 1. An early reference to these
23 compounds in the coffee bean was published in 1837, [4] although the term chlorogenic acid
24 was not used until 1846, [5] and the compound was not fully characterized until 1932.[3] While
25 chlorogenic acids are widely distributed in plants,[2] the coffee bean is one of the richest
26 sources (up to *ca.* 10% dry basis).

27 The chlorogenic acid content of the coffee beverage has been extensively studied [1,2,6] and the
28 transformation undergone by hydroxycinnamates during coffee roasting has been the subject
29 of a number of investigations.[7] It is well-established that these compounds are progressively
30 destroyed with increasing roast severity, resulting in a decreasing amount of the initial CGA
31 content. Nonetheless, coffee beverages remain the major source of dietary
32 hydroxycinnamates, with daily intakes of over 500 mg of CGAs easily attainable.

33 With a growing belief that dietary polyphenols, hydroxycinnamates and other phenolics have
34 the potential to provide beneficial effects on health, research has been focusing on
35 developing products with enhanced content by exploiting polyphenols recovered from food
36 waste or by-products to be used as natural additives. In contrast to green and roasted coffee
37 beans and instant coffees, there are scarce data for the chlorogenic acid contents of soluble
38 green coffee extracts.[8] There is also a lack of knowledge on the impact of important
39 parameters such as the type of coffee fruit, (i.e. Robusta or Arabica), and the geographical
40 origin of the fruit on the range of polyphenolic compounds present in whole coffee fruits.
41 The whole coffee fruits, or cherries (with seed intact), may provide a broad range of phenolic

42 and polyphenolic compounds not found in the beans; additionally, the husks of the coffee
43 fruits, normally discarded as waste products, could be a valuable source of polyphenolic and
44 related compounds. In this study, we analyzed the range of polyphenolic and
45 hydroxycinnamate compounds contained in six whole coffee fruits, grown in 3 different
46 countries (namely China, India and Mexico). We also report on the large variation in the
47 polyphenolic and hydroxycinnamate contents identified from the analysis of the separated
48 beans and husks of the whole coffee fruits.

49 MATERIALS AND METHODS

50 **Chemicals**. 5-*O*-caffeoylquinic acid, (–)-epicatechin, procyanidin dimer B2, quercetin-3-
51 *O*-glucoside and quercetin-3-*O*-rutinoside were obtained from AASC Ltd (Southampton,
52 UK). Methanol, ethanol and acetonitrile were obtained from Rathburn Chemicals
53 (Walkerburn, Scotland). Formic acid was obtained from Fisher Scientific (Loughborough,
54 UK). Folin reagent, sodium carbonate, aluminium chloride, potassium acetate, TPTZ (2,4,6-
55 tripyridyl-s-triazine) and ferric chloride ($FeCl_3 \cdot 6H_2O$) were obtained from Sigma (Sigma
56 Aldrich, Poole, Dorset, UK).

57 **Coffee material.** Coffee samples were provided by FutureCeuticals, Inc. (Momence, IL
58 USA) as air-dried whole fruits, beans (removed from the fruits) and husks. Arabica and
59 Robusta coffee samples from Mexico, India and China, each from the same harvest batch,
60 are included in this study. Mexican Robusta (*Coffea canephora* 'Robusta') and Arabica
61 (*Coffea arabica* 'Bourbon') samples originated from Coatepec in the Mexican state of
62 Veracruz (elevation 900-1200 m above sea level). Indian Robusta (*Coffea canephora*
63 'Robusta') and Arabica (*Coffea arabica* 'Typica') samples originated from Chikmagalur, in
64 the Karnataka province (elevation 1090 m above sea level). Chinese Robusta (*Coffea*
65 *canephora* 'Robusta') samples originated from the Fujian province (elevation 1100 m above

66 sea level), while Arabica samples (*Coffea arabica* 'Catimor') originated from the Yunnan
67 Province (elevation 1400-1600 m above sea level). All coffee fruits, except the Chinese
68 Robusta fruits, were harvested as immature (partially ripe) and ripe cherries; the Chinese
69 Robusta coffee fruits were harvested as unripe and immature (partially ripe) cherries.

70 **Sample preparation.** Triplicate samples were prepared from Arabica and Robusta
71 coffees originating from China, India and Mexico including, for each provenance, i) whole
72 coffee fruits, ii) beans removed from the coffee fruit, and iii) husks. Each coffee samples
73 were weighed (approximately 5 g), ground to a fine powder in coffee grinder and sieved (0.5
74 mm). Three aliquots (20-30 mg) were accurately weighed into 15 mL centrifuge tubes and
75 the powders were individually extracted with 50% ethanol/water (7 mL) by vortexing the
76 contents for 10 min followed by centrifugation at 2000 rpm for 2 min. The supernatant was
77 transferred to a 50 mL tube and the extraction repeated a total of 5 times. The final volume
78 of the extracts was made up to 50 mL, and 5 mL-aliquots were freeze-dried and reconstituted
79 in 500 µL methanol: 0.1% formic acid prior to LC-MS analysis. The original extracts were
80 frozen at -80 °C until further analysis.

81 **Total phenolic content and total flavonoid content.** The total phenolic content of the
82 whole coffee fruits were measured with the Folin-Ciocalteau assay[9] modified to be carried
83 out in a microtitre plate. Briefly, extracts (20 µL) were added in triplicate to the plate, along
84 with Folin reagent (100 µL, 1:10) and dH_2O (70 µL). The reaction was incubated at room
85 temperature for 5 min prior to addition of sodium carbonate solution (70 µL, NA_2CO_3, 1 M).
86 After 2 h incubation at room temperature, optical densities were measured at 765 nm
87 (Multiskan Spectrum Spectrophotometer, ThermoFisher). The total phenolic content of the
88 extracts was quantified as gallic acid equivalents (calibration curve linear in the range 0-400
89 µg/mL).

90 The total flavonoid content of the whole coffee fruits was measured with the aluminium
91 chloride method[10] adapted for microtitre plate. Briefly, extracts (100 µL) were added in
92 triplicate to the plate, along with 10% aluminium chloride (60 µL), 1 M potassium acetate
93 (30 µL) and dH_2O (95 µL). The reaction was incubated for 30 min at room temperature.
94 Optical densities were measured at 415 nm (Multiskan Spectrum Spectrophotometer,
95 ThermoFisher). The total flavonoid content of the extracts was quantified as quercetin
96 equivalents (calibration curve linear in the range 0-200 µg/mL).

97 **Antioxidant power.** The antioxidant power was measured in whole coffee fruits using
98 the method of Benzie and Stain[11] adapted for the microtitre plate. The FRAP reagent was
99 prepared fresh daily, by mixing sodium acetate pH 3.6 (25 mL, 300 mM), TPTZ (2.5 mL, 10
100 mM) and ferric chloride (2.5 mL, 20 mM). The coffee fruit extracts (25 µL) were added in
101 triplicate to the plate, along with FRAP reagent (225 µL, added to the whole plate within 30
102 s with a multi-channel pipette).

103 The reaction was incubated at room temperature for 4 min and optical densities were
104 measured at 593 nm (Multiskan Spectrum Spectrophotometer, ThermoFisher). The
105 antioxidant power of each sample was expressed as ferric acid equivalent based on the
106 standard calibration curve, which was linear in the range 0-1 mM.

107 **HPLC-PDA-Exact mass-MS.** Analysis was carried out on a Thermo Accela HPLC
108 system comprising of an autosampler with sampler cooler maintained at 6ºC, a photodiode
109 array detector scanning from 200-600 nm. Samples (5 or 10 µL) were injected onto a 150 x
110 3.0 mm C_{18} Accucore column (Thermo Fisher Scientific) maintained at 40ºC and eluted with
111 a 5-10-50% gradient of 1% formic acid and acetonitrile at 700 µL/min over 0-10-20 minutes.
112 After passing through the absorbance detector, the eluant was split and 200 µL/min was
113 directed to the electrospray interface of an Exactive Orbitrap mass spectrometer (Thermo

114 Fisher Scientific). Samples were run in negative ionization mode, the scan range was from
115 150-1200 *amu* with resolution set to 60,000.

116 Peak identifications were based on co-chromatography with authentic standards, when
117 available, as well as absorbance spectra and published MS^2 mass spectral data[12-14]. The
118 samples were also analyzed using the Orbitrap mass spectrometer operating with 15% in
119 source fragmentation energy to confirm exact mass of all the fragments of the compounds.

120 Quantification of hydroxycinnamic compounds was by comparison to an authentic standard
121 of 5-*O*-caffeoylquinic acid, range 5 to 750 ng monitored at 325 nm and caffeine at 275 nm in
122 the range 5 to 750 ng. Quantification of minor phenolic compounds was by exact mass
123 measurements of calibration standards over the range of 0.5 to 50 ng using (–)-epicatechin
124 for flavan-3-ol monomers and procyanidin B2 for dimeric, trimeric and tetrameric flavan-3-
125 ols. Quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside were quantified as quercetin-3-*O*-
126 glucoside equivalents.

127 **Statistical analysis.** Results are shown as means ± standard deviations of three true
128 replicates. Minor and major compounds were compared between the six coffee varieties
129 (either whole fruit, bean or husk) using one-way ANOVA. A two-way ANOVA was
130 conducted to examine the effect of country of origin (Mexico, India or China) and coffee
131 fruit type (Arabica or Robusta) on a selection of dependant variables, namely total phenol
132 and flavonoid contents, caffeine content, 5-CQA and total hydroxycinnamates in whole
133 coffee fruits. When an interaction occurred, the Tukey post-hoc test was used to identify the
134 effect of country of origin. Meanwhile, correlations among variables were assessed by means
135 of the Pearson's bivariate correlation test.

136

137 **RESULTS**

138 **Identification of hydroxycinnamic compounds.** Peak numbers, retention time, mass
139 spectral data and identification of the compounds described are listed in Table 1. The
140 chromatographic separation of the Arabica and Robusta samples from Mexico are shown in
141 Figure 2 and Figure 3.

142 *Peaks 1,2* and *4* All had similar absorbance spectra with λ_{max} at 325 nm. Mass spectral
143 analysis revealed they also had the same negatively charged molecular ion $[M-H]^-$ at *m/z*
144 353.088. In-source fragmentation of m/z 353 produced 3 different mass spectra (Figure 4)
145 which allowed identification of these compounds as the 3-, 5- and 4-*O*-caffeoylqunic acid
146 respectively.[12] In addition, peak 2 co-chromatographed with the authentic standard.

147 *Peaks 3 Had a* λ_{max} at 275 nm and produced no ions in the mass spectrometer. It co-
148 chromatographed with authentic standard of caffeine. Caffeine does not ionize in negative
149 ion mode.

150 *Peaks 5 and 7* both produced $[M-H]^-$ ions at *m/z* 577.135. Upon fragmentation both produced
151 a series of fragment ions at *m/z* 451, 425, 407 and 289 typical of that seen in dimeric (+)-
152 catechin / (–)-epicatechin. By co-chromatography peak 5 was identified as the epicatechin
153 (4-8) epicatechin dimer B2. By chromatographic elution profiles Peak 7 could be either the
154 catechin-epicatechin dimer B1 or the catechin-catechin dimer B3 (*15*) Examination of the
155 fragment ions would indicate that it is the dimer B1, since the fragment $[M-H^+-H_2O]$ at *m/z*
156 559 which was present in the B2 dimer is missing.[16]

157 *Peaks 6 and 8* both produced $[M-H]^-$ ions at *m/z* 289.071. Upon fragmentation both produced
158 a series of fragment ions at *m/z* 245, 205 and 179 typical of (+)-catechin / (–)-epicatechin
159 standard.[17] By co-chromatography peak 6 and 8 were identified as (+)-catechin and (–)-
160 epicatechin.

161 *Peak 9* produced an $[M-H]^-$ ion at *m/z* 863.183. Upon fragmentation it produced a series of
162 fragment ions at *m/z* 575.124, 289.071 typical of an A type procyanidin compound.[18]

163 *Peak 10* produced an $[M-H]^-$ at *m/z* 865.183, a two-mass units higher than that of Peak 9. It
164 produced fragmentation ions at m/z, 577.135, and 289.071. This fragmentation pattern is
165 typical of that seen in a procyanidin B type trimer. From the elution profile of the other
166 compounds, this peak could be the trimer C1.[15] However, without co-chromatography with
167 an authentic standard, this observation is only putative.

168 *Peak 11 and 12* followed the same pattern as that of Peaks 9 and 10, but with an $[M-H]^-$ an
169 additional 288 mass units higher at $[M-H]^-$ at *m/z* 1151.247 and 1153.263. This is typical of
170 the analysis of procyanidin tetramers. Therefore Peak 11 was identified as procyanidin A
171 type tetramer and Peak 12 was identified as a procyanidin B type tetramer.[19]

172 *Peaks 13 and 14.* Both had a $[M-H]^-$ at *m/z* 367.105 indicating the presence of feruloylquinic
173 acids. Peak 13 fragmented to produce a base ion at *m/z* 173 with another major ion at *m/z*
174 193. This pattern is in keeping with a 4-*O*-feruloylquinic acid conjugate. Fragmentation of
175 the parent ion in peak 14 produced a single fragment at *m/z* 191 which is in keeping with that
176 previously described for 5-*O*-feruloylquinic acid.[12]

177 *Peaks 15 and 17*: Both had an absorbance spectra with λ_{max} at 365 nm. Mass spectral
178 analysis revealed both peaks had a negatively charged molecular ion ($[M-H]^-$) at *m/z*
179 609.156. Fragmentation of the parent ions produced a mass spectral pattern which allowed
180 identification of these compounds as quercetin-*O*-rutinosides. Co-chromatography with a
181 standard allowed identification of peak 17 as quercetin-3-*O*-glucose-rhamnose conjugate
182 indicating that the earlier eluting peak 15 could be a quercetin-3-*O*-galactoside-rhamnose
183 conjugate.[20]

184 *Peaks 19 and 20*: Both had an absorbance spectra with λ_{max} at 365 nm. Mass spectral
185 analysis revealed both peaks had a negatively charged molecular ion ($[M-H]^-$) at *m/z*
186 463.093. Fragmentation produced a mass spectral pattern which allowed identification of
187 these compounds as quercetin-*O*-glucosides. Co-chromatography with a standard allowed
188 identification of peak 20 as quercetin-3-*O*-glucoside. Elution profile of these compounds
189 would indicate that peak 19 was quercetin-3-*O*-galactoside.[21]

190 *Peaks 16, 18 and 21.* All had a $[M-H]^-$ at *m/z* 515.120 indicating the presence of
191 dicaffeoylquinic acids. Fragmentation spectra and elution profile matched that previously
192 seen in coffee berry extracts.[12] The identity of peak 7 was 3,4-*O*-dicaffeoylquinic acid, peak
193 8 was the 3,5-*O*-dicaffeoylquinic acid and peak 9 the 4,5-*O*-dicaffeoylquinic acid.

194 *Peaks 22, 23 and 24*. All had the same $[M-H]^-$ at *m/z* 529.139. This parent ion is indicative of
195 an *O*-caffeoyl-*O*-feruloyl quinic acid conjugated compound, of which six have been
196 previously reported. Fragmentation spectra revealed the base daughter ion in peak 22 to be
197 *m/z* 367 with additional minor fragment ions at *m/z* 335, 193 and 173. This would suggest
198 that this is the 3-*O*-feruloyl-4-*O*-caffeoylquinic acid. Peak 23 and 24 differed in their
199 fragment spectra in that the base daughter ion was *m/z* 353 with major ions at *m/z* 367 (40
200 and 60% respectively) and minor ions at *m/z* 335 and 173 indicating that they may be 3-*O*-
201 caffeoyl-5-*O*-feruloylquinic acid and 4-*O*-caffeoyl-5-*O*-feruloylquinic acid respectively.
202 However, without authentic standards this identification must be seen as tentative.

203 The identities of the 3, 4 and 5-CQA compounds are normally confirmed by MS/MS analysis
204 as reported in Clifford et al.[12] When using high resolution accurate mass full scan analysis
205 this is not possible. However, it is possible to apply in source fragmentation during the
206 analysis, which allowed the confirmation of the three isomers of these and the other CGA

207 compounds in the extracts. The accurate mass of these minor ions of 3, 4 and 5-CQA are
208 presented in Figure 4.

209 **Quantification of major and minor compounds in the coffee samples.**

210 Quantification of identified compounds in whole coffee fruits, beans and husks is detailed as
211 follows. Quantification is by comparison to the most appropriate available standard.
212 However, the quantitative data is for comparison between samples and may not reflect the
213 true levels of compounds present, especially for the procyanindins which have a wide range
214 of ionisation efficiencies.

215 *Whole Coffee Fruits.* The quantitative data of the hydroxycinnamate and caffeine
216 content (major compounds) of the whole coffee fruit extracts are summarized in Table 2 (all
217 hydroxycinnamates are expressed as mg/g of 5-CQA equivalents ± SD). The Arabica and
218 Robusta samples from Mexico and India had similarly high quantities of CGAs (24±2.7,
219 21±1.6 and 19±0.7, 20±2.9 mg/g, respectively), whereas the samples from China were much
220 lower (0.6±0.1 and 3.5±0.6 mg/g, respectively). 5-CQA was the major CGA present in all
221 samples. The diversity of the hydroxycinnamate profile was the greatest in Mexican and
222 Indian samples, with all 12 compounds detected, whereas only 9 and 7 compounds where
223 identified in the Robusta and Arabica Chinese samples.

224 The caffeine levels in the Robusta samples were consistently higher than the Arabica
225 samples from all three countries, with samples from Mexico and India showing the highest
226 levels 8.2±1.7 and 7.5±1.5 mg/g respectively.

227 The polyphenolic content of the whole coffee fruits was also analyzed using non-targeted
228 high resolution accurate mass analysis. The quantitative data relative to the minor
229 polyphenolic compounds (procyanidins and flavonols) detected in the whole coffee fruit
230 extracts are summarized in Table 3. The result of this investigation presents a more complex

231 picture than the CGA: the main polyphenolics detected were flavan-3-ols and flavonols.
232 Arabica samples from Mexico and Arabica and Robusta samples from India presented the
233 greatest diversity, with 10 compounds identified each, followed by Arabica samples from
234 China (8 compounds), Robusta sample from Mexico (6 compounds) and Robusta sample
235 from China (4 compounds). Procyanidins were highest in Robusta samples from India
236 (176±24 μg/g) and lowest in Robusta samples from China (14±1 μg/g). Meanwhile, total
237 flavonols where highest in Arabica samples from Mexico (99±18 μg/g) and lowest in all
238 samples from India and China.

239 *Coffee Beans and husks.* The quantitative data of the coffee beans provided an almost
240 matching profile of CGAs to those reported in Table 2 for whole coffee fruits. These results
241 are presented in Table 4. None of the minor compounds reported in Table 3 were detected in
242 the coffee bean extracts.

243 The analyses of the husks samples revealed the presence of low levels of CGAs (major
244 compounds) and are presented in Table 5. The CGA levels in the husk were not detected at
245 all in the samples from China and were highest in Arabica Mexico and Robusta India
246 samples (2.6±0.9 and 2.2±0.8 mg/g, respectively).

247 The quantitative data on the minor flavonoid compounds found in the husks are presented in
248 Table 6. The high levels of combined flavonols and procyanidins quantified in the husks
249 indicate that the flavonoids found in the whole coffee fruit extracts originates from the husks.
250 The Arabica samples from Mexico had the highest levels of flavonols present (260±106
251 μg/g), much higher than any of the other samples (ranging between 5 and 34 μg/g), whereas
252 the Robusta samples from India had a high flavan-3-ol (procyanidins) concentration (534±61
253 μg/g) compared to all other samples (flavan-3-ols were not detected in Robusta samples

254 from Mexico). A typical flavan-3-ol profile (Arabica sample from India) is presented in
255 Figure 5.

256 *Whole coffee fruits - total phenol and flavonoid content and antioxidant*
257 *capacity*. The total phenol, total flavonoid and antioxidant capacity (FRAP) of the coffee
258 fruits are presented in Table 7. Total phenolic content, as well as 5-CQA content and total
259 hydroxycinnamates were strongly correlated to antioxidant power ($p<0.001$, $r2=0.98$, 0.75
260 and 0.86, respectively). The total flavonoid content did not correlate to the antioxidant
261 capacity. The antioxidant capacity of the Robusta samples from India was far superior to all
262 other samples, with both Arabica and Robusta samples from China at the lower end of the
263 scale. There was no statistical difference in the total flavonoid content of the samples
264 analyzed colorimetrically. The total phenol content of the Robusta sample from India was
265 the highest (1.5 to 2-folds higher than other coffee samples), in agreement with the FRAP
266 value.

267 *Whole coffee fruits - effect of the interaction between country of origin and*
268 *coffee fruit type on polyphenolic content*. A two-way ANOVA analysis revealed
269 significant interactions between country of origin and coffee fruit type on total phenol
270 ($p<0.01$), 5-CQA content ($p<0.01$) and antioxidant capacity ($p<0.01$), but not total flavonoid
271 content, or caffeine content or total hydroxycinnamates.

272 There was significant difference between countries of origin for total phenol ($p<0.01$, China
273 < Mexico < India), 5CQA ($p<0.01$, China < India < Mexico), total hydroxycinnamates
274 ($p<0.01$, China < India < Mexico) and antioxidant capacity ($p<0.01$, China lower than
275 Mexico and India). Meanwhile, coffee type only significantly influenced antioxidant power
276 ($p<0.05$, Arabica < Robusta), caffeine content ($p<0.01$, Arabica < Robusta) and 5CGA
277 content ($p<0.01$, Arabica > Robusta).

278 **DISCUSSION**

279 The term 'chlorogenic acid' (CGA) was first used in 1846 to describe a material crystallised
280 from a crude extract of green coffee beans.[5] This crystalline substance is now described,
281 using the IUPAC numbering,[22] as 5-*O*-caffeoylquinic acid (5-CQA), an ester of *trans*-caffeic
282 acid and quinic acid. It is now known that green coffee beans contain at least 69 structurally
283 related chlorogenic acids,[23] with 5-CQA accounting for approximately 50% of the total, and
284 along with another eight accounting for in excess of 95% of the total chlorogenic acid
285 content. The chlorogenic acids of the traditional roasted and solubilized coffee products have
286 been extensively analysed. Such reports on the polyphenolic content of green coffee beans
287 are less well documented.[24]

288 **Difference between these green coffees and roasted coffees (impact of absence of**
289 **processing).** From a quantitative aspect, the chlorogenic acid profile of these whole coffee
290 fruits is lower than that of a coffee prepared from roasted beans. Typical caffeoylquinic acids
291 content per serving of coffee can range between 70-200 mg per 200 mL cup of Arabica and
292 70-300 mg per 200 mL cup of Robusta.[25] This is assuming that 2 g of coffee powder is used
293 per serving. The simple ethanol/water extraction process used in this experiment was not
294 optimized for recovery of CGAs. However, it is possible to obtain 400 mg/g using
295 commercial extract processes on the whole coffee fruits.[26] Qualitatively, the main difference
296 is that these whole coffee fruits haves a much simpler CGA range of compounds than roasted
297 coffee, due to their reduced processing. None of the chlorogenic acid lactones were detected
298 in this analysis, either by absorbance detection or by using the mass spectral data. These
299 compounds are formed during the roasting process through elimination of a molecule of
300 water from the quinic acid resulting in the formation of a lactone ring.[8] Most noticeable,
301 however, was the presence of a range of flavan-3-ol compounds and flavonols in the μg/g

302 range, similar to concentrations found in a number of different berries and grapes.[27]
303 Procyanidins were previously reported in coffee pulp, in the range of 0.1-1.2% dry basis,
304 depending on the type of fraction extracted and whether the pulp was fresh or dried.[28]

305 **Variation between whole coffee fruit in terms of country of origin and coffee type.**
306 We have shown in this study that the CGA content of both Arabica and Robusta can vary
307 widely depending on the country of origin. This may be due to a number of factors other
308 than geographical location. No details regarding soil or climatic conditions were, however,
309 available. Both the Robusta and Arabica samples from China had very low CGA content
310 when compared to samples from India or Mexico, which may indicate the growing
311 conditions there are not favourable for CGA production. Indeed, Joet et al. reported that in
312 green Arabica coffee beans from the Reunion Island, average temperature and irradiance
313 level both influenced the CGA content, in particular caffeoyl quinic acids (but not feruloyl
314 quinic acids).[29] Ripening was similar (partially ripe to ripe) for all samples but Robusta
315 coffee from China, which could partially explain the lower CGA levels found in that sample.
316 However, the Arabica sample from China did have relatively high levels of flavanol-3-ols
317 present (81 μg/g), which was higher than the level found in the Mexican samples.

318 **Variations within the bean: compounds not found in the bean, compounds only**
319 **found in beans.** It was important to establish the source of flavan-3-ols and flavonols within
320 the whole coffee fruit. The whole coffee fruit samples described above were split into husks
321 and green beans and extracted in the same manner as the whole coffee fruits. This
322 established that the additional minor polyphenolic compounds were contained exclusively in
323 the husks. The CGA pattern of the extracted beans matched well with that found in the whole
324 coffee fruit.

325 **Variations within husks, compounds not found in husks, compound only found in**
326 **husk.** The variation in both the quantitative and qualitative profiles of the husk samples
327 analysed was however large. Robusta from India had the highest total polyphenolic content
328 of 553.1 µg/g, mainly due to the high level of flavan-3-ol procyanidin A type trimer.
329 However, the flavonol content only accounted for 19.5 µg/g. The Arabica sample from
330 Mexico had the highest level of flavonols, 260.6 µg/g, but only 114.6 µg/g of flavan-3-ols.
331 The husks did contain low levels of CGA and caffeine as well as the additional minor
332 polyphenolics compounds. This is of interest given that coffee husks are generally discarded
333 despite being potentially contributing to the diversity of polyphenolic compounds found in
334 coffee fruits.

335 **Opportunities for the food industry.** More recently extracts of green coffee beans have
336 been developed as rich sources of bioavailable chlorogenic acids and such extracts are
337 marketed as functional ingredients or health-supporting dietary supplements. The use of
338 these antioxidant rich extract ranges from dermatological treatments for skin damage by UV
339 exposure to blood pressure lowering effects on humans with mild hypertension.[30-33]

340 *Health.* Chlorogenic acids are for many people the main dietary (poly)phenols, and their
341 consumption, absorption, metabolism and excretion have been extensively studied in
342 volunteers,[31,34-37] animals[38-42] and *in vitro* [38,43,44] with a view to determining a possible
343 contribution to health and well-being. Specifically, recent trials have demonstrated the
344 impact of green coffee extract consumption on regulation of hypertensive state (drop of 10
345 mm Hg in the systolic blood pressure after 12 weeks in non-overweight human)[45] potentially
346 mediated by ferulic acid via the muscarinic acetylcholine receptors, as shown in rodents;[46]
347 impact on weight loss and improved lean to fat mass ratio in overweight humans (60-day
348 trial).[47] Potential mechanisms behind the effect of green coffee extract on weight loss, as

349 further examined in a meta-analysis by Onakpoya et al.[48] may include inhibition of human
350 hepatic glucose-6-phosphatase activity by caffeoylquinic and dicaffeoylquinic acids,[31] an
351 anti-diabetic mechanism which may account for the long-term effect of coffee drinking.[49]

352 A 1 g dose of a whole coffee fruit extract could easily attain 400 mg polyphenols,[26] with
353 caffeine levels remaining within the safe upper limit recommended during pregnancy by the
354 UK Food Standard Agency of 200 mg per day.[50] Indeed it has recently been demonstrated
355 that caffeine levels in a single serving of coffee can range between 51-322 mg,[51] despite
356 published guidelines suggesting a serving with levels ranging between 30-85 mg depending
357 on the type of coffee beverage.[52] Intoxication due to the high caffeine content of coffee
358 husks has been reported in horses where husk was used as stall bedding.[53] Potential
359 decaffeination of the husk based on processes already used in roasted coffee, such as organic
360 solvents or water or supercritical carbon dioxide[54] should be investigated. Although coffee is
361 a rich source of hydroxycinnamates, it lacks the wide range of polyphenolics offered by
362 other commonly consumed beverages[20] and may not be able to provide the wider range of
363 potential benefits associated with the wider array of dietary polyphenolic compounds.

364 *Valorisation of Waste.* The husks, as we report here, are a rich source of two
365 additional classes of polyphenolics, namely flavan-3-ols and flavonols, both of which have
366 been reported in bioactivity studies.[24] The presence of flavan-3-ols in whole coffee fruit
367 extracts and from the leaves has been previously reported.[55] Of interest in the report by De
368 Colmenares et al.[28] is that (epi)catechin dimers are the major flavan-3-osl present in the fresh
369 pulp and are converted to oligomers in the drying process. This could indicate that the
370 qualitative profile of the flavan-3-ols could be manipulated by alterations to the drying
371 process.

372 Coffee fruit husks are presently treated as a waste product, derived from dry processing
373 (versus wet processing, where the husk material is removed by fermentation). Dry
374 processing is a simpler methodology, with no demand for specific harvest condition or
375 uniform ripeness stage, applied widely to Robusta coffees worldwide as well as most
376 Arabica coffees in Brazil. Meanwhile, wet processing is widespread, especially for Arabica
377 coffees.[56,57] The processing type depends on the coffee, the region (and water access,
378 resources) and the expected quality and taste of the final produce. However, the excessive
379 amount of waste generated by the wet processing of coffee, as well as the environmental
380 issue posed by the contamination of the by-products, has prompted for alternative processing
381 techniques to be developed (such as ecological mechanical removal of the mucilage to
382 reduce water usage and contaminated by-products of the process).[57] It also highlights the
383 potential for dry processing and the revalorisation of the husks, which could be encouraged
384 via increased efficiency of extraction of polyphenolics.

385 Over the years, a number of industries have focused on optimising their waste handling, with
386 the revalorization of the waste product. There are a number of other examples of taking a by-
387 product and changing it from something that cost money to dispose of into a marketable
388 product. The wine industry has developed a market for grape seed and pomace, waste
389 products of the process, which are a powerful source of antioxidant compounds.[58-60] Grape
390 seed extracts have become an opportune and vital business for the wine industry.[61]

391 Similarly, the olive oil industry has revalorised the mill wastewater by evaluating the
392 recovery process of polyphenolic compounds from waste waters. Successful approaches
393 included the use of absorbent vegetable material to recover a range of flavan-3-ols in
394 significant quantities.[62] The phenolic compounds present in olive mill wastewater are major
395 contributors to its toxicity and antibacterial properties. Therefore extraction of these
396 compounds again produces a marketable product and also reduces the toxicity and pollution

397 of the mill waste water. Apple peel is often a part of the fruit that is discarded during
398 processing though it can contain the bulk of the antioxidants in the whole apple. As 25-40%
399 of the fruit processed ends up as waste there is an obvious need to recover and reuse it. A
400 review of the recovery and uses of the carbohydrates and polysaccharides from apple
401 pomace as well as the bioactive molecules such as proteins, vitamin, minerals and
402 antioxidants is provided by Bhushan et al.[63] The recovery in apple waste of antioxidant
403 compounds was investigated by Tow et al.[64] This investigation also raised the issue of
404 extractable and non-extractable polyphenolics and their potential antioxidant activities and *in*
405 *vitro* antiproliferation abilities. The results of the investigation would indicate that there are
406 over 500 mg gallic acid equivalents per gram dry weight of non- extractable polyphenols
407 compared to only 77 mg/g extractable polyphenols. These polyphenols are thought to be
408 predominantly procyanidins that are bound to the cell wall material. Pistachio nuts are
409 another source of antioxidant compounds and also have a waste product that could be used as
410 source of polyphenolic antioxidants. In most food products the nuts are used after removal of
411 the skin. The discarded skins of the pistachio nuts account for about 10% of the total shelled
412 weight of the pistachio.[65] However, the skin accounts for over 90% of the total flavonoid
413 content of the pistachio.

414 We have analyzed the CGA content of whole coffee fruits and described a simpler profile of
415 compounds present than seen in processed coffee products. We have also shown that the
416 husks of whole coffee fruits can be a potential source of flavan-3-ols and flavonols. The
417 potential content of these compounds in the husks may be greatly underestimated as the
418 extraction efficiency of our process may be as low as 3%, as was reported by Andrade et
419 al.,[66] which focussed on the extraction of antioxidants from coffee grounds and husks,
420 reporting the presence of epicatechin and a number of phenolic acids (but no flavonols).
421 Such low extraction efficiency highlights the level of unextractable procyanidin to be

422 recovered from the husks and the scope for alternative extraction methods to be developed.
423 While, at present, grape-derived extract with high polyphenolic contents may represent a
424 more readily available source (with a lesser non-extractable fraction), the continued
425 development of extraction methods for phenolics in coffee husks and other waste products
426 issued from coffee waste-processing offer potential for revalorisation as well as reduction of
427 toxic by-products.[57] Beyond coffee husks only, Murthy and Naidu highlighted the high
428 polyphenol content of other coffee by-products (including coffee pulp, silver skin and spent
429 waste). Along their high phenolic content, coffee by-products, especially husk, silver skin
430 and spent waste, contained high amount of soluble (8-26%) and insoluble dietary fiber (16-
431 35%).[67] This combined high fiber and antioxidant make coffee by-products attractive for use
432 as nutraceuticals and ingredients in functional foods, since combined antioxidant and fibre
433 content are likely to impact positively on health, including colonic health.[67,68]

434 Investigations such as the aforementioned could serve to further enhance the use of coffee
435 fruits, material that was once considered an industrial waste product and that has recently
436 emerged as a commercially viable commodity.

437

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614

615 **FIGURE CAPTIONS**

616 **Figure 1.** Structure of major hydroxycinnamates, flavan-3-ols and flavonols detected in CBE
617 samples

618 **Figure 2.** Gradient reverse phase HPLC absorbance analysis at 325 nm of CGA compounds
619 in Arabica samples. Peak numbers as listed in Table 1

620 **Figure 3.** Gradient reverse phase HPLC absorbance analysis at 325 nm of CGA compounds
621 in Robusta samples. Peak numbers as listed in Table 1

622 **Figure 4.** Accurate mass fragments for identification of peaks 1, 2 and 4

623 **Figure 5.** Gradient reverse phase HPLC full scan accurate mass analysis of Arabica India
624 flavan-3-ol compounds. Peak number as listed in Table 1

625

Table 1. Peak Numbers, Spectral Properties and Identities of Compounds in Samples Arabica and Robusta Extracts [a]

Peak No	Rt	$[M\text{-}H]^-$ *(m/z)*	**Fragment ions** *(m/z)*	**Compound**
1	2.3	353.088	191.057,179.036	3-*O*-Caffeoylquinic acid
2	4.6	353. 088	191.057	5-*O*-Caffeoylquinic acid
3	4.8	195.087^+	-	Caffeine
4	4.9	353. 088	173.046,179.036	4-*O*-Caffeoylquinic acid
5	5.2	577.135	451,107, 425.091, 407.080,289.071	Procyanidin dimer
6	5.9	289.071	245.083, 205.151	(+)-Catechin
7	7.0	577.135	451,107, 425.091, 407.080,289.071	Procyanidin dimer B2
8	7.3	289.071	245.083, 205.151	(–)-Epicatechin
9	8.8	863.183	575.123	Procyanidin A type trimer
10	8.9	865.198	577.135, 425.091, 407.080	Procyanidin B type trimer
11	9.4	1151.247	n.d.	Procyanidin A type tetramer
12	9.6	1153.262	865.198	Procyanidin B type tetramer
13	9.7	367.105	191,173	4-*O*-Feruloylquinic acid
14	9.9	367.105	191.057	5-*O*-Feruloylquinic acid
15	11.4	609.156	301.035	Quercetin-*O*-rutinoside*
16	11.5	515.120	353.088	3,4-*O*-Dicaffeoylquinic acid
17	11.6	609.156	301.035	Quercetin-3-*O*-rutinoside
18	11.6	515.120	353.088	3.5-*O*-Dicaffeoylquinic acid
19	11.7	463.096	301.035	Quercetin-3-*O*-galactoside
20	11.8	463.096	301.035	Quercetin-3-*O*-glucoside
21	11.9	515.120	353.088	4,5-*O*-Dicaffeoylquinic acid
22	12.1	529.139	367.105, 335.225	3-*O*-Feruloyl-4-*O*-caffeoylquinic acid
23	12.3	529.139	367.105, 335.225	3-*O*-Caffeoyl-5-*O*-feruloylquinic acid
24	12.5	529.139	367.105, 335.225	4-*O*-Caffeoyl-5-*O*-feruloylquinic acid

[a] + Indicates identification in positive ionisation mode; * Same spectral properties as peak 17; n.d., non detected

Table 2: Quantification of Major Compounds Found in Whole Coffee Fruits. Peak Identities Described in Table 1 [a,b]

Compound	Peak	Mexico		India		China	
		Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
3-*O*-Caffeoylquinic acid	1	0.4 ± 0.1 [a]	1 ± 0.1 [b]	1.6 ± 0.1 [c]	0.9 ± 0.1 [bc]	n.d.	0.4 ± 0 [a]
5-*O*-Caffeoylquinic acid	2	18 ± 1.7 [c]	11 ± 0.7 [b]	12 ± 0.4 [b]	9.9 ± 1.4 [b]	0.3 ± 0 [a]	1.6 ± 0.2 [a]
Caffeine	3	5.2 ± 1.2 [b]	8.2 ± 1.7 [c]	1.3 ± 0.1 [a]	7.5 ± 1.5 [bc]	1.3 ± 0.1 [a]	4.9 ± 0.2 [b]
4-*O*-Caffeoylquinic acid	4	1.1 ± 0.2 [c]	1.7 ± 0.1 [d]	2.3 ± 0.1 [e]	1.5 ± 0.2 [cd]	<0.1 ± 0 [a]	0.5 ± 0.1 [b]
4-*O*-Feruloylquinic acid	13	0.1 ± 0 [b]	0.2 ± 0 [c]	0.1 ± 0 [b]	0.2 ± 0 [bc]	n.d.	<0.1 ± 0 [a]
5-*O*-Feruloylquinic acid	14	1.1 ± 0.2 [b]	2.4 ± 0.3 [c]	0.8 ± 0 [b]	2.1 ± 0.3 [c]	0.1 ± 0 [a]	0.2 ± 0 [a]
3,4-*O*-Dicaffeoylquinic acid	16	0.3 ± 0.1 [a]	1.3 ± 0.1 [c]	0.7 ± 0 [b]	1.3 ± 0.2 [c]	<0.1 ± 0 [a]	0.2 ± 0 [a]
3.5-*O*-Dicaffeoylquinic acid	18	2.6 ± 0.5 [a]	2.1 ± 0.2 [b]	1.1 ± 0 [b]	1.8 ± 0.3 [c]	0.2 ± 0 [a]	0.2 ± 0.1 [a]
4,5-*O*-Dicaffeoylquinic acid	21	0.2 ± 0 [a]	1 ± 0.1 [b]	0.8 ± 0.1 [b]	1.3 ± 0.3 [c]	0.1 ± 0 [a]	0.3 ± 0.1 [a]
3-*O*-Feruloyl-4-*O*-caffeoylquinic acid	22	0.1 ± 0 [b]	0.3 ± 0 [c]	<0.1 ± 0 [a]	0.2 ± 0.1 [c]	n.d.	n.d.
3-*O*-Caffeoyl-5-*O*-feruloylquinic acid	23	0.1 ± 0 [a]	0.3 ± 0 [c]	0.1 ± 0 [a]	0.3 ± 0.1 [b]	n.d.	n.d.
4-*O*-Caffeoyl-5-*O*-feruloylquinic acid	24	0.1 ± 0 [a]	0.2 ± 0 [b]	<0.1 ± 0 [a]	0.2 ± 0.1 [b]	n.d.	n.d.
Total major compounds	**-**	**29 ± 3.7 [c]**	**29 ± 3.2 [c]**	**21 ± 0.6 [b]**	**27 ± 4.3 [bc]**	**2 ± 0.1 [a]**	**8.4 ± 0.7 [a]**
Total minus caffeine	**-**	**24 ± 2.7 [b]**	**21 ± 1.6 [b]**	**19 ± 0.7 [b]**	**20 ± 2.9 [b]**	**0.6 ± 0.1 [a]**	**3.5 ± 0.6 [a]**

[a] Results are displayed as mean ± SD (n=3 true replicates), in mg/g; n.d., non detected

[b] Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p<0.05$

Table 3: Quantification of Minor Compounds Found in Whole Coffee Fruits [a,b]

Compound	Peak	Mexico		India		China	
		Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
Procyanidin dimer	5	n.d.	n.d.	n.d.	59 ± 8.1	n.d.	n.d.
(+)-Catechin	6	11 ± 1.9 [a]	27 ± 0.2 [c]	25 ± 2 [bc]	22 ± 3 [b]	n.d.	14 ± 1 [a]
Procyanidin dimer B2	7	4.5 ± 0.8 [b]	n.d.	23 ± 1.8 [d]	1.3 ± 0.2 [a]	9.1 ± 1.4 [c]	n.d.
(–)-Epicatechin	8	7.1 ± 1.3 [a]	n.d.	2.5 ± 0.2 [a]	2.9 ± 0.4 [a]	26 ± 3.8 [b]	n.d.
Procyanidin A type trimer	9	5.3 ± 1 [a]	7.2 ± 0.6 [a]	30 ± 2.4 [b]	80 ± 11 [c]	11 ± 1.7 [a]	n.d.
Procyanidin B type trimer	10	3 ± 0.5 [a]	n.d.	22 ± 1.7 [c]	11 ± 1.5 [b]	26 ± 3.8 [c]	n.d.
Procyanidin A type tetramer	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin B type tetramer	12	0.3 ± 0 [a]	n.d.	4.4 ± 0.3 [b]	n.d.	9.3 ± 1.4 [c]	n.d.
Quercetin-*O*-rutinoside	15	8.9 ± 1.6 [c]	9.5 ± 0.1 [c]	0.5 ± 0 [a]	1.2 ± 0.2 [ab]	2.7 ± 0.4 [b]	n.d.
Quercetin-3-*O*-rutinoside	17	55 ± 9.9 [b]	3.6 ± 0 [a]	2.5 ± 0.2 [a]	2.7 ± 0.4 [a]	3.5 ± 0.5 [a]	3.6 ± 0.3 [a]
Quercetin-3-*O*-galactoside	19	0.5 ± 0.1 [b]	7.9 ± 0.1 [d]	0.2 ± 0 [a]	1.2 ± 0.2 [c]	0.1 ± 0 [a]	0 ± 0 [a]
Quercetin-3-*O*-glucoside	20	34 ± 6.2 [b]	7.8 ± 0.1 [a]	3 ± 0.2 [a]	2.7 ± 0.4 [a]	n.d.	3.8 ± 0.3 [a]
Total PCA [*]	**-**	**31 ± 5.6 [a]**	**34 ± 0.8 [a]**	**107 ± 8.4 [b]**	**176 ± 24 [c]**	**81 ± 12 [b]**	**14 ± 1 [a]**
Total flavonol	**-**	**99 ± 18 [c]**	**29 ± 0.2 [b]**	**6.2 ± 0.5 [a]**	**7.8 ± 1.1 [a]**	**6.3 ± 0.9 [a]**	**7.5 ± 0.6 [a]**
Total	**-**	**129 ± 23 [c]**	**63 ± 1 [ab]**	**113 ± 8.9 [c]**	**184 ± 25 [d]**	**87 ± 13 [bc]**	**21 ± 1.6 [a]**

[a] Results are displayed as mean ± SD (n=3 true replicates), in µg/g; [*] PCA: procyanidin; n.d., non detected

[b] Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p<0.05$

Table 4: Quantification of Major Compounds Found in the Beans Removed From the Coffee Fruits [a,b]

Compound	Peak	Mexico		India		China	
		Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
3-*O*-Caffeoylquinic acid	1	0.9 ± 0.1 b	1.3 ± 0.2 bc	1.5 ± 0.2 c	0.9 ± 0 b	<0.1 ± 0 a	0.2 ± 0.1 a
5-*O*-Caffeoylquinic acid	2	9.7 ± 1.2 b	11 ± 1.4 b	10.1 ± 1.6 b	10 ± 0.5 b	0.1 ± 0.1 a	1.3 ± 0.3 a
Caffeine	3	3.1 ± 0.3 ab	7.9 ± 1.1 e	5.4 ± 1.2 cd	7.2 ± 0.4 de	1.3 ± 0.1 a	4.9 ± 0.3 bc
4-*O*-Caffeoylquinic acid	4	2 ± 0.3 b	2.5 ± 0.4 b	2.7 ± 0.5 b	2.1 ± 0.1 b	n.d.	0.4 ± 0.1 a
4-*O*-Feruloylquinic acid	13	0.1 ± 0 b	n.d.	0.1 ± 0 b	0.2 ± 0 c	n.d.	<0.1 ± 0 a
5-*O*-Feruloylquinic acid	14	0.7 ± 0.1 b	n.d.	0.7 ± 0.1 b	1.8 ± 0.3 c	n.d.	n.d.
3,4-*O*-Dicaffeoylquinic acid	16	0.4 ± 0.1 b	1.2 ± 0.1 c	0.6 ± 0.1 b	1.3 ± 0.1 c	<0.1 ± 0 a	0.1 ± 0 a
3.5-*O*-Dicaffeoylquinic acid	18	0.9 ± 0.1 b	1.7 ± 0.2 c	0.8 ± 0.1 b	1.5 ± 0.1 c	<0.1 ± 0 a	0.2 ± 0 a
4,5-*O*-Dicaffeoylquinic acid	21	0.8 ± 0.1 b	0.8 ± 0.1 b	0.8 ± 0.1 b	1.2 ± 0.1 c	<0.1 ± 0 a	0.1 ± 0.1 a
3-*O*-Feruloyl-4-*O*-caffeoylquinic acid	22	<0.1 ± 0 a	0.1 ± 0 b	<0.1 ± 0 a	0.2 ± 0 c	n.d.	n.d.
3-*O*-Caffeoyl-5-*O*-feruloylquinic acid	23	<0.1 ± 0 a	0.1 ± 0 b	<0.1 ± 0 a	0.2 ± 0 b	n.d.	<0.1 ± 0 a
4-*O*-Caffeoyl-5-*O*-feruloylquinic acid	24	<0.1 ± 0 a	0.7 ± 0.1 b	0.1 ± 0 a	0.9 ± 0.1 c	n.d.	n.d.
Total major compounds	**-**	**18.8 ± 2.3 b**	**27.3 ± 3 b**	**22.9 ± 3.9 b**	**27.4 ± 1.7 b**	**1.5 ± 0.2 a**	**7.2 ± 0.9 a**
Total minus caffeine	**-**	**15.7 ± 2 b**	**19.4 ± 2.6 c**	**17.4 ± 2.8 bc**	**20.2 ± 1.3 c**	**0.2 ± 0.1 a**	**2.3 ± 0.6 a**

[a] Results are displayed as mean ± SD (n=3 true replicates), in mg/g; n.d., non detected

[b] Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p<0.05$

Table 5: Quantification of Major Compounds Found in Coffee Fruit Husks [a,b]

Compound	Peak	Mexico		India		China	
		Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
3-*O*-Caffeoylquinic acid	1	0.1 ± 0 [b]	n.d.	<0.1 ± 0 [a]	0.1 ± 0 [b]	n.d.	n.d.
5-*O*-Caffeoylquinic acid	2	1.9 ± 0.8 [b]	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	1.4 ± 0.8 [ab]	n.d.	n.d.
Caffeine	3	1.3 ± 0.4 [ab]	0.9 ± 0.1 [a]	2.2 ± 0.7 [b]	1 ± 0.5 [a]	n.d.	n.d.
4-*O*-Caffeoylquinic acid	4	0.2 ± 0.1 [b]	n.d.	<0.1 ± 0 [a]	0.2 ± 0.1 [b]	n.d.	n.d.
4-*O*-Feruloylquinic acid	13	0.1 ± 0 [b]	n.d.	<0.1 ± 0 [a]	0.1 ± 0 [b]	n.d.	n.d.
5-*O*-Feruloylquinic acid	14	<0.1 ± 0 [b]	n.d.	<0.1 ± 0 [a]	<0.1 ± 0 [b]	n.d.	n.d.
3,4-*O*-Dicaffeoylquinic acid	16	<0.1 ± 0 [a]	n.d.	<0.1 ± 0 [a]	0.1 ± 0.1 [b]	n.d.	n.d.
3.5-*O*-Dicaffeoylquinic acid	18	0.1 ± 0 [a]	n.d.	0.1 ± 0 [a]	0.2 ± 0.1 [b]	n.d.	n.d.
4,5-*O*-Dicaffeoylquinic acid	21	0.1 ± 0 [b]	n.d.	<0.1 ± 0 [a]	0.1 ± 0 [a]	n.d.	n.d.
3-*O*-Feruloyl-4-*O*-caffeoylquinic acid	22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-*O*-Caffeoyl-5-*O*-feruloylquinic acid	23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-*O*-Caffeoyl-5-*O*-feruloylquinic acid	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total major compounds	**-**	3.9 ± 0.6 [b]	1.1 ± 0.2 [a]	2.6 ± 0.8 [ab]	3.2 ± 1.1 [b]	n.d.	n.d.
Total minus caffeine	**-**	2.6 ± 0.9 [b]	0.2 ± 0.1 [a]	0.4 ± 0.1 [a]	2.2 ± 0.8 [b]	n.d.	n.d.

Results are displayed as mean ± SD (n=3 true replicates), in mg/g; n.d., non detected

Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p<0.05$

Table 6: Quantification of Minor Compounds Found in Coffee Husks [a,b]

Compound	Peak	Mexico		India		China	
		Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
Procyanidin dimer	5	0.3 ± 0.5 ^{a}	n.d.	1 ± 0.5 ^{a}	134.4 ± 1.9 ^{b}	1.8 ± 1.5 ^{a}	n.d.
(+)-Catechin	6	32.4 ± 7.8 ^{c}	n.d.	37.3 ± 15.6 ^{c}	7.7 ± 1.3 ^{ab}	21.1 ± 6.2 ^{bc}	0.1 ± 0.1 ^{a}
Procyanidin dimer B2	7	18.7 ± 1.4 ^{a}	n.d.	60.6 ± 23.4 ^{b}	16.6 ± 0.2 ^{a}	14.4 ± 2.2 ^{a}	0.7 ± 0.6 ^{a}
(–)-Epicatechin	8	17.9 ± 2.6 ^{b}	n.d.	4.6 ± 2.1 ^{a}	n.d.	16 ± 2.5 ^{b}	0.5 ± 0.5 ^{a}
Procyanidin A type trimer	9	32.4 ± 2.9 ^{ab}	n.d.	67 ± 24.4 ^{b}	306.1 ± 52.8 ^{c}	38.4 ± 6.7 ^{ab}	0.1 ± 0.1 ^{a}
Procyanidin B type trimer	10	10.1 ± 0.4 ^{a}	n.d.	62.2 ± 8.1 ^{c}	42.2 ± 5.2 ^{b}	n.d.	n.d.
Procyanidin A type tetramer	11	< 0.1± 0 ^{a}	n.d.	0 ± 0 ^{a}	26.6 ± 2.9 ^{c}	10.7 ± 1.5 ^{b}	n.d.
Procyanidin B type tetramer	12	2.7 ± 0.1 ^{a}	n.d.	19.4 ± 7.4 ^{b}	n.d.	1.7 ± 1.5 ^{a}	n.d.
Quercetin-3-*O*-rutinoside*	15	23.1 ± 9.9 ^{b}	8.1 ± 0.9 ^{a}	3.9 ± 2.2 ^{a}	6.4 ± 3.5 ^{a}	2 ± 0.5 ^{a}	0.7 ± 0.2 ^{a}
Quercetin-3-*O*-rutinoside	17	153.8 ± 62.4 ^{b}	3.7 ± 0.2 ^{a}	16.1 ± 9.2 ^{a}	4.7 ± 2.5 ^{a}	9.8 ± 2.2 ^{a}	2.6 ± 0.5 ^{a}
Quercetin-3-*O*-galactoside	19	1.4 ± 0.7 ^{abc}	2.9 ± 0.3 ^{c}	0.8 ± 0.3 ^{ab}	2.9 ± 1.7 ^{bc}	0.2 ± 0 ^{a}	0.1 ± 0 ^{a}
Quercetin-3-*O*-glucoside	20	82.2 ± 33.4 ^{b}	4.5 ± 0.2 ^{a}	13.1 ± 6.6 ^{a}	5.5 ± 3 ^{a}	4 ± 0.7 ^{a}	1.6 ± 0.3 ^{a}
Total PCA*	**-**	**115 ± 14.2 ^{a}**	**n.d.**	**252 ± 80 ^{b}**	**534 ± 61 ^{c}**	**104 ± 20 ^{a}**	**1.3 ± 1.2 ^{a}**
Total flavonol	**-**	**261 ± 106.4 ^{b}**	**20 ± 1.4 ^{a}**	**34 ± 18 ^{a}**	**19 ± 11 ^{a}**	**16 ± 3.3 ^{a}**	**5 ± 0.9 ^{a}**
Total	**-**	**375 ± 110.8 ^{cd}**	**20 ± 1.4 ^{a}**	**286 ± 93 ^{bc}**	**553 ± 72 ^{d}**	**120 ± 24 ^{ab}**	**6.3 ± 1.6 ^{a}**

[a] Results are displayed as mean ± SD (n=3 true replicates), in µg/g; *PCA: procyanidin; n.d., non detected

[b] Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p<0.05$

Table 7: Total Phenol and Total Flavonoid Content of the Six Whole Coffee Fruit Samples, With Associated Antioxidant Power [a,b]

		Total Phenol (mg/g)	Total Flavonoid (mg/g)	FRAP (µmole/g)
Mexico	***Arabica***	50 ± 4.3 [b]	6.3 ± 1.0 [a]	267 ± 24 [b]
	Robusta	49 ± 5.0 [b]	5.7 ± 1.6 [a]	257 ± 26 [b]
India	***Arabica***	44 ± 4.8 [b]	6.9 ± 0.7 [a]	227 ± 19 [b]
	Robusta	84 ± 18 [c]	7.5 ± 0.6 [a]	386 ± 30 [c]
China	***Arabica***	33 ± 3.0 [ab]	6.4 ± 0.9 [a]	112 ± 17 [a]
	Robusta	16 ± 1.8 [a]	7.3 ± 1.0 [a]	59 ± 4.0 [a]

[a] Results are displayed as mean ± SD (n=3 true replicates)

[b] Differences between samples, for each measurement, are highlighted by a letter (same letter in same column, no difference) at $p<0.05$

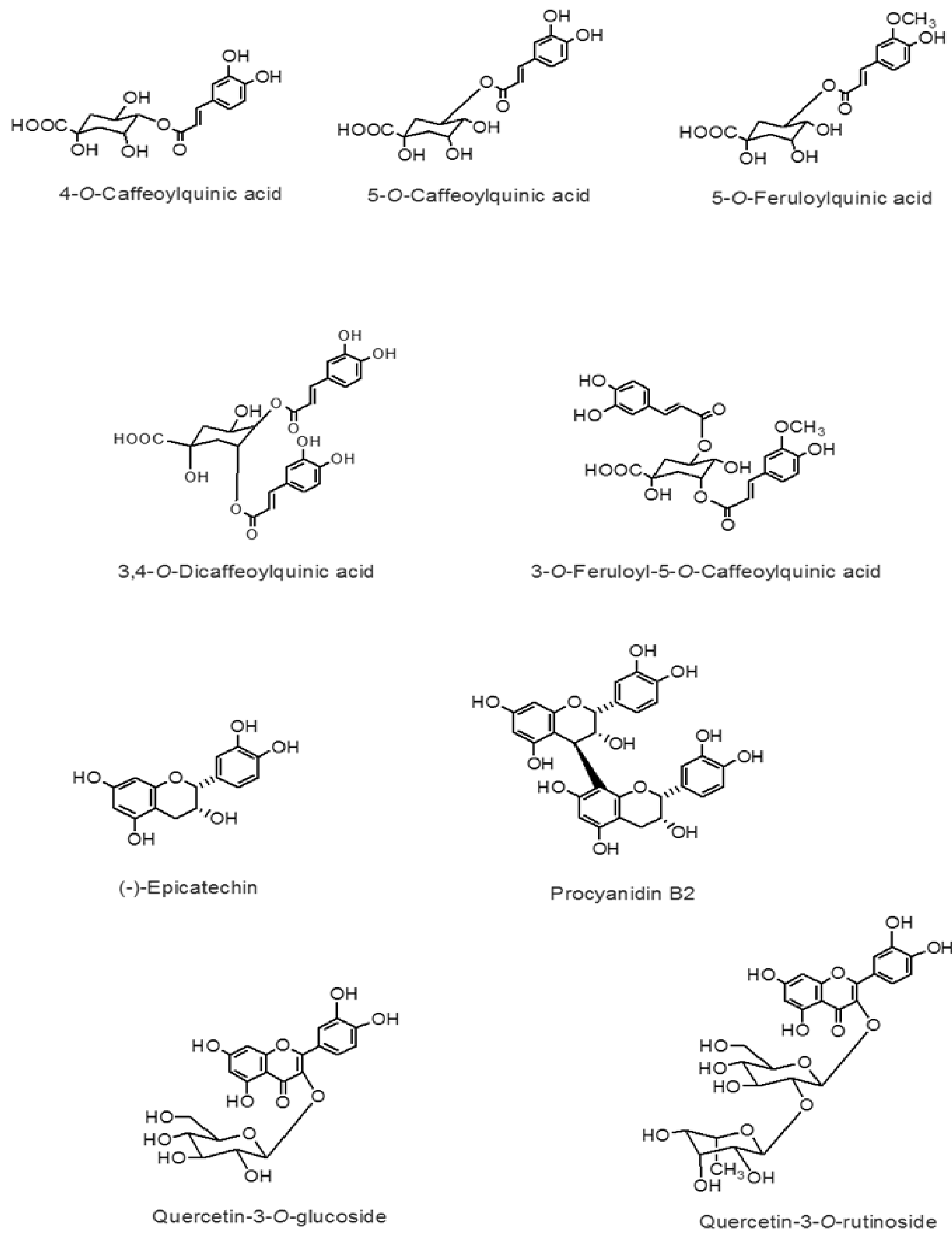

Figure 1

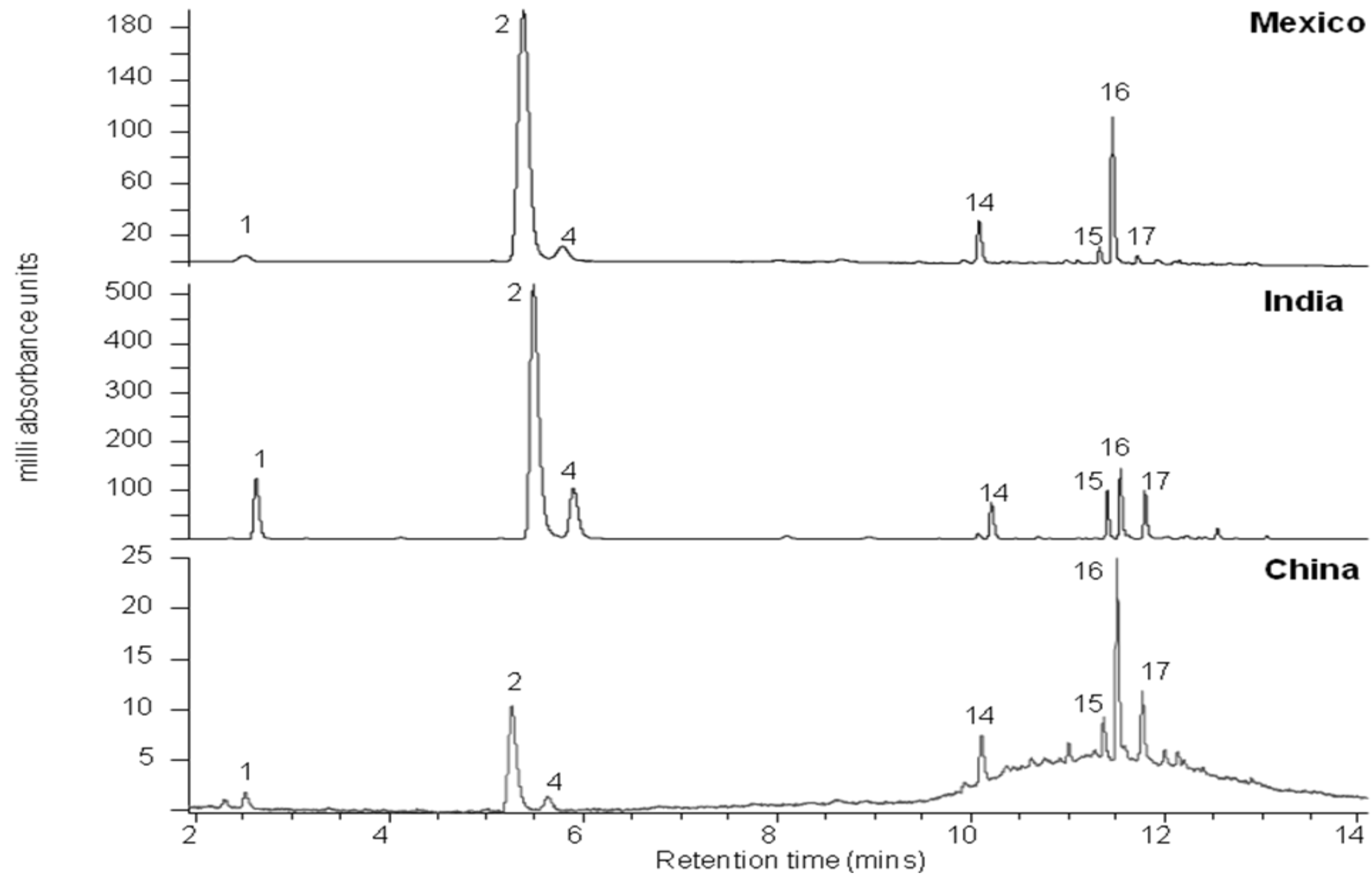

Figure 2

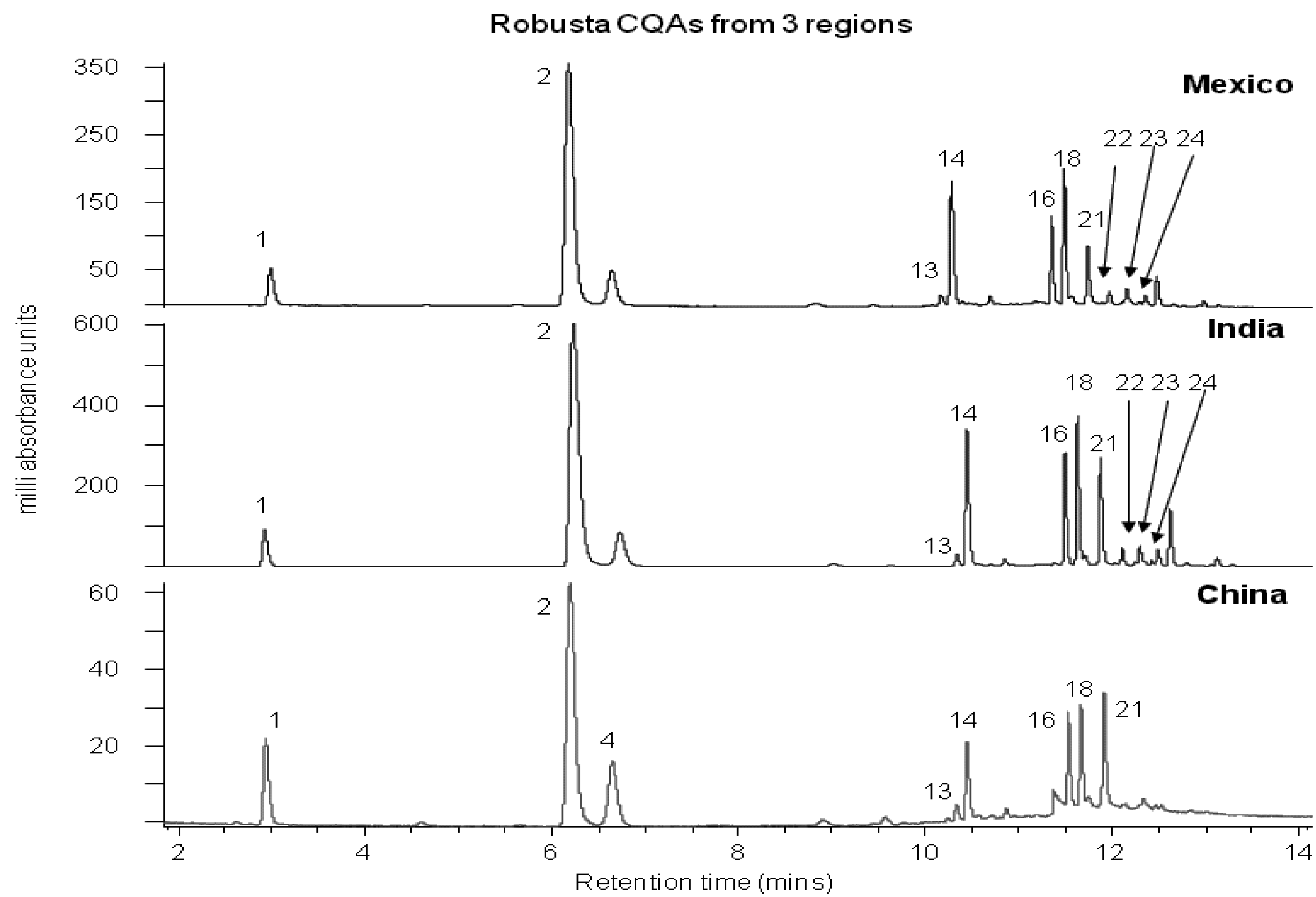

Figure 3

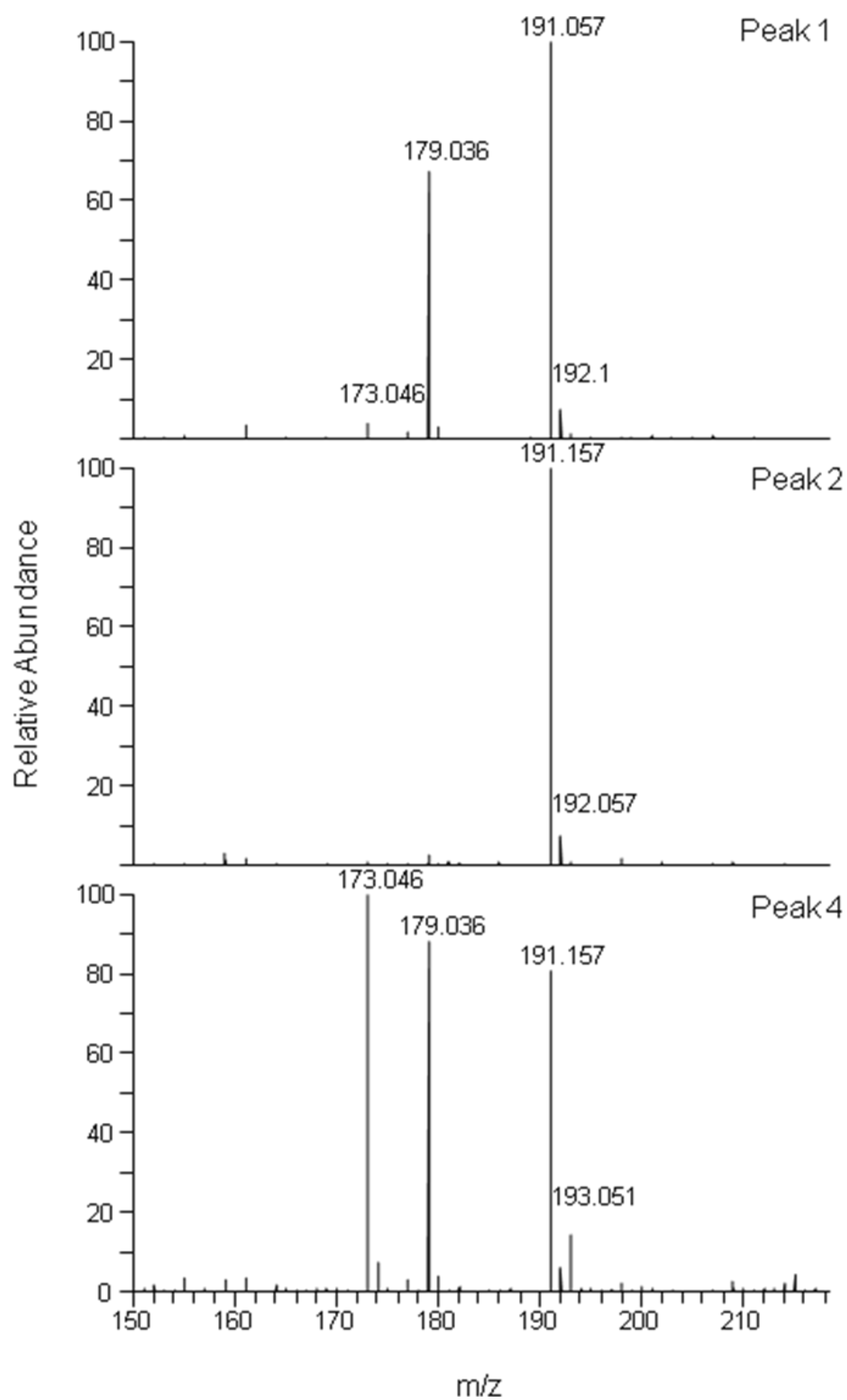

Figure 4

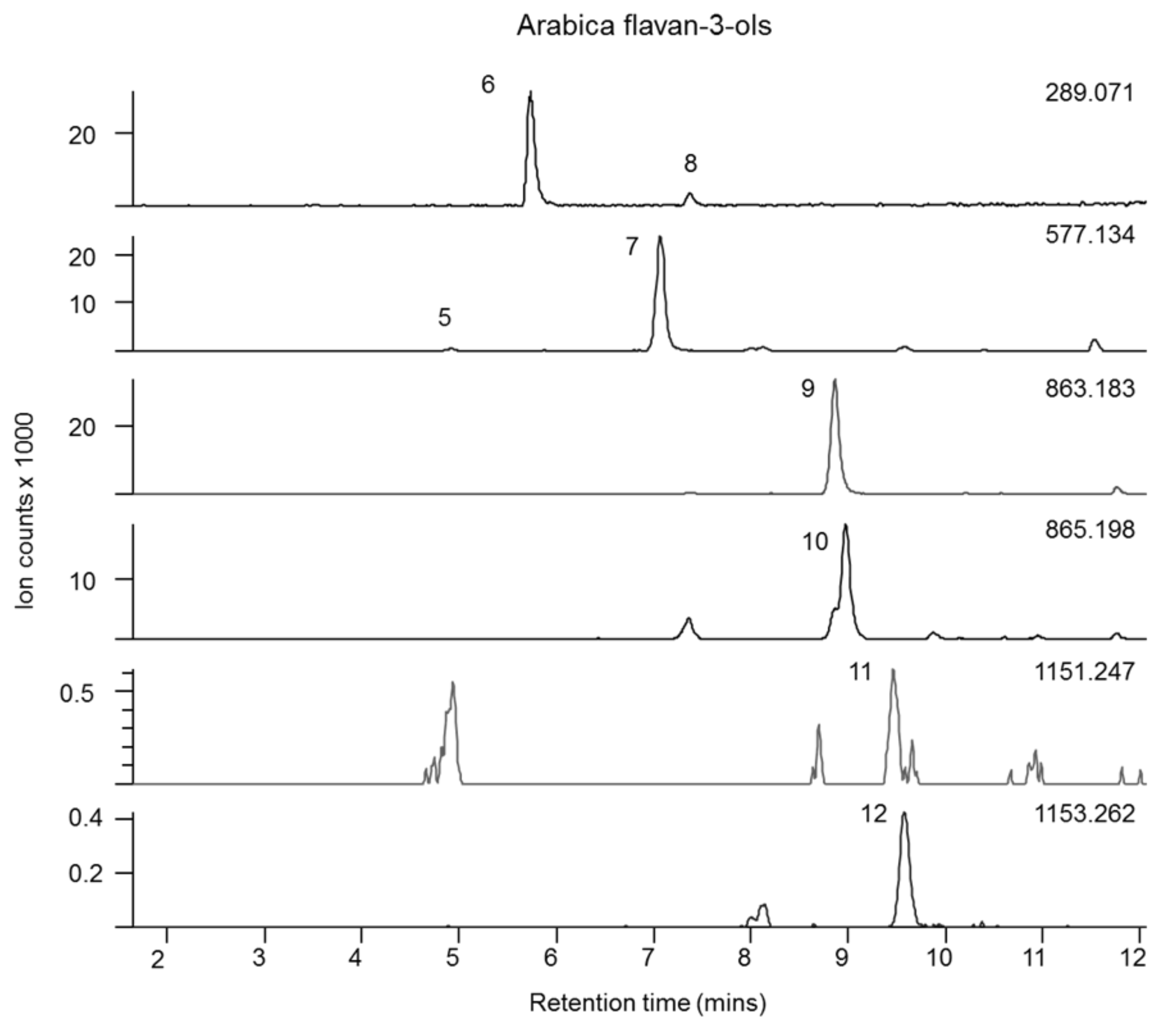

Figure 5

TOC Graphic