



Mass spectrometry screening of Arabica coffee roasting: A non-target and non-volatile approach by EASI-MS and ESI-MS



Jeane Santos da Rosa^{a,b,*}, Otniel Freitas-Silva^a, Janaina Ribeiro Costa Rouws^c, Iris Gonçalves da Silva Moreira^b, Fabio Junior Moreira Novaes^b, Débora de Almeida Azevedo^b, Nicolas Schwab^d, Ronoel Luiz de Oliveira Godoy^a, Marcos Nogueira Eberlin^d, Claudia Moraes de Rezende^{b,*}

^a Embrapa Food Technology, Avenida das Américas, 29501 Rio de Janeiro, RJ, Brazil

^b Federal University of Rio de Janeiro (UFRJ), Institute of Chemistry, Avenida Athos da Silveira Ramos, 149 Rio de Janeiro, RJ, Brazil

^c Embrapa Agrobiologia, BR-465, KM7, Ecologia, Seropédica, RJ, Brazil

^d ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas (UNICAMP), Rua Josué de Castro 126, Cidade Universitária, Campinas, SP, Brazil

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ABSTRACT

Coffee roasting needs precise control and innovative techniques that are economically viable to monitor and improve its consistency. In this study, mass spectrometry was used as a tool to screen chemical markers that appear on the surface of coffee beans (whole bean) along the roasting process. A non-target and non-volatile approach was used with an ambient technique (EASI) coupled to a single quadrupole mass analyzer to monitor roasting chemical changes in the coffee bean. Green (raw), soft, medium, dark and very dark roasted coffee beans showed a decrease in ions in the range of m/z 500–600, whereas an increase in abundance in the m/z 800–900 range was clearly observed in the most roasted coffees. A multivariate approach through PCA separated the different roasts in 70% of the variance using PC1 and PC2. The major ions in the range of m/z 500–600 were characterized by ESI-MS and also HPLC-fluorescence as the *N*-alkanoyltryptamides, surface constituents of coffee wax layer which are almost fully degraded in darker roasts. The ions in the range of m/z 800–900 were characterized as di- and triacylglycerols and its increase during the roasting process was systematically observed. For these classes of chemical markers of the roasting process, ESI-MS showed also the sodium and potassium adducts with good relative abundances.

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1. Introduction

Coffee is the second global commodity nowadays only behind petroleum and thus plays an important role in world economy. Coffee as a drink is appreciated worldwide and is no longer seen just as a stimulant, but has recognized functional properties and a growing gourmet market. There is a complexity of aromas and flavors in a cup of good quality coffee, which result from the extensive technology employed in planting, harvesting, fruit processing, storing, roasting and even grinding the roasted grain (Dórea & da Costa, 2005; Esquivel & Jiménez, 2012).

From the food processing point of view, coffee roasting consists of a transfer of mass triggered by heating and the consequent endothermic and exothermic reactions. In general, coffee beans are roasted using hot combustion gases that heat the roaster drum (conduction) or hot air (convection) at temperatures that can reach up to 250 °C (Bottazzi, Farina, Milani, & Montorsi, 2012).

The roasted coffee bean has a peculiar shape and its structure is heterogeneous. On heating, its volume swells and the inner structure

collapses, releasing cell contents. The increase in the bean size, number and size of pores and internal cavities and the decrease in weight and density are due to the amount of steam and CO₂ released during the process. Carbon dioxide is the main gas produced during roasting. The CO₂ comes from sugar pyrolysis and secondary reactions from the Maillard reaction. The inside amount of CO₂ is also responsible for the extended shelf life of coffee beans until grinding (Wang & Lim, 2015).

A brief literature review on studies of roasting consistency, reproducibility and quality shows there is a focus on volatile organic compounds (VOCs) or target analytes, mainly caffeine, chlorogenic acids, trigonelline, kahweol and cafestol (kaurene diterpenes) and some sugars. Most of these studies use rapid analytical techniques that are less time consuming in sample preparation, but require more complex analytical systems as mass spectrometry, soft ionization sources that are often employed to provide mass spectra that closely represent the molecular information with low fragmentation. In the same way the use of multivariate analysis techniques such as the Principal Component Analysis (PCA) is almost mandatory (Amorim et al., 2009; Hertz-Schünemann, Dorfner, Yeretian, Streibel, & Zimmermann, 2013; Wieland et al., 2012).

Ambient ionization MS consists of several techniques that are gaining attention in the scientific community due to simplicity of operation and free sample preparation practicality. DESI (Desorption

* Corresponding authors.

E-mail addresses: jeane.rosa@embrapa.br (J.S. Rosa), crezende@iq.ufrj.br (C.M. Rezende).

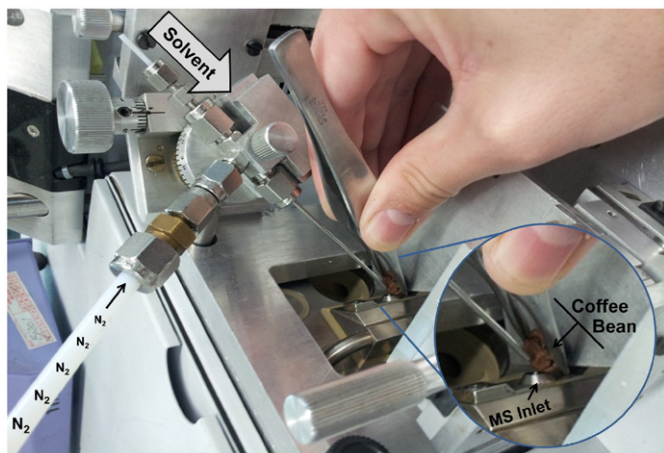


Fig. 1. EASI-MS experiment. Ten beans per sample were taken and evaluated randomly.

Electrospray) in 2004 and DART (Direct Analysis in Real Time) in 2005 started this new age in mass spectrometry analysis (Cody, Laramée, & Durst, 2005; Cooks, Ouyang, Takats, & Wiseman, 2006). These ambient techniques basically produce a flow of ions directly from the sample under investigation to the mass analyzer, requiring no vacuum or special chambers containing a high gas flow. In fact, its principal advantage is the direct analysis with practically no sample preparation. Huang,

Cheng, Cho, and Shiea (2011) presented a very logical and simple approach to the classification of ambient source techniques based on their ionization principles. Thus, three major groups of techniques that lead to the production of ions from target analytes were established: a) direct ionization technique, b) direct desorption/ionization techniques and c) two-step ionization techniques.

In the direct ionization techniques a high electric field induces an electro spray ionization from an analyte solution, solid or droplet. In the direct desorption/ionization techniques, the sample surface suffers an impact of species such as charged solvent droplets, ions and metastable gases. The analyte molecules are then desorbed and ionized from the sample surface. In the two-step ionization techniques, there is first the formation of charged species such as solvents, ions and metastable gases and the formation of analytes of interest through laser irradiation, thermal energy, shock waves or nebulization. The analytes then react with the charged species or metastable gases, producing analyte ions.

The EASI source used in this work is one of the milder ionizations available nowadays and is a direct desorption/ionization technique that uses a voltage-free apparatus (Haddad, Sparrapan, & Eberlin, 2006). EASI hardware is simple and inexpensive as is made up of a Swagelok T-connector, fittings, a fused silica capillary at the solvent entrance and a stainless steel duct at the exit to the sample where nebulization occurs (Fig. 1). No voltage or high temperatures are used and the high-velocity sprayer from nitrogen and charged solvent produce small charged droplets. Soft ionization occurs due to a random distribution of positive/negative ions in such droplets (Harris, Galhena, & Fernandez, 2011).

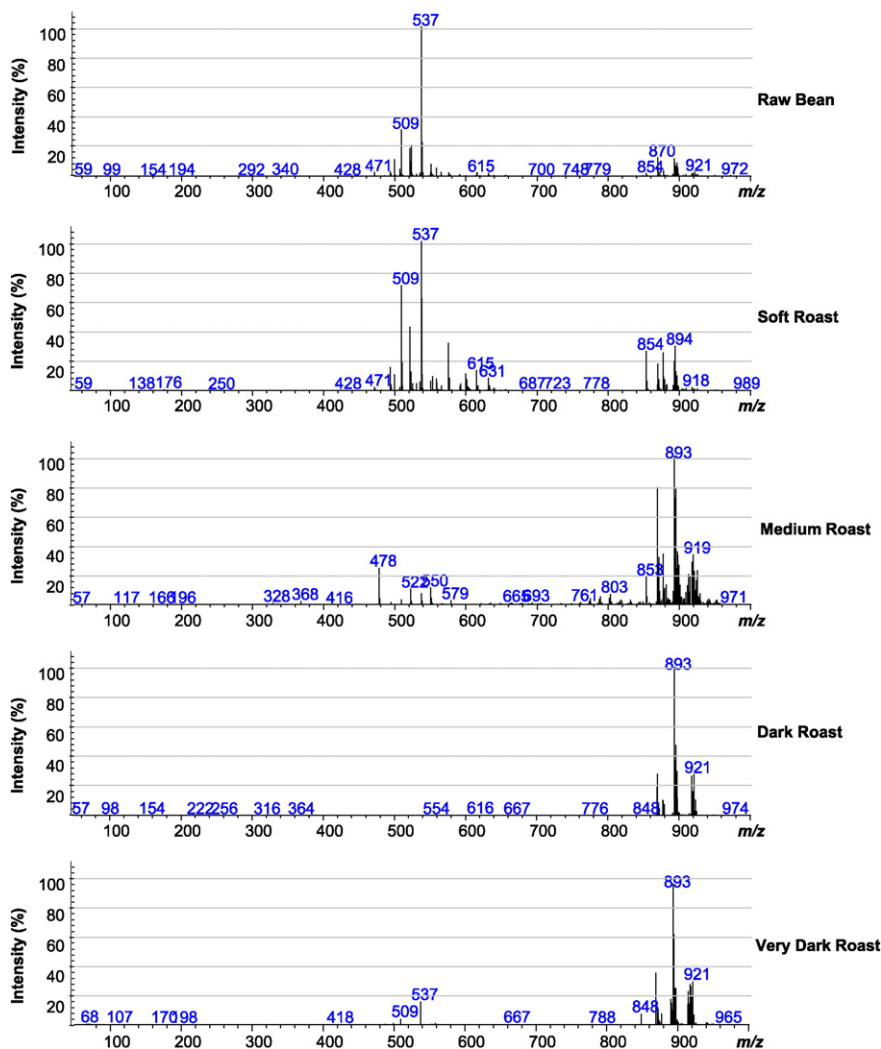


Fig. 2. Mass spectra of beans from Sample 9: green beans (raw), soft, medium, dark and very dark roasts from an EASI-MS experiment in positive ionization mode: EASI(+)-MS.

The aim of this study was to employ non-target compounds techniques to monitor changes in coffee bean surfaces during roasting, using rapid and direct methods in a “press button” way. The goals were to identify roasting levels of the studied samples, establishing a chemical fingerprint of the roast degree and its chemical markers through EASI-MS, and to monitor and suggest the identification of the possible chemical markers by ESI-MS-, using the exact mass of information as the guide.

2. Material and methods

2.1. Coffee blends and roasting process

Three Arabica coffee samples from the South of Minas Gerais state in Brazil were roasted at light, medium, dark and very dark levels. The samples were donated by Minas Sul coffee producer cooperative and

were from plants growing at medium/high altitudes (800 to 1400 m), all the plants were *Coffea arabica* L. cultivars Mundo Novo, Catuaí and Bourbon. The samples were from the 2011 harvest.

The roasting of the samples was performed in a semi industrial roaster at Grão Mestre Café, a training center run by ABIC (Brazilian Association of Coffee Industries). The roasting process was operator-dependent as in the industry. The three coffee blends were identified by their levels of defective beans (50, 15 and 9). Thus, 15 samples were generated after roasting these three samples at the four roasting levels (light to very dark) plus green (raw) beans.

In order to eliminate possible effects of the defective beans, they were removed generating clean samples that were identified as: 050, 015 and 09. All defective beans removed from the samples also made a sample, a pool of defective beans, identified as sample 100. In this way, after roasting at the four levels plus raw beans, twenty new samples were generated. Thus, there were a total of 35 samples evaluated

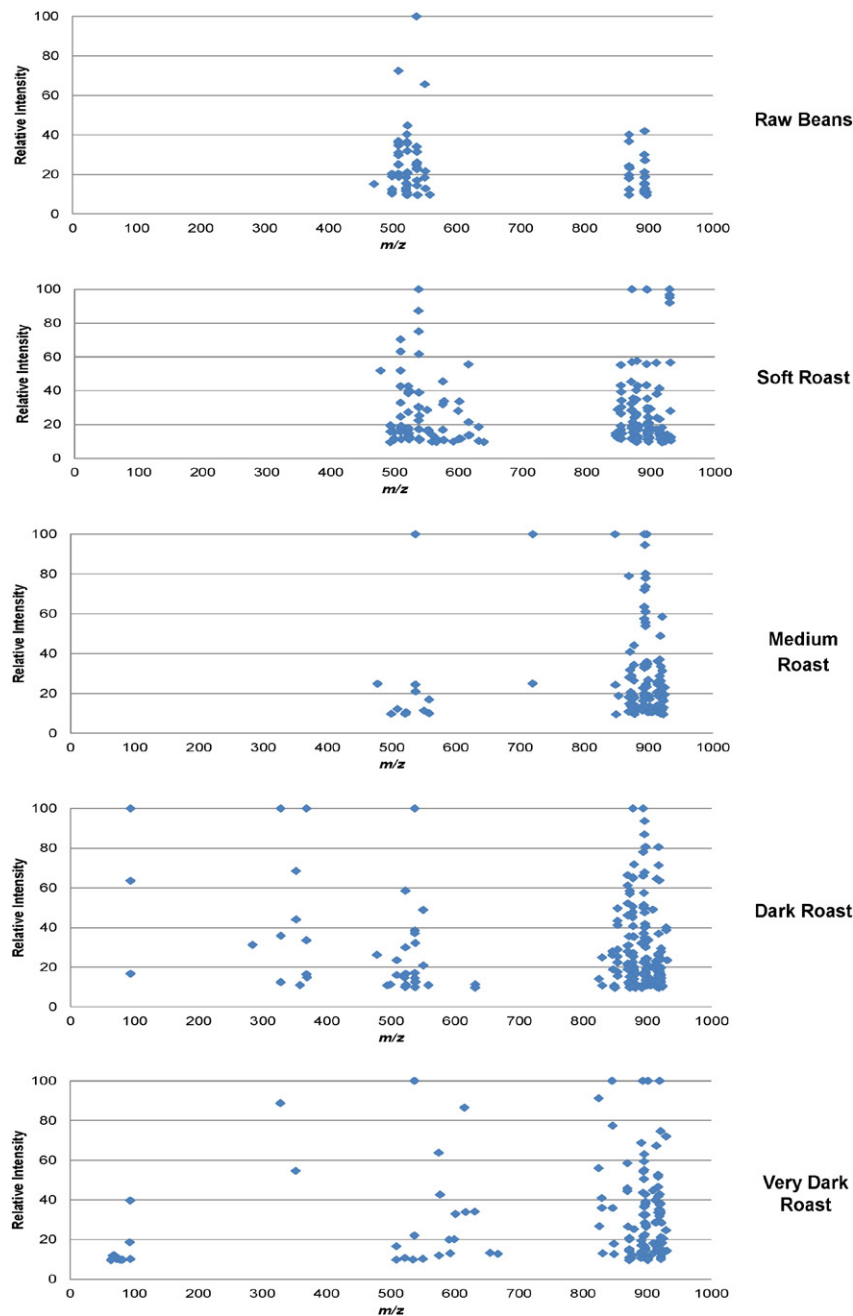


Fig. 3. Ions (*m/z*) distribution versus relative intensity for the roast levels of Sample 9 (9% of defective beans): green (raw) beans, soft, medium, dark and very dark roasts on an (+)-EASI-MS.

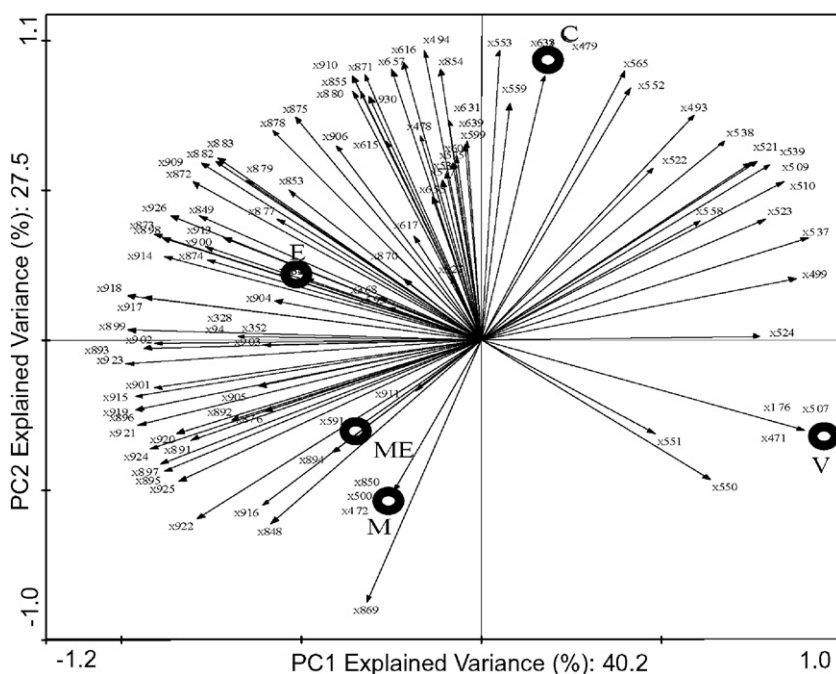


Fig. 4. PCA graph generated for Sample 9 (sample with 9 % of defectives beans) with well-defined roasting regions. Where: V = green beans (raw), C = soft roast, M = medium roasting, E = dark roast and ME = very dark roasting. PC1 × PC2 graphs for the other samples are in the Supplementary material.

by this study. The samples were: 9, 09, 15, 015, 50, 050 and 100 each one with four roasting levels and its green beans (raw). Approximately 500 g of each coffee sample were roasted to the four desired levels. The samples were kept in the dark wrapped in plastic bags.

2.2. Screenings

To establish the roast level and fingerprinting of the roast markers, beans from each sample were placed in front of the mass analyzer entrance, generating mass spectra. The EASI source, operating in the positive mode, was from the the ThoMSon Laboratory, Institute of Chemistry at the University of Campinas and the mass spectrometer was a single quadrupole Shimadzu model 2010A. The coffee bean samples were analyzed by positioning them between the EASI source jet (methanol/formic acid (1%)) under a perpendicular high flow of nitrogen) and the entrance of the mass analyzer.

Ten beans from each sample were evaluated generating three hundred and fifty mass spectra from the bean surfaces. No repetitions were performed for individual bean, but ten different beans per sample were taken and evaluated randomly. This was the maximum number of evaluations possible due to equipment availability. The data were displayed on an Excel® spreadsheet where one hundred and ten significant ions were tabled as variables and their relative abundances. The

data were transferred to the Canoco® program version 4.5 for multivariate analysis. The samples were evaluated separately on their corresponding degrees of roast and defective beans effects were not considered in this study.

2.3. ESI-MS screenings

In order to identify the chemical markers of the roasting levels, screening experiments on ESI-QTOF were performed with 1 g of whole beans. The extracts were made in an ultrasound bath for 1 min with a solution of methanol and 0.1% formic acid (5 mL). A QTOF Waters Synapt® G1 mass spectrometer was used mainly in ESI (+). The experimental conditions were: capillary temperature: 150 °C; desolvation: 40 °C; capillary energy 3.0 kV; cone: 22 V; cone extraction: 7.0 V; N₂ flow: cone: 60 mL/min; desolvation: 300 mL/min and default energy in collision cell. Screenings were performed for 5 min in the direct infusion mode.

2.4. HPLC fluorescence (FLD) and diode array (PDA) analysis of the N-alcanoyl-5-hydroxytryptamides

A HPLC fluorescence (FLD) and a diode array detector (PDA) method were also developed to qualify tryptamide derivatives in the samples. Using the same sample extraction method as for the QTOF screening,

Table 1
Relevant ions found by (+) EASI-MS for the different roast degrees of each studied samples.

Sample	Roast degree				
	Green	Soft	Medium	Dark	Very dark
9	537	553, 493, 615, 853, 657	593, 615	923, 893, 917, 901, 869	869 ^a
09	521, 537, 493, 509, 551, 499	471 ^a	284	923 ^a	893, 877, 919, 898, 853
15	649, 521, 615, 631, 493, 655	849, 853, 877, 899	921 ^a	902, 913, 877	896
015	537, 509, 553	558, 575, 615, 599, 848, 903, 871	893, 919, 94, 915	923 ^a , 911 ^a	923
50	649, 639, 591, 577, 559, 521, 509, 499, 617, 369	916, 912, 899, 927	869 ^a	923 ^a , 877 ^a , 893 ^a	925
050	655, 631, 176, 521, 537	561 ^a	537, 575	877 ^a , 893 ^a , 869 ^a , 918 ^a	869, 479
100	537	930 ^a , 593 ^a , 284 ^a , 911 ^a	853	558, 104	896, 873, 923

^a Ions in PC3 and PC4.

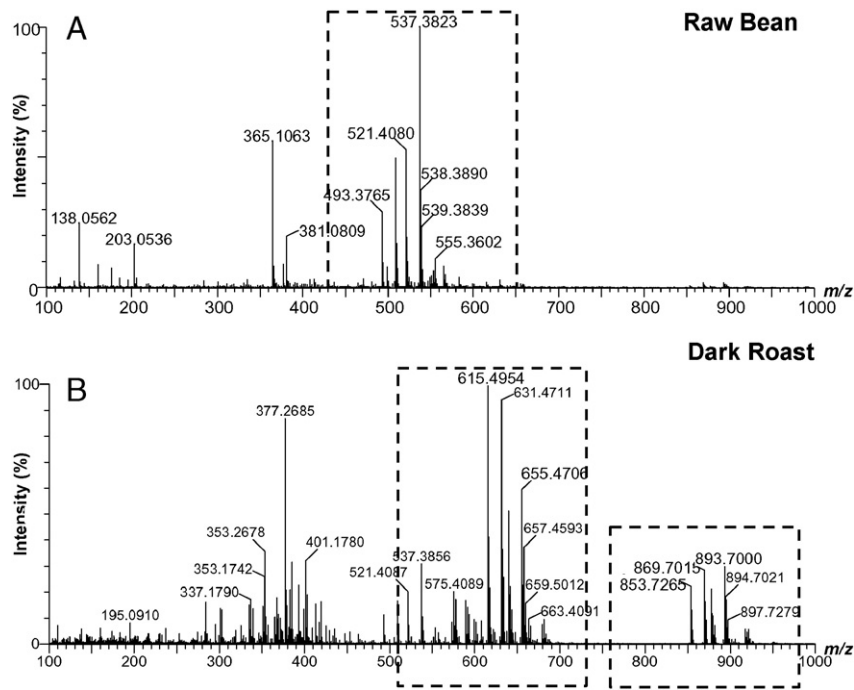


Fig. 5. ESI (+) QTOF spectra of raw (A) and very dark (B) methanolic extracts of the whole coffee bean in dark roast.

samples were injected into a Liquid Chromatograph Waters Alliance® e2695 equipped with a Waters 2475 fluorescence detector (EX = 280 nm and EM = 340 nm). Excitation and emission wavelengths were based on serotonin spectra. Waters 2998 PDA detector was used separately. The standards of C₁₄, C₁₆, C₁₈ and C₂₀5HT were previously synthesized in the Aroma Laboratory of Federal University of Rio de Janeiro (Lang et al., 2009). A Thermo C18 column 5 cm × 4.6 mm and 2.6 μm was used. The injection volume was of 1 μL. A linear gradient was performed with methanol/formic acid of 1%. The initial composition 27/63 changed to 100/0 in 15 min and stayed in this proportion for 25 min. After 30 min the composition returned to the initial conditions.

3. Results and discussion

3.1. Fingerprinting and ion markers of roasting degree from EASI-MS screenings

EASI-MS experiment in a coffee bean surface is shown in Fig. 1 and produced spectra such as the ones showed in Fig. 2. These spectra are from green (raw), soft, medium, dark and very dark roastings and from sample 9 (9% of defective beans) and shows that an increase in roasting degree decreases ions in the m/z 500–600 range whereas increasing ions in the range of m/z 800–900.

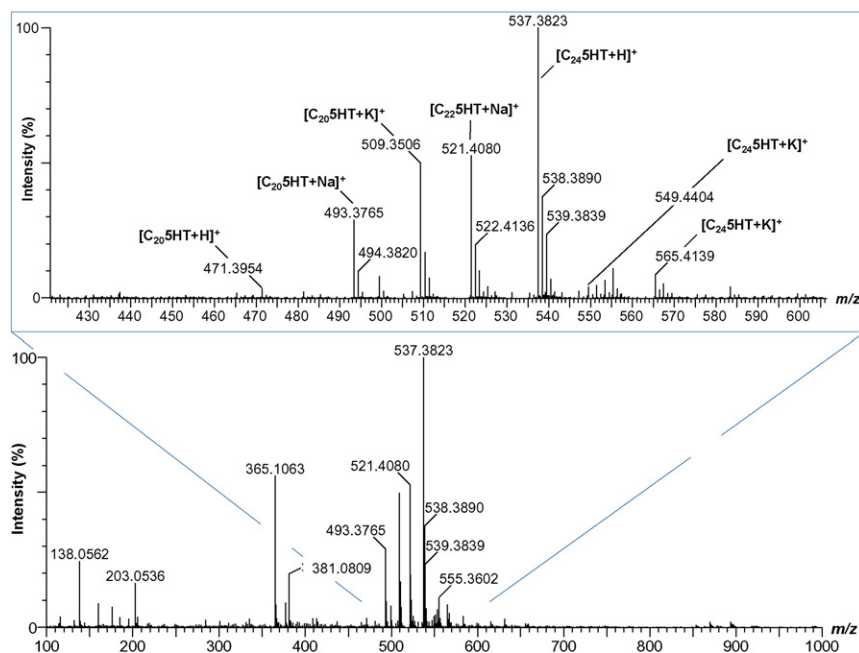


Fig. 6. Zoom in the region of tryptamides on a ESI spectrum of raw beans.

Fig. 3 shows the ions (m/z) versus relative intensity variation in Sample 9 at their levels of roast: green beans (raw), soft, medium, dark and very dark. Each sample represents spectra of 10 beans. This univariate evaluation confirms the tendency of the two major ions groups mentioned above along the roast levels.

A multivariate approach through Principal Component Analysis (PCA) was used to reduce the large number of variables and evaluate the separation of roasting patterns from relevant ions formed on the surface of the coffee beans. Ion peaks with relative intensity below 5% in a sample were considered as noise for the study and were therefore discarded. Another exclusion criterion was when an ion was not present in at least 10 of the 350 generated spectra (Amorim et al., 2009).

Separate roasting level patterns between the four types of roasted coffees and green coffee was found in all samples, thus there is a relationship between the principal component 1 (PC1) and principal component 2 (PC2) in the PCA analysis. The variability of relative intensities for each ion peak found in each sample was cumulatively explained with at least 67.3% for the first two components. The ions loaded in this study can be treated as chemical fingerprints to match ideal/safe coffee roasting levels for the samples studied.

As most of the variance information was found in the first two principal components, the graphs were generated on PC1 and PC2 functions only. Fig. 4 shows an example of the PC1 \times PC2 graph generated for Sample 9 (for the other samples, PC1 \times PC2 graphs are in the Supplementary material), where it is possible to discriminate the roasting level regions and the ions responsible for them. Arrows help show the effect of each ion on the values of the principal components; the greater the length of the arrow the greater the effect. The angles between two arrows indicate the correlation between two ions.

The most relevant ions (in ascending order) per roasting degree for each sample are listed in Table 1. To achieve more ions in some roasts, information of lower relevance in PC3 and PC4 were used. The ions in PC3 and PC4 regions correspond to the smaller arrows in the graph. The selection of ions was also based on the streamlining of isotopic varieties.

The results confirm that ions in the m/z 500–600 range appear mostly in raw (green) beans. In the soft and medium roasts, less of the higher MW species were observed, but they are predominant in the dark and very dark roasts.

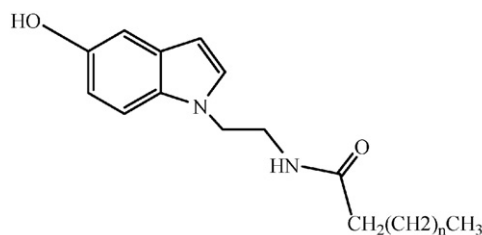
3.2. Chemical markers identified by ESI-MS screenings

In this study, methanolic extracts of samples were injected directly into an ESI-QTOF high resolution mass analyzer in scan mode. Fig. 5 shows two positive ESI-QTOF spectra of raw (A) and very dark extracts (B). The regions highlighted in the spectra have relatively abundant ions that are not well characterized in the literature by similar techniques. In agreement with our previous reports (Amorim et al., 2009), the mass spectra generated by ESI (+) QTOF showed molecules for coffee matrix such as caffeine m/z 195 ($M+H$)⁺; trigonelline m/z 138 ($M+H$)⁺ and m/z 176 ($M+K$)⁺; sucrose m/z 365 ($M+Na$)⁺ and 381 ($M+K$)⁺; chlorogenic acid m/z 377 ($M+Na$)⁺ and 393 ($M+K$)⁺, all related to lower MW fraction.

3.2.1. Scans in the m/z 400–600 region.

Fig. 6 shows a zoom-in of the m/z 400–600 region in a scan of a sample of raw beans. The scale was changed to the ion of highest abundance in the zoom window. The ions marked correspond to the ions identified by exact mass in this study. It is important to notice that continuous calibration of the TOF analyzer (Lock Spray) was used in exact mass measurements. In this region of the spectra, 5-hydroxytryptamide (C5HT) fatty acid derivatives were seen.

Fig. 7 represents the general chemical structure of these substances. The more intense fatty acid derivatives were: *N*- β -arachinoyl-5-hydroxytryptamide (C₂₀5HT) and *N*- β -behenoyl-5-hydroxytryptamide



Where: $n=18 \rightarrow \beta$ -*N*-arachinoyl-5-hydroxytryptamide (C₂₀5HT)
 $n=20 \rightarrow \beta$ -*N*-behenoyl-5-hydroxytryptamide (C₂₂5HT)
 $n=22 \rightarrow \beta$ -*N*-lignoceryl-5-hydroxytryptamide (C₂₄5HT)

Fig. 7. General chemical structure of 5-hydroxytryptamide fatty acid derivatives.

(C₂₂5HT) of m/z 471.3954 and 499.4263, respectively (Garrett et al., 2014; Speer & Kölling-Speer, 2006).

Some ions detected in the tryptamide region remained to be identified for all roast levels and in the raw coffee. The ions with greater peak intensity were of m/z 509.3506; m/z 521.4080 and m/z 537.3823 (the most intense of all). Garrett et al. (2014) suggested identification of the ion of m/z 537 as a diterpene ester of cafestol (palmitate) obtained by $[M+H-H_2O]^+$. The fragmentation pattern obtained for this same ion in the experiments of this study (data not showed), differs from that found by Garrett et al., so it may be another substance.

The ions in the m/z 400–600 range (correlating data with studies from the EASI source) of the roasts studied showed that their peak intensities decreased mainly in the dark and very dark roasts. Despite the discrimination phenomena at the source, this trend may suggest that these analytes are in the wax layer of the bean, partially consumed during the roasting process (Buchmann, Zahm, Kölling-Speer, & Speer, 2009; Lang et al., 2009).

Fatty acid 5-hydroxy tryptamides (C_n5HTs) are serotonin derivatives and the principal constituents of coffee wax (a thin outer coating of the bean). Most stomach friendly coffees have had this layer totally removed by dewaxing processes or partially removed by steam treatment because C_n5HTs (besides caffeine, chlorogenic acids and also di/trihydroxybenzenes from the roasting) can cause irritation in the digestive tract and has an important ulcerogenic potential (Chu, 2012; Farah, 2012). However, C_n5HTs have been the object of important studies for the treatment of Parkinson's disease (Lee et al., 2013). Furthermore, these molecules have an excellent potential to be markers for roasting levels. In fact, the C_n5HT levels drop by approximately 70% during the roasting process and are decomposed to serotonin and then to 5-hydroxyindole derivatives (Rubach et al., 2014; Zahm & Speer, 2009).

In Table 2, the principal m/z and molecular formulas founded in this study are listed for the region of C_n5HTs (Couch & Williams, 1972; Lang et al., 2009; Lang & Hofmann, 2005).

Potassium adducts have not yet been described for fatty acid tryptamide derivatives. All errors found were well below the 5 ppm limit of the equipment.

Table 2
Identification of the more relevant ions founded in the m/z 400–600 for coffee beans.

Experimental m/z	Theoretical m/z	Error (ppm)	Molecular formula	Suggested identification
471.3954	471.3951	0.3	C ₃₀ H ₅₀ N ₂ O ₂	C ₂₀ 5HT (M+H) ⁺
499.4263	499.4264	-0.2	C ₃₂ H ₅₄ N ₂ O ₂	C ₂₂ 5HT (M+H) ⁺
493.3765	493.3770	-1.0	C ₃₀ H ₅₀ N ₂ O ₂	C ₂₀ 5HT (M+Na) ⁺
509.3506	509.3509	-0.6	C ₃₀ H ₅₀ N ₂ O ₂	C ₂₀ 5HT (M+K) ⁺
521.4080	521.4083	-0.6	C ₃₂ H ₅₄ N ₂ O ₂	C ₂₂ 5HT (M+Na) ⁺
537.3823	537.3822	0.2	C ₃₂ H ₅₄ N ₂ O ₂	C ₂₂ 5HT (M+K) ⁺
527.4696	527.4577	-0.2	C ₃₄ H ₅₈ N ₂ O ₂	C ₂₄ 5HT (M+H) ⁺
549.4404	549.4396	-0.8	C ₃₄ H ₅₈ N ₂ O ₂	C ₂₄ 5HT (M+Na) ⁺
565.4139	565.4135	-0.4	C ₃₄ H ₅₈ N ₂ O ₂	C ₂₄ 5HT (M+K) ⁺

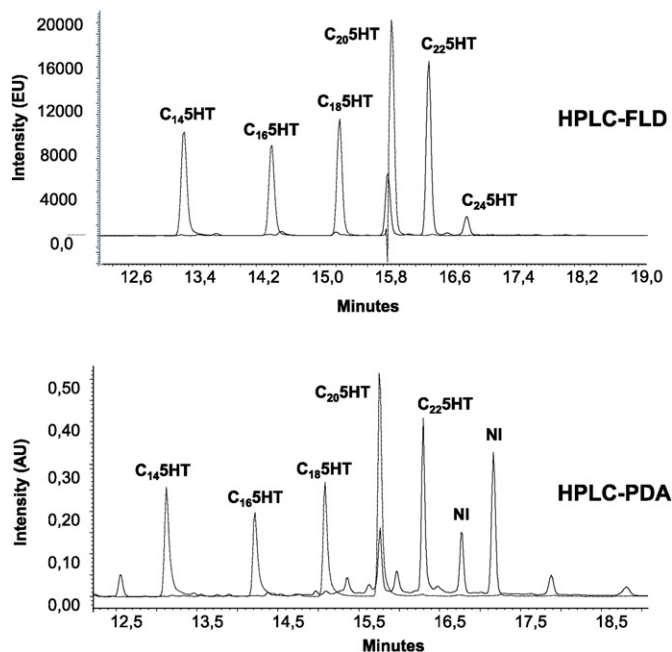


Fig. 8. HPLC chromatograms for FLD and PDA detectors and the qualification of 5-hydroxytryptamide fatty acid derivatives in a light roast sample. The dash lines are from standards synthesized in Aroma Laboratory of Federal University of Rio de Janeiro. Where: NI = not identified.

3.2.2. Verification of 5-hydroxytryptamides fatty acid derivatives by HPLC

Fig. 8 shows the zoomed in and overlay of chromatograms obtained using fluorescence and PDA detectors. The data were not made sequentially. In the fluorescence signal, the dash line is from the tryptamide standards synthesized and the continuous line from an extract of a light roast sample. The spike at the peak of $C_{20:5HT}$ in the standard is due to its higher concentration. The ESI-MS data confirmed the presence of $C_{22:5HT}$ and $C_{24:5HT}$. $C_{n:5HT}$ s were not quantified, but its decrease with elevation of roasting degree was confirmed in the data found by the EASI-MS technique.

In the noisy, less sensitive and less selective PDA signals, there is an overlay between the light roast sample and standard (dash line). In the UV-VIS spectra (in the Supplementary material), it is possible to observe the similarity among $C_{20:5HT}$ profile in standard and in sample. $C_{22:5HT}$ suspect peak in sample also has a similar profile, while $C_{24:5HT}$ could not be observed in the PDA data, probably due to its low concentration. The next relevant vicinal peak has a different spectra profile, and was classified as non-identified (Chattopadhyay, Rukmini, & Mukherjee, 1996; Martins & Gloria, 2010).

3.2.3. Scans in the m/z 600–800 region.

The next important region at the ESI-QTOF mass spectra was from m/z 600 to 900, also observed in EASI-MS, is shown in zoom in Fig. 9. Diacylglycerols (DAGs) were identified in A and triacylglycerols (TAGs) in B spectra. Table 3 lists the principal m/z and molecular formulas for these regions of the spectra.

DAGs and TAGs $(M+H)^+$ species were not found in the spectra. All $(M+K)^+$ ions were more intense than $(M+Na)^+$ ions and LL $(M+Na)^+$ was the least intense ion among them, which can explain the higher error that still is below 5 ppm, the error limit of the equipment.

Segall, Artz, Raslan, Jham, and Takahashi (2005) found thirteen different triacylglycerols in coffee oil. The major ones were PLL (dilinoleoyl-palmitoyl-glycerol – 25.90%), and PPL (dipalmitoyl-linoleoyl-glycerol – 13.74%) and diacylglycerols. They were observed on MS^2 experiments as fragments of TAGs.

Bosco, Toffanin, Palo, Zatti, and Segre (1999) did not find any DAGs in espresso and mocha coffee brews even in strong roasts. Ellis, Brown, in het Panhuis, Blanksby, and Mitchell (2013), reviewed surface MS analysis of lipids and stated that desorption/ionization of TAGs in a DART source resulted in the formation of DAG-like ions, with relative abundance greater than the ions arising from intact TAG molecules, thus making the detection of DAGs originating from tissue matrix difficult.

In this EASI-MS study (a softer ionization source), the samples had been recently roasted and the data showed not only ions in the m/z 600–700 range, but also in the m/z 800–900 range, which were more intense and representative. In the QTOF screenings, the ESI source temperatures and as well as low CID energies stayed at the minimum levels required by the equipment manufacturer. Therefore, it is believed that

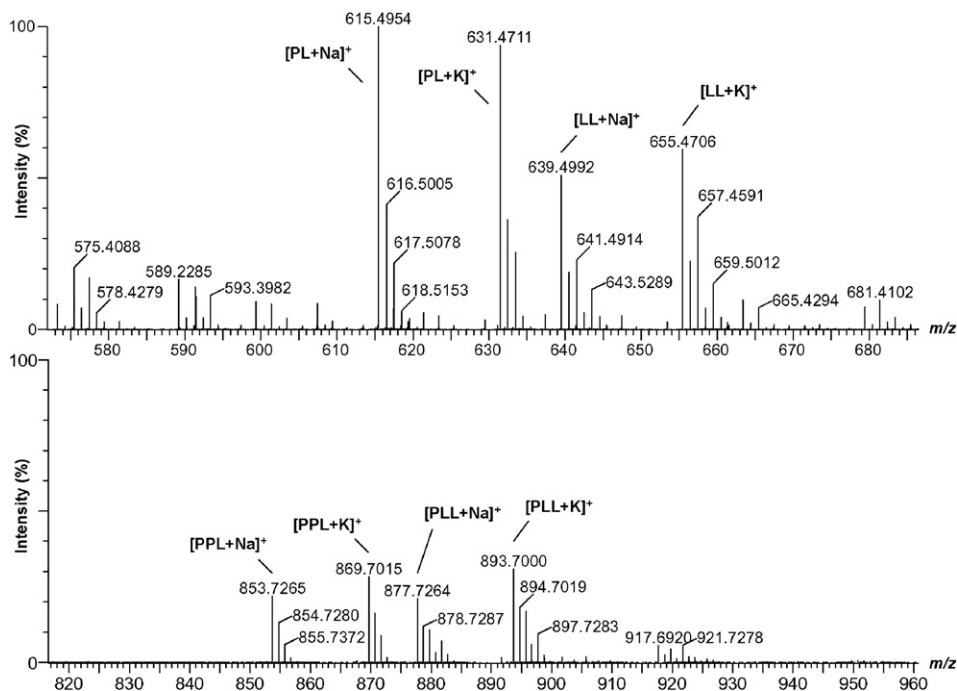


Fig. 9. ESI-QTOF zoom at scans of the acylglycerol region: diacylglycerols (m/z 600–700) and triacylglycerols (m/z 800–900) from a dark roast sample.

Table 3
Identifications suggested for the more relevant ions in the *m/z* 600–800 range.

Experimental <i>m/z</i>	Theoretical <i>m/z</i>	Error (ppm)	Molecular formula	Suggested identification
615.4954	615.4964	-1.6	C ₃₇ H ₆₈ O ₅	Diacylglycerols: palmitoyl-linoleoyl (PL) – (M + Na) ⁺
631.4711	631.4704	1.1	C ₃₇ H ₆₈ O ₅	Diacylglycerols: palmitoyl-linoleoyl (PL) – (M + K) ⁺
639.4992	639.4964	4.4	C ₃₉ H ₆₈ O ₅	Diacylglycerols: dilinoleoyl (LL) – (M + Na) ⁺
655.4706	655.4704	0.3	C ₃₉ H ₆₈ O ₅	Diacylglycerols: dilinoleoyl (LL) – (M + K) ⁺
853.7265	853.7261	0.5	C ₅₃ H ₉₈ O ₆	Triacylglycerols: dipalmitoyl-linoleoyl-glycerol (PPL) – (M + Na) ⁺
869.7015	869.7000	1.7	C ₅₃ H ₉₈ O ₆	Triacylglycerols: dipalmitoyl-linoleoyl-glycerol (PPL) – (M + K) ⁺
877.7264	877.7261	0.3	C ₅₅ H ₉₈ O ₆	Triacylglycerols: palmitoyl-dilinoleoyl-glycerol (PLL) – (M + Na) ⁺
893.7000	893.7000	1.0	C ₅₅ H ₉₈ O ₆	Triacylglycerols: palmitoyl-dilinoleoyl-glycerol (PLL) – (M + K) ⁺

the DAGs found were from the coffee matrix and not from fragmentations of TAGs from the ESI source or collision cells. Novaes, Oigman, de Souza, Rezende, and de Aquino Neto (2015) corroborate this conclusion using a high temperature gas chromatography technique coupled with a mass detector (CGHT-MS) to evaluate DAGs in coffee oil; palmitoyl-linoleoyl (PL) and dilinoleoyl (LL) were the major peaks founded.

Toci, Neto, Torres, and Farah (2013), however, discussed TAG hydrolyses of diacylglycerols (and also monoacylglycerols) during storage of roasted coffees. Therefore, the origin of the DAGs founded in QTOF screening may also be due to TAG decomposition in the coffee matrix. Although some studies have shown that the position of the acyl groups on TAG affects their hydrolysis (Christie, 2011; Michalski et al., 2013), the thermal effects in this study seems to be responsible for the presence of the TAGs and DAGs, because they appear mainly in medium to very dark roasts in samples studied, probably due to the collapse of vegetable cells and the release of lipids onto the bean surface.

4. Conclusions

The use of the direct desorption/ionization EASI-MS ambient technique offers a promising and relatively simple protocol to determine the degree of roast and thus contribute to the reproducibility and consistency of this complex unitary operation which is coffee roasting.

As a non-target study, not all ions monitored could be identified, especially those in roasting fingerprinting by EASI-MS. However, the screenings via ESI-QTOF and HPLC–fluorescence/HPLC–PDA supplemented information, providing identity for the most relevant ions found in both mass spectrometry techniques employed.

As a study focused on non-volatiles the aim was to complement the large number of data available in the literature on the volatile composition of roasted coffee, which is highly dependent on low molecular weight constituents with selective and intrinsic procedures of extraction. Here, we could see the great contribution of the heaviest constituents present in the coffee bean to differentiate between the degrees of roasting most commonly used in coffee market.

In the samples studied, the *N*-alkanoyl-5-hydroxytryptamides were seen as chemical markers for a light roast and that the presence of diacylglycerols and triacylglycerols (higher MW species) can a dark to a very dark roast.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.foodres.2016.03.021>.

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