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Polyphenolic and Hydroxycinnamate Contents of Whole Coffee Fruits from China, India, and Mexico

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ABSTRACT: Air-dried whole coffee fruits, beans, and husks from China, India, and Mexico were analyzed for their chlorogenic acids (CGA), caffeine, and polyphenolic content. Analysis was by HPLC and Orbitrap exact mass spectrometry. Total phenol, total flavonol, and antioxidant capacity were measured. The hydroxycinnamate profile consisted of caffeoylquinic acids, feruloyquinic acids, dicaffeoylquinic acids, and caffeoyl-feruloylquinic acids. A range of flavan-3-ols as well as flavonol conjugates were detected. The CGA content was similar for both Mexican and Indian coffee fruits but was much lower in the samples from China. Highest levels of flavan-3-ols were found in the Indian samples, whereas the Mexican samples contained the highest flavonols. Amounts of CGAs in the beans were similar to those in the whole fruits, but flavan-3-ols and flavonols were not detected. The husks contained the same range of polyphenols as those in the whole fruits. The highest levels of caffeine were found in the Robusta samples.

KEYWORDS: *chlorogenic acid, caffeine, quercetin, procyanidins, total flavonol, total phenol and antioxidant*

INTRODUCTION

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

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

With a growing belief that dietary polyphenols, hydroxycinnamates, and other phenolics have the potential to provide beneficial effects on health, research has been focusing on developing products with enhanced content by exploiting polyphenols recovered from food waste or byproducts to be used as natural additives. In contrast to green and roasted coffee beans and instant coffees, there are scarce data for the chlorogenic acid contents of soluble green coffee extracts.[8] There is also a lack of knowledge on the impact of important parameters such as the type of coffee fruit, (i.e., Robusta or Arabica) and the geographical origin of the fruit on the range of polyphenolic compounds present in whole coffee fruits. The whole coffee fruits, or cherries (with seed intact), may provide a broad range of phenolic and polyphenolic compounds not found in the beans; additionally, the husks of the coffee fruits, normally discarded as waste products, could be a valuable source of polyphenolic and related compounds. In this study, we analyzed the range of polyphenolic and hydroxycinnamate compounds contained in six whole coffee fruits, grown in 3 different countries (namely, China, India, and Mexico). We also report on the large variation in the polyphenolic and hydroxycinnamate contents identified from the analysis of the separated beans and husks of the whole coffee fruits.

MATERIALS AND METHODS

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

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

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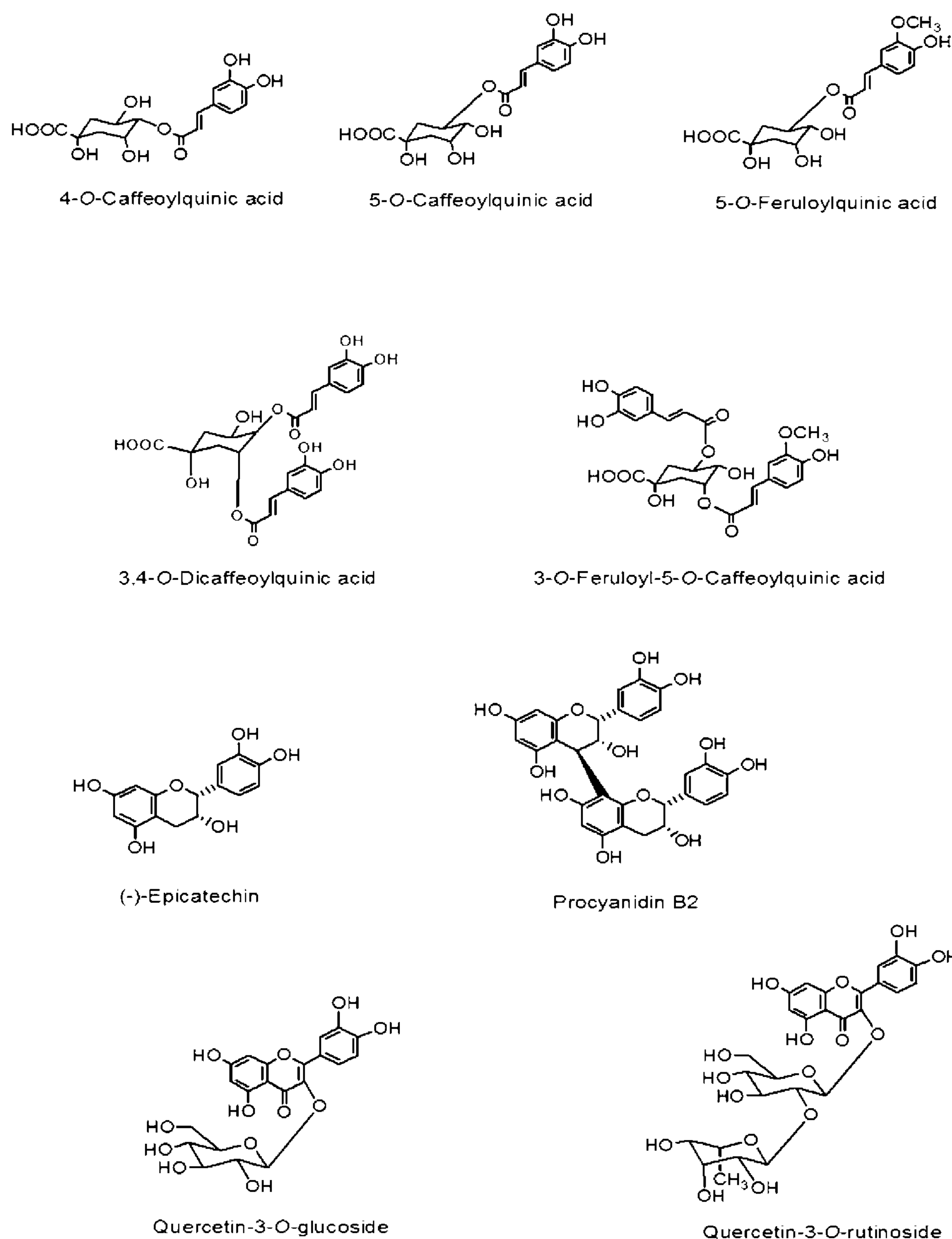

Figure 1. Structure of major hydroxycinnamates, flavan-3-ols, and flavonols detected in CBE samples.

samples (*Coffea arabica* 'Catimor') originated from the Yunnan province (elevation 1400−1600 m above sea level). All coffee fruits, except the Chinese Robusta fruits, were harvested as immature (partially ripe) and ripe cherries; the Chinese Robusta coffee fruits were harvested as unripe and immature (partially ripe) cherries.

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

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

The total flavonoid content of the whole coffee fruits was measured with the aluminum chloride method[10] adapted for a microtiter plate. Briefly, extracts (100 μL) were added in triplicate to the plate, along with 10% aluminum chloride (60 μL), 1 M potassium acetate (30 μL), and dH_2O (95 μL). The reaction was incubated for 30 min at room temperature. Optical densities were measured at 415 nm (Multiskan Spectrum Spectrophotometer, ThermoFisher). The total flavonoid

content of the extracts was quantified as quercetin equivalents (calibration curve linear in the range 0−200 μg/mL).

Antioxidant Power. The antioxidant power was measured in whole coffee fruits using the method of Benzie and Stain[11] adapted for the microtiter plate. The FRAP reagent was prepared fresh daily, by mixing sodium acetate pH 3.6 (25 mL, 300 mM), TPTZ (2.5 mL, 10 mM), and ferric chloride (2.5 mL, 20 mM). The coffee fruit extracts (25 μL) were added in triplicate to the plate, along with the FRAP reagent (225 μL, added to the whole plate within 30 s with a multichannel pipet).

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

HPLC-PDA-Exact Mass-MS. Analysis was carried out on a Thermo Accela HPLC system comprising an autosampler with a sampler cooler maintained at 6 °C, a photodiode array detector scanning from 200 to 600 nm. Samples (5 or 10 μL) were injected onto a 150 × 3.0 mm C_{18} Accucore column (Thermo Fisher Scientific) maintained at 40 °C and eluted with a 5−10−50% gradient of 1% formic acid and acetonitrile at 700 μL/min over 0−10−20 min. After passing through the absorbance detector, the eluant was split, and 200 μL/min was directed to the electrospray interface of an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific). Samples were run in negative ionization mode, and the scan range was from 150 to 1200 amu with resolution set to 60,000.

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

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

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

RESULTS

Identification of Hydroxycinnamic Compounds. Peak numbers, retention time, mass spectral data, and identification of the compounds described are listed in Table 1. The chromatographic separation of the Arabica and Robusta samples from Mexico are shown in Figures 2 and 3.

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

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

peak no.	Rt	$[M - H]^-$ (*m/z*)	fragment ions (*m/z*)	compd
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[b]
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 ionization mode. [b] Same spectral properties as peak 17; n.d., nondetected.

Peak 3 had a λ_{max} at 275 nm and produced no ions in the mass spectrometer. It cochromatographed with the authentic standard of caffeine. Caffeine does not ionize in negative ion mode.

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

Peaks 6 and 8 both produced $[M - H]^-$ ions at *m/z* 289.071. Upon fragmentation, both produced a series of fragment ions at *m/z* 245, 205, and 179 typical of the (+)-catechin/(−)-epicatechin standard.[17] By cochromatography, peaks 6 and 8 were identified as (+)-catechin and (−)-epicatechin.

Peak 9 produced an $[M - H]^-$ ion at *m/z* 863.183. Upon fragmentation, it produced a series of fragment ions at *m/z*

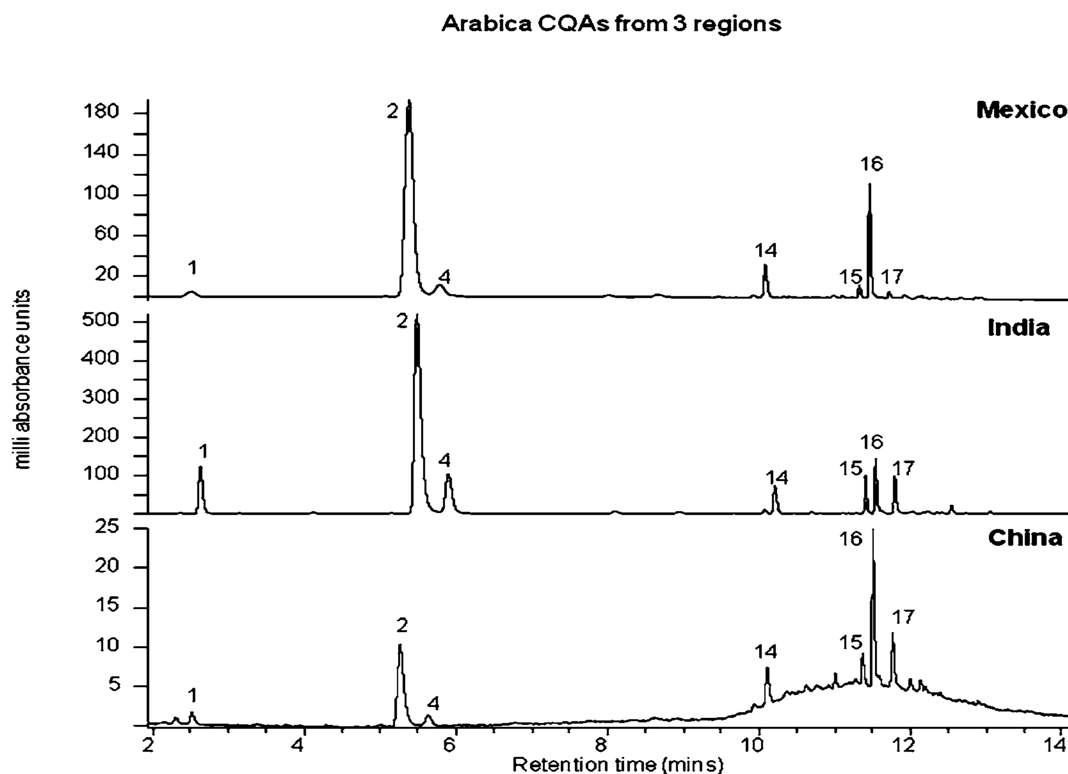

Figure 2. Gradient reverse phase HPLC absorbance analysis at 325 nm of CGA compounds in Arabica samples. Peak numbers are as listed in Table 1.

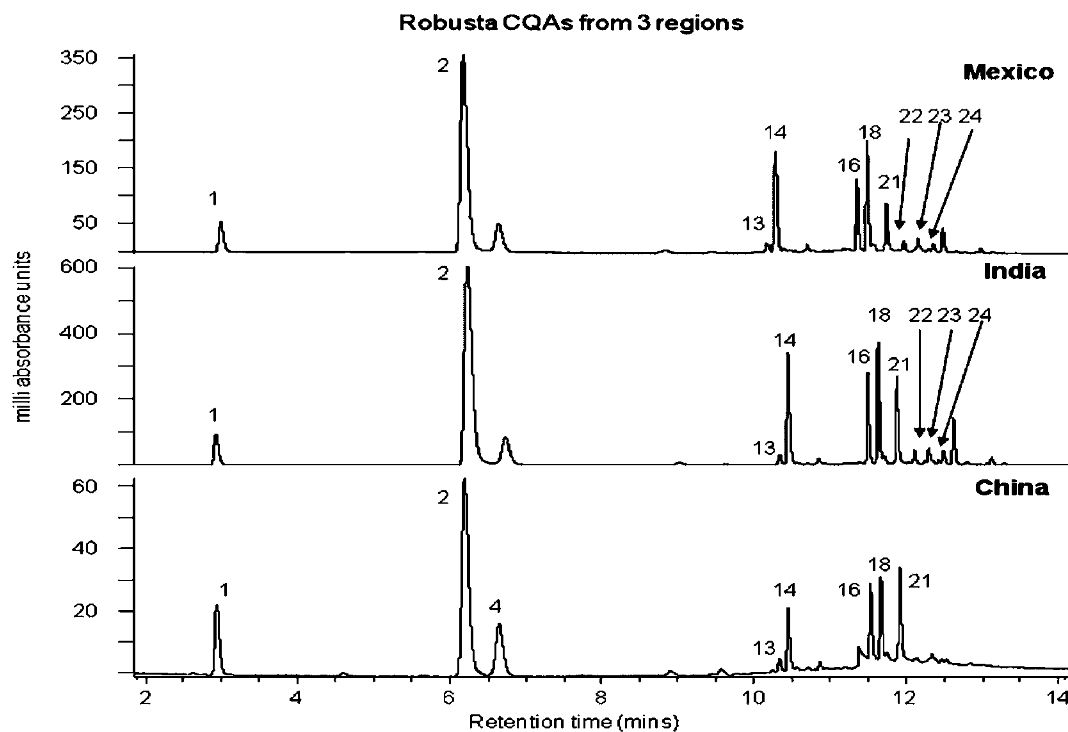

Figure 3. Gradient reverse phase HPLC absorbance analysis at 325 nm of CGA compounds in Robusta samples. Peak numbers are as listed in Table 1.

575.124 and 289.071 typical of an A type procyanidin compound.[18]

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

Peaks 11 and 12 followed the same pattern as that of peaks 9 and 10 but with an $[M - H]^-$ an additional 288 mass units higher at $[M - H]^-$ at m/z 1151.247 and 1153.263. This is typical of the analysis of procyanidin tetramers. Therefore, peak 11 was

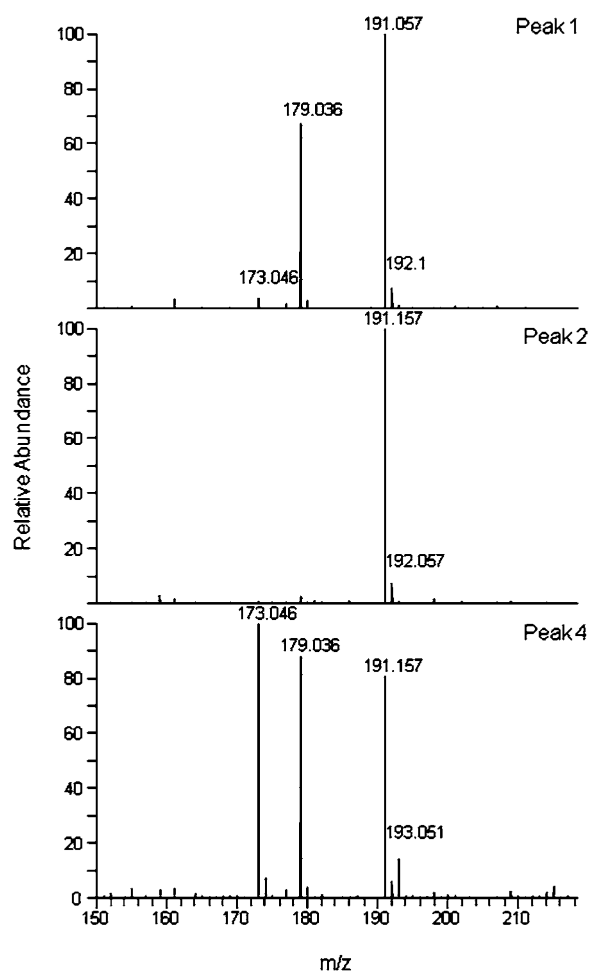

Figure 4. Accurate mass fragments for identification of peaks 1, 2, and 4.

identified as a procyanidin A type tetramer, and peak 12 was identified as a procyanidin B type tetramer.[19]

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

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

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

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

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

Table 2. Quantification of Major Compounds Found in Whole Coffee Fruits[a]

		Mexico		India		China	
compd	peak	Arabica	Robusta	Arabica	Robusta	Arabica	Robusta
3-*O*-caffeoylquinic acid	1	0.4 ± 0.1 a	1 ± 0.1	1.6 ± 0.1 c	0.9 ± 0.1 b,c	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 compds		**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]Peak identities are described in Table 1. Results are displayed as the mean ± SD (n = 3 true replicates), in mg/g; n.d., nondetected. 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]

compd	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[b]		**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 the mean ± SD (n = 3 true replicates), in μg/g. Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p < 0.05$. [b]PCA: procyanidin; n.d., nondetected.

Table 4. Quantification of Major Compounds Found in the Beans Removed from Coffee Fruits[a]

compd	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 c	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 compds		**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 the mean ± SD (n = 3 true replicates), in mg/g; n.d., nondetected. Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p < 0.05$.

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

Quantification of Major and Minor Compounds in the Coffee Samples. Quantification of identified compounds in whole coffee fruits, beans, and husks is detailed as follows. Quantification is by comparison to the most appropriate available standard. However, the quantitative data are for comparison between samples and may not reflect the true levels of compounds present, especially for the procyanindins, which have a wide range of ionization efficiencies.

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

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

The polyphenolic content of the whole coffee fruits was also analyzed using nontargeted high resolution accurate mass analysis. The quantitative data relative to the minor polyphenolic compounds (procyanidins and flavonols) detected in the whole coffee fruit extracts are summarized in Table 3. The result of this investigation presents a more complex picture than the CGA: the main polyphenolics detected were flavan-3-ols and flavonols.

Table 5. Quantification of Major Compounds Found in Coffee Fruit Husks[a]

compd	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 compds		**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.**

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

compd	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[b]		**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 the mean ± SD (n = 3 true replicates), in μg/g. Differences in concentrations between samples, for each compound, are highlighted by a letter (same letter, no difference) at $p < 0.05$. [b]PCA: procyanidin; n.d., nondetected.

Arabica samples from Mexico and Arabica and Robusta samples from India presented the greatest diversity, with 10 compounds identified each, followed by Arabica samples from China (8 compounds), a Robusta sample from Mexico (6 compounds), and a Robusta sample from China (4 compounds). Procyanidins were highest in Robusta samples from India (176 ± 24 μg/g) and lowest in Robusta samples from China (14 ± 1 μg/g). Meanwhile, total flavonols where highest in Arabica samples from Mexico (99 ± 18 μg/g) and lowest in all samples from India and China.

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

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

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

Whole Coffee Fruits: Total Phenol and Flavonoid Contents and Antioxidant Capacity. The total phenol and total flavonoid contents and antioxidant capacity (FRAP) of the coffee fruits are presented in Table 7. Total phenolic content, as well as 5-CQA content, and total hydroxycinnamates were strongly correlated to antioxidant power ($p < 0.001$, r^2 = 0.98, 0.75, and 0.86,

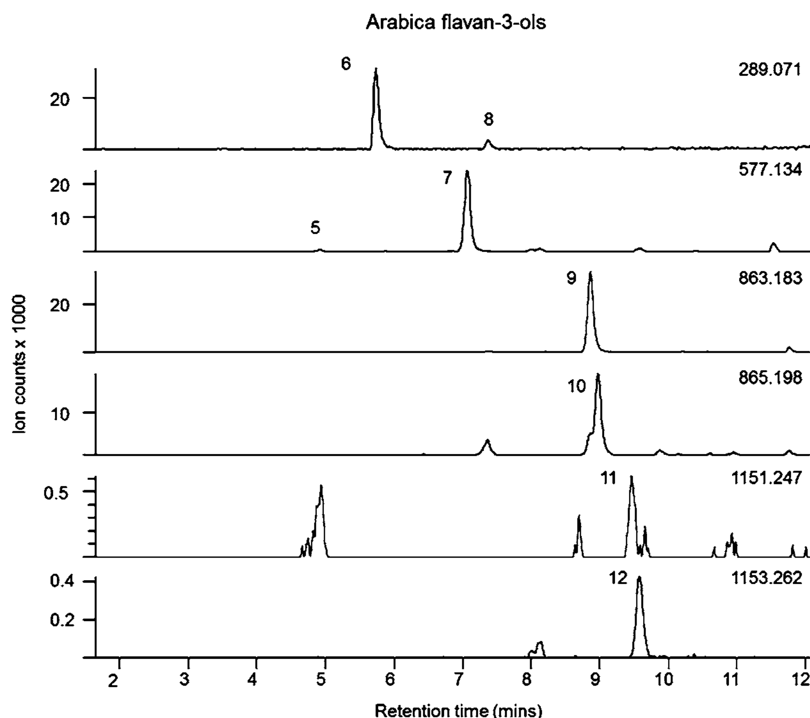

Figure 5. Gradient reverse phase HPLC full scan accurate mass analysis of Arabica India flavan-3-ol compounds. Peak numbers are as listed in Table 1.

Table 7. Total Phenol and Total Flavonoid Contents of the Six Whole Coffee Fruit Samples with Associated Antioxidant Power[a]

		total phenol (mg/g)	total flavonoid (mg/g)	FRAP (μmol/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 the mean ± SD (n = 3 true replicates). Differences between samples, for each measurement, are highlighted by a letter (same letter in same column, no difference) at $p < 0.05$.

respectively). The total flavonoid content did not correlate to the antioxidant capacity. The antioxidant capacity of the Robusta samples from India was far superior to that of all other samples, with both Arabica and Robusta samples from China at the lower end of the scale. There was no statistical difference in the total flavonoid content of the samples analyzed colorimetrically. The total phenol content of the Robusta sample from India was the highest (1.5- to 2-fold higher than that of other coffee samples), in agreement with the FRAP value.

Whole Coffee Fruits: Effect of the Interaction between Country of Origin and Coffee Fruit Type on Polyphenolic Content. A two-way ANOVA analysis revealed significant interactions between country of origin and coffee fruit type on total phenol ($p < 0.01$), 5-CQA content ($p < 0.01$), and antioxidant capacity ($p < 0.01$) but not total flavonoid content, caffeine content, or total hydroxycinnamates.

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

DISCUSSION

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

Difference between These Green Coffees and Roasted Coffees (Impact of Absence of Processing). From a quantitative aspect, the chlorogenic acid profile of these whole coffee fruits is lower than that of a coffee prepared from roasted beans. Typical caffeoylquinic acid content per serving of coffee can range between 70 and 200 mg per 200 mL cup of Arabica and 70−300 mg per 200 mL cup of Robusta.[25] This is assuming that 2 g of coffee powder is used per serving. The simple ethanol/water extraction process used in this experiment was not optimized for the recovery of CGAs. However, it is possible to obtain 400 mg/g using commercial extract processes on the whole coffee fruits.[26] Qualitatively, the main difference is that these whole coffee fruits haves a much simpler CGA range of compounds than roasted coffee, due to their reduced processing. None of the chlorogenic acid lactones were detected in this analysis, either by absorbance detection or by using the mass spectral data. These compounds are formed during the roasting process through elimination of a

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molecule of water from the quinic acid resulting in the formation of a lactone ring.[8] Most noticeable, however, was the presence of a range of flavan-3-ol compounds and flavonols in the μg/g range, similar to concentrations found in a number of different berries and grapes.[27] Procyanidins were previously reported in coffee pulp, in the range of 0.1−1.2% dry basis, depending on the type of fraction extracted and whether the pulp was fresh or dried.[28]

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

Variations within the Bean: Compounds Not Found in the Bean and Compounds Only Found in Beans. It was important to establish the source of flavan-3-ols and flavonols within the whole coffee fruit. The whole coffee fruit samples described above were split into husks and green beans and extracted in the same manner as the whole coffee fruits. This established that the additional minor polyphenolic compounds were contained exclusively in the husks. The CGA pattern of the extracted beans matched well with that found in the whole coffee fruit.

Variations within Husks: Compounds Not Found in Husks and Compounds Only Found in Husks. The variation in both the quantitative and qualitative profiles of the husk samples analyzed was, however, large. Robusta from India had the highest total polyphenolic content of 553.1 μg/g, mainly due to the high level of flavan-3-ol procyanidin A type trimer. However, the flavonol content only accounted for 19.5 μg/g. The Arabica sample from Mexico had the highest level of flavonols, 260.6 μg/g, but only 114.6 μg/g of flavan-3-ols. The husks did contain low levels of CGA and caffeine as well as the additional minor polyphenolics compounds. This is of interest given that coffee husks are generally discarded despite potentially contributing to the diversity of polyphenolic compounds found in coffee fruits.

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

Health. Chlorogenic acids are for many people the main dietary (poly)phenols, and their consumption, absorption, metabolism, and excretion have been extensively studied in volunteers,[31,34−37] animals,[38−42] and in vitro[38,43,44] with a view to determining a possible contribution to health and well-being. Specifically, recent trials have demonstrated the impact of green coffee extract consumption on the regulation of hypertensive state (drop of 10 mmHg in the systolic blood pressure after 12 weeks in a nonoverweight human)[45] potentially mediated by ferulic acid via the muscarinic acetylcholine receptors, as shown in rodents:[46] impact on weight loss and improved lean to fat mass ratio in overweight humans (60-day trial).[47] Potential mechanisms behind the effect of green coffee extract on weight loss, as further examined in a meta-analysis by Onakpoya et al.,[48] may include inhibition of human hepatic glucose-6-phosphatase activity by caffeoylquinic and dicaffeoylquinic acids,[31] an antidiabetic mechanism which may account for the long-term effect of coffee drinking.[49]

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

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

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

Over the years, a number of industries have focused on optimizing their waste handling, with the revalorization of the

waste product. There are a number of other examples of taking a byproduct and changing it from something that costs money to dispose of into a marketable product. The wine industry has developed a market for grape seed and pomace, waste products of the process, which are a powerful source of antioxidant compounds.[58−60] Grape seed extracts have become an opportune and vital business for the wine industry.[61]

Similarly, the olive oil industry has revalorized mill wastewater by evaluating the recovery process of polyphenolic compounds from waste waters. Successful approaches included the use of absorbent vegetable material to recover a range of flavan-3-ols in significant quantities.[62] The phenolic compounds present in olive mill wastewater are major contributors to its toxicity and antibacterial properties. Therefore, extraction of these compounds again produces a marketable product and also reduces the toxicity and pollution of mill wastewater. Apple peel is often a part of the fruit that is discarded during processing, though it can contain the bulk of the antioxidants in the whole apple. As 25−40% of the fruit processed ends up as waste, there is an obvious need to recover and reuse it. A review of the recovery and uses of the carbohydrates and polysaccharides from apple pomace as well as the bioactive molecules such as proteins, vitamin, minerals, and antioxidants is provided by Bhushan et al.[63] The recovery in apple waste of antioxidant compounds was investigated by Tow et al.[64] This investigation also raised the issue of extractable and nonextractable polyphenolics and their potential antioxidant activities and in vitro antiproliferation abilities. The results of the investigation would indicate that there are over 500 mg of gallic acid equivalents per gram dry weight of nonextractable polyphenols compared to only 77 mg/g extractable polyphenols. These polyphenols are thought to be predominantly procyanidins that are bound to the cell wall material. Pistachio nuts are another source of antioxidant compounds and also have a waste product that could be used as a source of polyphenolic antioxidants. In most food products, the nuts are used after removal of the skin. The discarded skins of the pistachio nuts account for about 10% of the total shelled weight of the pistachio.[65] However, the skin accounts for over 90% of the total flavonoid content of the pistachio.

We have analyzed the CGA content of whole coffee fruits and described a simpler profile of compounds present than seen in processed coffee products. We have also shown that the husks of whole coffee fruits can be a potential source of flavan-3-ols and flavonols. The potential content of these compounds in the husks may be greatly underestimated as the extraction efficiency of our process may be as low as 3%, as was reported by Andrade et al.,[66] who focused on the extraction of antioxidants from coffee grounds and husks, reporting the presence of epicatechin and a number of phenolic acids (but no flavonols). Such low extraction efficiency highlights the level of unextractable procyanidin to be recovered from the husks and the scope for alternative extraction methods to be developed. While, at present, a grape-derived extract with high polyphenolic contents may represent a more readily available source (with a lesser nonextractable fraction), the continued development of extraction methods for phenolics in coffee husks and other waste products issued from coffee waste processing offers potential for revalorization as well as reduction of toxic byproducts.[57] Beyond coffee husks only, Murthy and Naidu highlighted the high polyphenol content of other coffee byproducts (including coffee pulp, silver skin, and spent waste). Along with their high phenolic content, coffee byproducts, especially the husk, silver skin, and spent waste, contained high amounts of soluble (8−26%) and insoluble dietary fiber (16−35%).[67] This combined high fiber and antioxidant contents make coffee byproducts attractive for use as nutraceuticals and ingredients in functional foods since combined antioxidant and fiber contents are likely to impact positively on health, including colonic health.[67,68]

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

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Notes

The authors declare no competing financial interest.

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