Arabica and Robusta Coffees: Identification of Major Polar Compounds and Quantification of Blends by Direct-Infusion Electrospray Ionization−Mass Spectrometry

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Ⓢ *Supporting Information*

ABSTRACT: Considering that illegal admixture of robusta coffee into high-quality arabica coffee is an important task in coffee analysis, we evaluated the use of direct-infusion electrospray ionization−mass spectrometry (ESI−MS) data combined with the partial least-squares (PLS) multivariate calibration technique as a fast way to detect and quantify arabica coffee adulterations by robusta coffee. A total of 16 PLS models were built using ESI(±) quadrupole time-of-flight (QTOF) and ESI(±) Fourier transform ion cyclotron resonance (FT-ICR) MS data from hot aqueous extracts of certified coffee samples. The model using the 30 more abundant ions detected by ESI(+) FT-ICR MS produced the most accurate coffee blend percentage prediction, and thus, it was later successfully employed to predict the blend composition of commercial robusta and arabica coffee. In addition, ESI(±) FT-ICR MS analysis allowed for the identification of 22 compounds in the arabica coffee and 20 compounds in the robusta coffee, mostly phenolics.

KEYWORDS: *Coffee, electrospray ionization, FT-ICR MS, QTOF, PLS*

■ INTRODUCTION

Coffee is one of the most consumed beverages in the world and an important commodity for many developing countries. Its world consumption was ca. 132.5 million bags in 2010.[1] There are several species of the genus *Coffea* (Rubiaceae), but the world's commercial coffee come from only two species: *Coffea arabica* L. and *Coffea canephora* var. *robusta*. These species are most commonly known as arabica and robusta coffee, respectively. Arabica beans provide a high-quality brew with intense aroma and a finer taste than robusta, representing approximately 70% of the total world coffee production.[2,3] Arabica beans are also more appreciated by consumers; hence, its market prices are about 2−3 times higher than robusta coffee. For economical reasons, therefore, proof of authenticity and the detection of frauds involving illegal admixture of cheaper robusta coffee beans into high-quality arabica coffee are crucial analytical tasks in coffee analysis.

Different methods are described to distinguish arabica from robusta coffee. Usually, these methods are based on the quantification of chemical markers, such as caffeine, trigonelline, and chlorogenic acids,[4] fatty acids,[5] sugars,[6] and diterpene alcohols.[7] Although these methods seem to provide reliable results, pretreatment steps and elaborated methodologies make them time-consuming and somewhat limited in terms of fraud screening because few components are monitored. Methods based on near infrared (NIR), Fourier transform infrared (FTIR), and Raman spectroscopy analyses have been used to quickly distinguish between coffee varieties according to more comprehensive chemical profiles of nonvolatile compounds.[8,9] In addition, studies in coffee authentication combining NIR and

FTIR with multivariate calibration methods to quantify the content of robusta coffee in arabica are also described in the literature.[10,11] However, information about the chemical composition of the samples and the compounds responsible for differentiation between the varieties is normally not available or poorly described by these methods.

Time-of-flight (TOF) and Fourier transform ion cyclotron resonance (FT-ICR) mass analyzers combined with atmospheric pressure ionization techniques, such as electrospray ionization (ESI), have become one of the most efficient techniques to directly investigate complex natural mixtures. No pre-separation methods and simple protocols for sample preparation are required when direct-infusion ESI is employed. These instruments provide high mass resolving power and mass accuracy and have been widely applied in areas such as metabolomics,[12,13] proteomics,[14] petroleomics,[15] and natural product structure determination.[16]

In this study, we evaluated for the first time the applicability of direct-infusion ESI quadrupole time-of-flight (QTOF) and ESI FT-ICR mass spectrometry (MS) data treated by a partial least-squares (PLS) multivariate calibration technique as a fast method to quantify blends of robusta and arabica coffee, as well as to investigate the identity of the major compounds responsible for the distinction between the coffee varieties by ESI FT-ICR MS.

MATERIALS AND METHODS

Chemicals. Two certified samples of ground and roasted arabica and robusta coffee provided by the Brazilian Agricultural Research Corporation (EMBRAPA) were mixed in different proportions to obtain representative blends. The pure samples and the blends were then subjected to hot-water extraction in a commercial paper filter. Samples with 1 g of pure arabica, pure robusta, and mixtures of 20, 25, 40, 50, 60, 75, and 80% of robusta coffee in arabica were brewed, in triplicate, with 10 mL of ultrapure hot water (temperature of around 90 °C) in a small size cone-shaped filter paper (100% of cellulose fiber) inside a cone-shaped holder. The hot aqueous extracts (1.0 mL) were centrifuged at 13 400 rpm for 5 min using a microcentrifuge (Minispin, Eppendorf), and 100 μL of the upper phase was diluted in methanol/water (1:1) and used for the direct-infusion ESI−MS analysis.

In addition to the certified coffee samples, one unknown coffee blend from a local supermarket and six blends (10, 20, 30, 40, 50, and 70% of robusta coffee in arabica) made by mixing five robusta with six arabica coffee, purchased from different Brazilian coffee vendors, were extracted in duplicate in the same way as described above.

MS. Mass spectra were acquired using a QTOF Micro mass spectrometer (Waters, Manchester, U.K.) with sample introduction performed by a syringe pump (Harvard Apparatus, Pump 11) and an ESI source operating in positive- or negative-ion modes. General conditions were as follows: source temperature of 100 °C, capillary voltage of 3.1 kV, and cone voltage of 30 V. FT-ICR MS data were collected using a 7.2 T LTQ FT Ultra mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a chip-based direct-infusion nanoelectrospray ionization source (Advion BioSciences, Ithaca, NY) operating in positive- or negative-ion modes. General conditions were as follows: capillary voltage of 3.1 kV, tube lens of 140 V, and temperature of 270 °C. Mass spectra were acquired by scanning along the m/z 100−1000 range. Identification of the ions was performed comparing the m/z values obtained by ESI FT-ICR MS with a homemade library of coffee compounds. We considered a match between the experimental m/z value and the theoretical m/z value from our library when the mass error was <3 ppm. An isotope distribution pattern of the ions identified was also considered with the proposed chemical formula.

Data Handling and Statistical Treatment. Data acquisition was performed using the software MassLynx 4.0 (Waters, Manchester, U.K.) and Xcalibur 2.0 (Thermo Scientific, Bremen, Germany) for QTOF and FT-ICR MS, respectively. The abundance readings were normalized to the maximum abundance value, and the 50, 40, 30, and 20 of the most abundant ions were selected and aligned, with their m/z values used to generate four data matrices for each mode of ionization. In these matrices, each line represents a sample and each column represents a variable (m/z value and relative abundances of selected ions). Multivariate analysis was performed by PLS using the software The Unscrambler, version 9.1 (CAMO Software AS). The variables that appeared in less than three samples in each calibration data set were removed and not used in the PLS analysis.[17] To check and compare the performance of the developed PLS models, internal cross-validation, coefficient of determination (R^2), root-mean-square error of calibration (RMSEC), and root-mean-square error of prediction (RMSEP) were used. The regression coefficients of the PLS model were used to indicate the most important variables (ions) in the coffee blend quantification.

RESULTS AND DISCUSSION

Figures 1 and 2 show the ESI FT-ICR MS for the hot aqueous coffee extracts in the positive- and negative-ion mode of ionization, respectively. Although basically the same sets of ions are detected for both arabica and robusta coffee, it is clear from visual inspection that substantial and characteristic differences in their relative abundances occur and are therefore useful for their distinction, as demonstrated below via chemometric
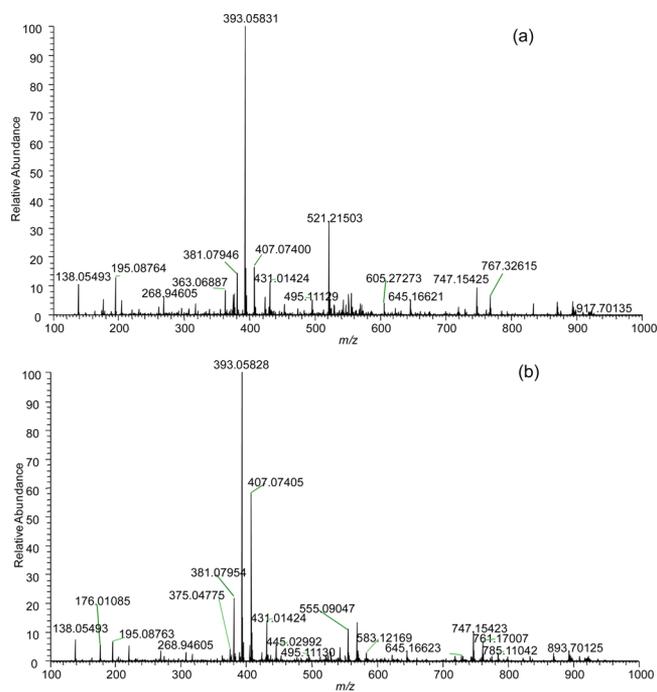

Figure 1. Typical ESI(+) FT-ICR MS for the aqueous extracts of (a) arabica and (b) robusta coffee.
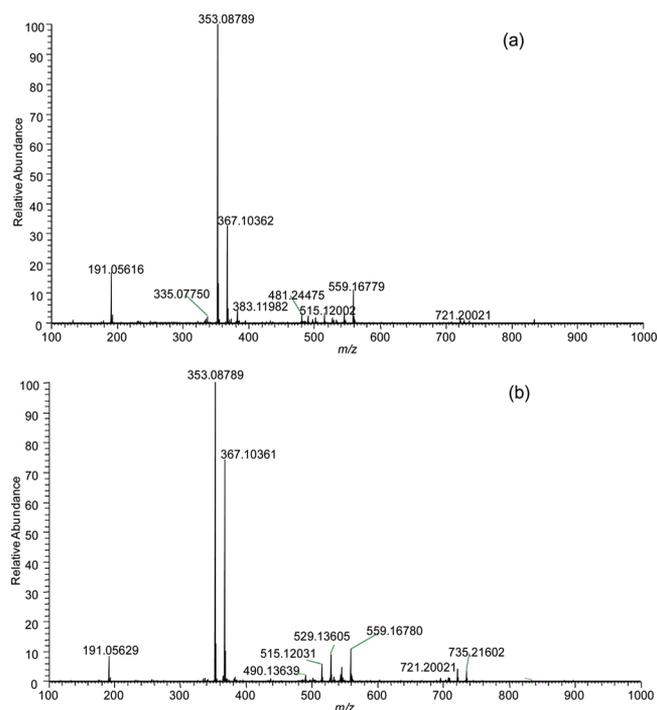

Figure 2. Typical ESI(−) FT-ICR MS for the aqueous extracts of (a) arabica and (b) robusta coffee.

analysis. This profile difference, of ion relative abundance, was also observed in the ESI QTOF analysis.

Quantitative Analysis. Using only the certified arabica and robusta coffee, five samples were run in triplicate as the calibration set (0, 25, 50, 75, and 100% robusta coffee) and four samples were run in triplicate as the test set (20, 40, 60, and 80% robusta coffee) to yield predicted percent compositions. Four PLS regression models were built for each ionization mode (positive and negative) using the m/z values of the 50,

40, 30, and 20 more abundant ions detected by ESI QTOF and ESI FT-ICR MS to verify which model would generate the best robusta and arabica blend percentage prediction, as well as to compare the capability of coffee blend percentage prediction by the two different mass spectrometers.

Tables 1 and 2 show the number of variables retained in each model and the final number of variables after removing those

Table 1. Number of Variables Used in Each PLS Model and Results from the PLS Regressions and Predictions for ESI FT-ICR MS Data

models	ions used	variables retained	final variables	calibration		prediction
				R^2	RMSEC (%)	RMSEP (%)
ESI(+) FT-ICR MS						
1POS	50	176	71	0.993	2.92	3.67
2POS	40	145	55	0.990	3.47	3.79
3POS	30	106	42	0.995	2.50	2.54
4POS	20	74	30	0.991	3.37	3.43
ESI(−) FT-ICR MS						
1NEG	50	124	66	0.985	4.28	9.92
2NEG	40	99	50	0.985	4.28	9.98
3NEG	30	75	38	0.985	4.36	10.33
4NEG	20	43	25	0.985	4.37	10.23

Table 2. Number of Variables Used in Each PLS Model and Results from the PLS Regressions and Predictions for ESI QTOF Data

models	ions used	variables retained	final variables	calibration		prediction
				R^2	RMSEC (%)	RMSEP (%)
ESI(+) QTOF						
5POS	50	150	70	0.997	2.50	20.28
6POS	40	112	56	0.998	1.90	21.00
7POS	30	75	42	0.996	2.76	23.03
8POS	20	50	26	0.984	6.36	17.20
ESI(−) QTOF						
5NEG	50	91	65	0.997	2.50	8.43
6NEG	40	74	53	0.997	2.26	8.94
7NEG	30	54	38	0.997	2.72	8.98
8NEG	20	30	22	0.997	2.56	9.31

that appeared in less than three samples for FT-ICR MS and QTOF data, respectively. Two PLS−latent variables (LVs) were used for each model built with both positive and negative ions detected by FT-ICR MS, and four PLS−LVs were used for each model built with both positive and negative ions detected by QTOF. Tables 1 and 2 also show the summarized results from the PLS regression models.

FT-ICR MS provides a high mass accuracy with mass error <1 ppm, and it is able to reach resolution greater than 100 000 fwhm. Hence, we aligned the FT-ICR MS data with a ±5 ppm mass error range. Their PLS models were built using m/z values with three decimal places. Although the QTOF employed in this work can provide a mass accuracy of around 5 ppm when the lock mass is used, its resolution is only 5000 full width at half maximum (fwhm). It does not possess the adequate high resolving power to analyze direct infusions of complex samples, such as coffee extracts. As a result, we aligned the QTOF data using ±100 ppm mass error range and built their PLS models using m/z values with one decimal place.

The PLS models built with m/z generated by ESI(+) FT-ICR MS showed lower RMSEP (%) than those PLS models built with m/z generated by ESI(−) FT-ICR MS, with therefore better percentage predictions of the robusta and arabica by ESI(+). The opposite was observed by the ESI QTOF PLS models, where the ESI(−) showed lower RMSEP (%) than ESI(+). Among all PLS models, the 3POS (Table 1) built with the m/z values of the 30 more abundant ions detected by ESI(+) FT-ICR MS produced the most accurate blend percentage prediction with a RMSEP value of 2.54%, being considered the best model. All ESI QTOF PLS models showed high RMSEP (%) and could not accurately predict the blend percent composition of robusta coffee in arabica.

Figure 3 shows a plot of predicted versus reference blend percent composition of robusta coffee in arabica for the 3POS
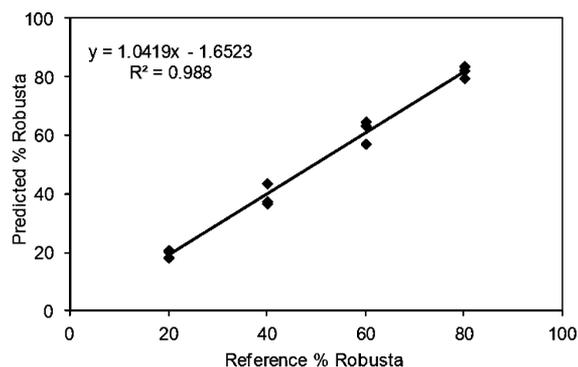

Figure 3. Predicted versus reference blend percent composition of robusta coffee in arabica for the test set of the 3POS PLS model.

model. The R^2 value obtained in this plot was 0.998, showing a very good linear correlation. Prediction errors near to value of 2.54% found in the ESI(+) FT-ICR MS model were also observed in other coffee PLS models using FTIR.[8,11]

Once the best PLS model was found, it was employed to predict the blend composition of commercial coffee. The samples with 20 and 50% of robusta coffee in arabica made by commercial coffee were inserted into the 3POS model to enlarge the coffee variability of the model. Four blends made by 10, 30, 40, and 70% of commercial robusta and arabica coffee were used as the test set. Figure 4 shows the plot of predicted versus reference blend percent composition of the commercial coffee for this modified 3POS model. The RMSEP calculated
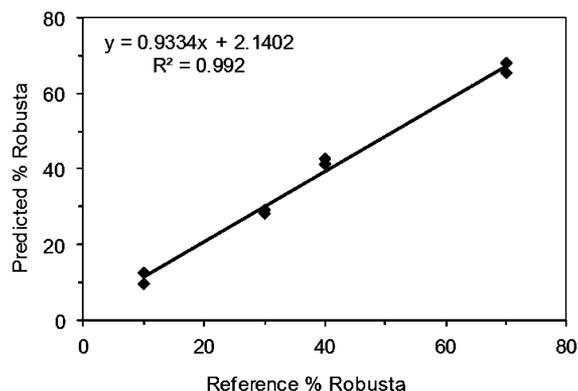

Figure 4. Predicted versus reference blend percent composition for the commercial robusta and arabica coffee samples using the modified PLS model 3POS.

Table 3. Compounds Identified in the Aqueous Extract of Pure Arabica Coffee by ESI(+) FT-ICR MS

			arabica coffee, ESI(+) FT-ICR MS		
compounds	formula	adduct	theoretical m/z	experimental m/z	error (ppm)
trigonelline	$C_7H_7NO_2$	$[M + H]^+$	138.05496	138.05493	0.22
		$[M + K]^+$	176.01084	176.01082	0.11
caffeic acid	$C_9H_8O_4$	$[M + H - H_2O]^+$	163.03898	163.03893	0.31
caffeine	$C_8H_{10}N_4O_2$	$[M + H]^+$	195.08765	195.08764	0.05
quinic acid	$C_7H_{12}O_6$	$[M + K]^+$	231.02655	231.02654	0.04
		$[2M + K]^+$	423.08993	423.09006	−0.31
caffeoylquinic acid	$C_{16}H_{18}O_9$	$[M + H]^+$	355.10236	355.10246	−0.28
		$[M + Na]^+$	377.08430	377.08432	−0.05
		$[M + K]^+$	393.05824	393.05831	−0.18
		$[2M + K]^+$	747.15332	747.15426	−1.26
caffeoylshikimic acid or caffeoylquinide	$C_{16}H_{16}O_8$	$[M + K]^+$	375.04768	375.04778	−0.27
coumaroylquinic acid	$C_{16}H_{18}O_8$	$[M + K]^+$	377.06333	377.06337	−0.11
sucrose	$C_{12}H_{22}O_{11}$	$[M + K]^+$	381.07937	381.07946	−0.24
ferulic acid hexoside	$C_{16}H_{20}O_9$	$[M + K]^+$	395.07389	395.07397	−0.20
feruloylquinic acid	$C_{17}H_{20}O_9$	$[M + K]^+$	407.07389	407.07400	−0.27
acetyl-caffeoylquinic acid	$C_{18}H_{20}O_{10}$	$[M + K]^+$	435.06881	435.06893	−0.28
dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M + K]^+$	555.08993	555.09030	−0.67
feruloyl-caffeoylquinic acid	$C_{26}H_{26}O_{12}$	$[M + K]^+$	569.10558	569.10596	−0.67
atractyloside analogue II	$C_{25}H_{38}O_9$	$[M + K]^+$	521.21474	521.21498	−0.46
atractyloside analogue III	$C_{30}H_{46}O_{10}$	$[M + K]^+$	605.27226	605.27273	−0.78
atractyloside analogue I	$C_{36}H_{56}O_{15}$	$[M + K]^+$	767.32508	767.32615	−1.39

Table 4. Compounds Identified in the Aqueous Extract of Pure Robusta Coffee by ESI(+) FT-ICR MS

			robusta coffee, ESI(+) FT-ICR MS		
compounds	formula	adduct	theoretical m/z	experimental m/z	error (ppm)
trigonelline	$C_7H_7NO_2$	$[M + H]^+$	138.05496	138.05493	0.22
		$[M + K]^+$	176.01084	176.01085	−0.06
caffeine	$C_7H_7NO_2$	$[M + H]^+$	195.08765	195.08763	−0.10
caffeoylshikimic acid or caffeoylquinide	$C_{16}H_{16}O_8$	$[M + K]^+$	375.04768	375.04775	−0.19
caffeoylquinic acid	$C_{16}H_{18}O_9$	$[M + H]^+$	355.10236	355.10243	−0.20
		$[M + Na]^+$	377.08430	377.08447	−0.45
		$[M + K]^+$	393.05879	393.05828	1.30
		$[2M + Na]^+$	731.17938	731.18051	−1.55
		$[2M + K]^+$	747.15332	747.15423	−1.22
coumaroylquinic acid	$C_{16}H_{18}O_8$	$[M + K]^+$	377.06333	377.06342	−0.24
sucrose	$C_{12}H_{22}O_{11}$	$[M + K]^+$	381.07937	381.07954	−0.45
caffeoyltyrosine	$C_{18}H_{17}NO_6$	$[M + K]^+$	382.06875	382.06882	−0.18
caffeoyltryptophan	$C_{20}H_{18}N_2O_5$	$[M + K]^+$	405.08473	405.08492	−0.47
feruloylquinic acid	$C_{17}H_{20}O_9$	$[M + Na]^+$	391.09995	391.10004	−0.23
		$[M + K]^+$	407.07389	407.07405	−0.39
		$[2M + K]^+$	775.18462	775.18588	−1.63
quinic acid	$C_7H_{12}O_6$	$[2M + K]^+$	423.08993	423.09014	−0.50
acetyl-caffeoylquinic acid	$C_{18}H_{20}O_{10}$	$[M + K]^+$	435.06881	435.06896	−0.34
dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M + K]^+$	555.08993	555.09047	−0.97
feruloyl-caffeoylquinic acid	$C_{26}H_{26}O_{12}$	$[M + K]^+$	569.10558	569.10597	−0.69
diferuloylquinic acid	$C_{27}H_{28}O_{12}$	$[M + K]^+$	583.12123	583.12185	−1.06

was 2.76%, which is similar to the previous 3POS model. We also used this modified model to predict the composition of an unknown coffee blend, and the value obtained was 10.44% of robusta coffee in arabica.

The modified PLS model 3POS was built using a restricted number of Brazilian coffee samples and may not well represent the large variability expected for commercial coffee from different origins and processes. However, the clear distinction achieved between both coffee varieties and the quite linear quantification of the blends indicate the reliability of ESI FT-ICR MS to characterize and properly quantify robusta and

arabica coffee blends via rapid analysis of simple hot-water extracts.

Ion Identification. As Tables 3 and 4 summarize, ESI(+) FT-ICR MS data allowed for the identification via chemical composition of 16 compounds in arabica coffee and 14 in robusta with a mass error <2 ppm. The compounds identified in larger amounts for both coffee varieties by ESI(+) FT-ICR MS were, as expected, most abundant polar constituents of coffee aqueous extracts, such as caffeine, trigonelline, caffeoylquinic acid, feruloylquinic acid, and sucrose. The

compounds caffeine and trigonelline were only observed by the ESI(+) mode.

Three diterpene glycosides, namely, atractyloside analogues I, II, and III, were only found in the arabica coffee. These compounds were first found in the aqueous extract of green and roasted coffee in the 1970s.[18−20] The sum of atractyloside analogues appears to be considerably higher in arabica than in robusta beans.[21]

The cinnamoyl−amino acid conjugates caffeoyltryptophan and caffeoyltyrosine were only found in the extract of robusta coffee. The abundance of caffeoyltryptophan in robusta coffee is much higher than in arabica and contributes to the botanical characterization of green coffee beans.[22] Caffeoyltyrosine has not yet been found in arabica coffee, only in robusta.[22−24]

The larger regression coefficients of the PLS model 3POS pointed to feruloylquinic acid, feruloyl-caffeoylquinic acid, caffeoyltryptophan, and dicaffeoylquinic acid as the most important polar compounds for the quantification of the robusta and arabica coffee blends using hot aqueous extracts. These compounds showed higher relative abundances in the robusta coffee compared to the arabica coffee.

The ESI(−) FT-ICR MS data were able to reveal 20 compounds in arabica and 18 compounds in robusta coffee, respectively (see Tables 1S and 2S of the Supporting Information). The polar compounds identified in a larger amount for both coffee varieties by ESI(−) FT-ICR MS were caffeoylquinic acid, feruloylquinic acid, dicaffeoylquinic acid, and quinic acid.

The compounds ferulic acid, dimethoxycinnamoylquinic acid, 3,4-dimethoxycinnamic acid, a hexose, palmitic acid, and stearic acid were only found by the ESI(−) FT-ICR analysis in the arabica coffee. In the robusta coffee, the compounds found were dimethoxycinnamoylquinic acid, ferulic acid, ferulic acid hexoside, a hexose, p-coumaroyl-caffeoylquinic acid, and caffeoylphenylalanine or p-coumaroyltyrosine. These two last compounds are only found in robusta coffee and have been mentioned to be characteristic markers for Ugandan robusta coffee.[22,24] The exact mass obtained (326.10363, $[M − H]^-$) agrees with the structural assignment but is unable to differentiate these two constitutional isomers. This differentiation could be achieved, for example, by liquid chromatography (LC)−MS^n experiments.[25,26]

From both ESI(+) and ESI(−) FT-ICR MS data, a total of 22 compounds could be identified in the arabica and 20 compounds could be identified in the robusta coffee. Several isotopologue ions with very close m/z values were observed in the spectra: for instance, the m/z ion 394.06172 corresponds to $[M + K]^+$ of caffeoylquinic acid ($C_{15}^{13}CH_{18}O_9$; error, −0.30 ppm); the m/z ion 376.05096 corresponds to $[M + K]^+$ of caffeoylshikimic acid or caffeoylquinide ($C_{15}^{13}CH_{16}O_8$; error, 0.19 ppm), the m/z ion 382.08281 corresponds to $[M + K]^+$ of sucrose ($C_{11}^{13}CH_{22}O_{11}$; error, −0.24 ppm); and the m/z ion 408.07734 corresponds to $[M + K]^+$ of feruloylquinic acid ($C_{16}^{13}CH_{20}O_9$; error, −0.22 ppm).

To our knowledge, this is the first report on coffee blend quantification using direct-infusion ESI−MS data. Moreover, ESI FT-ICR MS proved to be suitable for the identification of major polar compounds of coffee aqueous extracts and to quantify blends of the two most common coffee varieties: robusta and arabica.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Chemical structures proposed for the compounds identified in the arabica and robusta coffee by ESI(±) FT-ICR MS (Figure 1S), compounds identified in the aqueous extract of pure arabica coffee by ESI(−) FT-ICR MS (Table 1S), and compounds identified in the aqueous extract of pure robusta coffee by ESI(−) FT-ICR MS (Table 2S). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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