Food Chemistry 111 (2008) 490–497

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Chemical characterisation of non-defective and defective green arabica and robusta coffees by electrospray ionization-mass spectrometry (ESI-MS)

Juliana C.F. Mendonça [a], Adriana S. Franca [a,*], Leandro S. Oliveira [a], Marcella Nunes [b]

[a] Programa de Pós-Graduação em Ciência de Alimentos, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6623, 31270-901 Belo Horizonte, MG, Brazil
[b] Instituto de Ciências Biológicas/UFMG, Avenida Antônio Carlos, 6623, 31270-901 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 1 December 2007
Received in revised form 8 January 2008
Accepted 31 March 2008

Keywords:
Coffee
Defective beans
Fingerprinting
Chemometrics

ABSTRACT

The coffee roasted in Brazil is considered to be of low quality, due to the presence of defective coffee beans that depreciate the beverage quality. These beans, although being separated from the non-defective ones prior to roasting, are still commercialized in the coffee trading market. Thus, it was the aim of this work to verify the feasibility of employing ESI-MS to identify chemical characteristics that will allow the discrimination of Arabica and Robusta species and also of defective and non-defective coffees. Aqueous extracts of green (raw) defective and non-defective coffee beans were analyzed by direct infusion electrospray ionization mass spectrometry (ESI-MS) and this technique provided characteristic fingerprinting mass spectra that not only allowed for discrimination of species but also between defective and non-defective coffee beans. ESI-MS profiles in the positive mode (ESI(+)-MS) provided separation between defective and non-defective coffees within a given species, whereas ESI-MS profiles in the negative mode (ESI(−)-MS) provided separation between Arabica and Robusta coffees.

1. Introduction

Defective coffee beans have gained much attention in the research community recently due to an increasing awareness regarding the negative aspects they impart to the quality of the roasted and ground coffee used for beverage preparation and consumption (Farah, Monteiro, Calado, Franca, & Trugo, 2006; Franca, Mendonça, & Oliveira, 2005b; Franca, Oliveira, Mendonça, & Silva, 2005a; Mancha Agresti, Franca, Oliveira, & Augusti, 2008; Mazzafera, 1999; Oliveira, Franca, Mendonça, & Barros-Júnior, 2006; Ramalakshmi, Kubra, & Rao, 2007; Vasconcelos, Franca, Glória, & Mendonça, 2007). The intrinsic defects (sour, black and immature beans) are the ones that when roasted contribute the most to the depreciation of the coffee beverage quality. Such defects represent about 15–20% of the total production of coffee in Brazil (Oliveira et al., 2006) and similar amounts are expected in other producing areas around the world (Ramalakshmi et al., 2007). Since to the producers they represent an investment in growing, harvesting, handling and processing, these beans are currently being traded in international markets, thus, contributing to the depreciation of the quality of coffee beverages consumed worldwide. In order to propose alternative profitable uses for these beans, there is an evident need for a rigorous chemical characterization of defective and non-defective (graded) beans that allows for their complete discrimination/differentiation. However, being part of the plant kingdom, their extracts are expected to be highly complex in composition, and analytical methodologies that encompass the diversity of chemicals in an extract should be sought.

Hyphenated techniques, such as gas or liquid chromatography coupled to mass spectrometry, are suitable for the analysis of such complex extracts. However, these techniques usually require a derivatization reaction in order to assure detection and identification of all chemical components in the extract. Hence, an increasing interest is being placed in techniques that do not require chromatographic separation but rather emphasize the recognition of patterns in chemical compositions that will allow for the discrimination of biological materials by species, varieties, maturity stages, geographic origins and other characteristics. These techniques are usually termed 'fingerprinting' (Goodacre, York, Heald, & Scott, 2003) and usually require the employment of chemometrics for a suitable interpretation of the complex chemical profiles of biological extracts. Electrospray ionization mass spectrometry (ESI-MS) is one of such techniques that have only recently been employed for fingerprinting of biological materials (plant extracts, food, beverages, etc.). Cooper and Marshall (2001) were probably the first authors to suggest that ESI-MS in the negative mode could be employed for wine discrimination and classification. Goodacre et al. (2003) demonstrated by application of various chemometric methods that it was possible to discriminate the metabolic fingerprints obtained from unfractionated Pharbitis nil leaf sap by direct

* Corresponding author. Tel.: +55 31 32381777; fax: +55 31 32381789.
E-mail addresses: adrianafranca@ufmg.br, adrianafranca@pesquisador.cnpq.br (A.S. Franca).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.03.080

infusion into an ESI(−)-MS. Araújo et al. (2005) demonstrated the feasibility of discriminating beers from different countries by ESI-MS fingerprinting spectra. Santos, Catharino, Aguiar, Tsai, and Eberlin (2006) applied ESI-MS and chemometrics to provide fast soybean typification by characteristic fingerprinting mass spectra. Liu et al. (2007) utilized HPLC-DAD and LC–MS to develop a fingerprint for the quality of Danshen (roots of Salvia miltiorrhiza) and four related preparations. De Souza et al. (2007) successfully applied direct infusion ESI(−)MS to discriminate Brazilian artisan cachaças (sugarcane spirit) stored in different wood casks and also to monitor changes in chemical composition during the aging period. There are no literature reports on the application of ESI-MS for fingerprinting of coffee.

In view of the aforementioned, the objectives of this work were to employ ESI-MS for the characterization of green Arabica and Robusta coffees and to verify the feasibility of this technique for the discrimination of defective and non-defective coffee beans.

2. Methodology

2.1. General

Arabica and Robusta green coffee samples (2003/2004 crop) were obtained from Samambaia Farm (Santo Antônio do Amparo, Minas Gerais, Brazil). Coffee beans were subjected to selection in an electronic sorter. The beans rejected by the sorting machine were used in this study and are designated as PVA mixture, which stands for "Preto, Verde e Ardido", the Brazilian denominations for black, immature and sour beans, respectively. For the Arabica samples, this mixture consisted in average of 1% black, 12% immature,

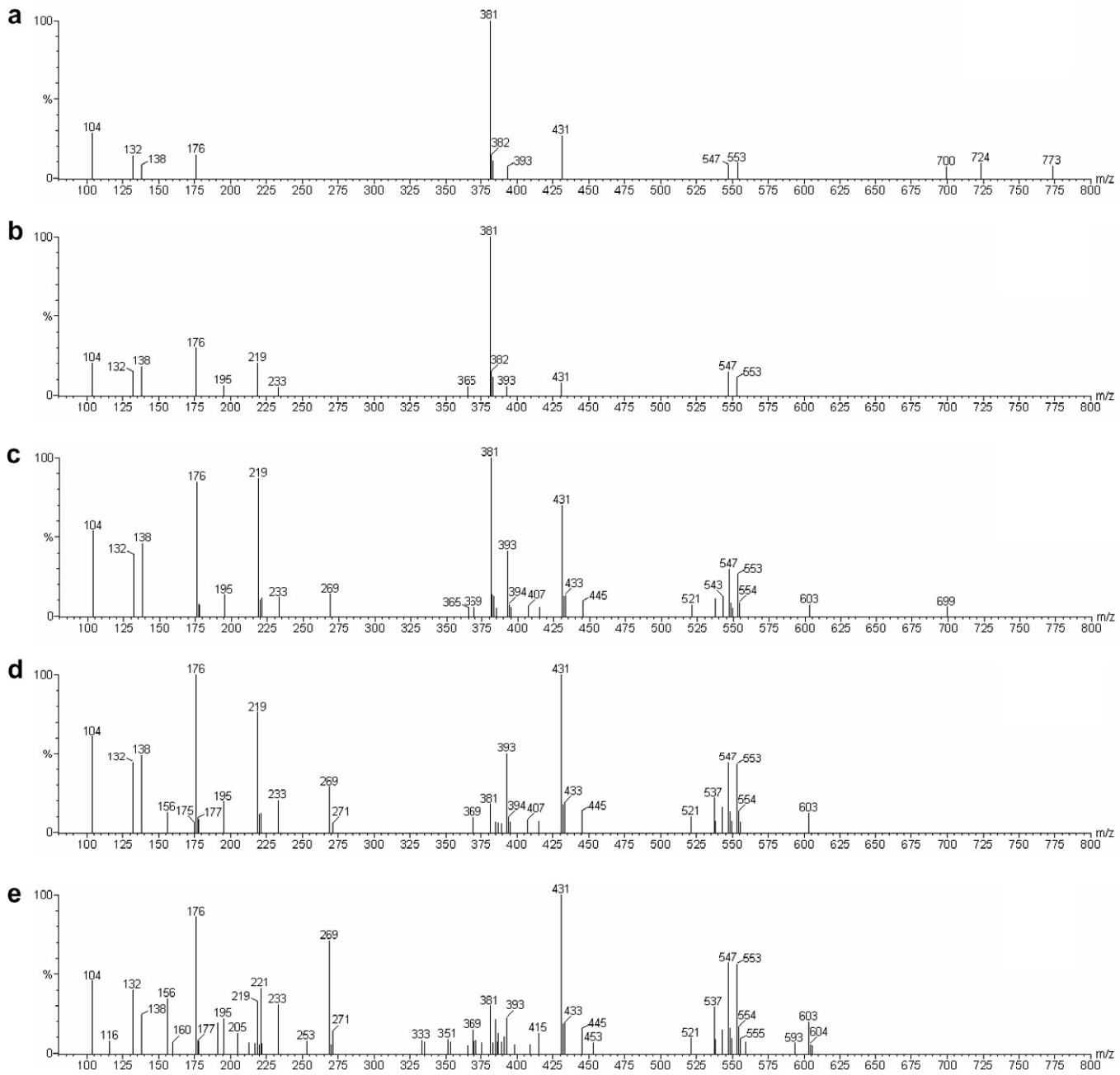

Fig. 1. ESI(+)-MS of Arabica (A) coffee samples: (a) non-defective (ND); (b) immature (IM); (c) light sour (LS); (d) dark sour (DS); (e) black (BL).

20% sour (7% dark coloured and 13% light coloured) and 67% non-defective beans in weight. For the Robusta samples, the PVA mixture consisted in average of 5% black, 25% immature, 51% sour (19% dark coloured and 32% light coloured) and 19% non-defective beans in weight. Black, sour (light and dark), immature and non-defective beans were manually separated from the Arabica and Robusta PVA mixtures to constitute five sampling lots (non-defective, black, immature, dark sour and light sour) for each coffee species (Arabica, Robusta).

2.2. Electrospray-mass spectrometry (ESI-MS) analysis

Ground coffee samples were defatted by ether extraction for 6 h in a Sohxlet apparatus (Tecnal, São Paulo, Brazil). In view of a possible thermal degradation of potential chemical markers such as carbohydrates during hot water extraction, some preliminary tests were performed in order to verify extraction efficiency. Water extraction at 90 °C for 15 min, which is the methodology commonly used in studies employing coffee samples, was compared to extraction in an ultrasonic bath at ambient temperature (Mecozzi, Amici, Pietrantonio, & Acquistucci, 1999). Tests were performed for both non-defective and black coffee beans, since such samples are the ones with the highest and lowest carbohydrate concentrations, respectively. No significant differences were observed in terms of extraction efficiency, but the ultrasound extraction resulted in slightly higher concentrations of the detected compounds, and therefore such methodology was selected. Also, tests were performed for extraction

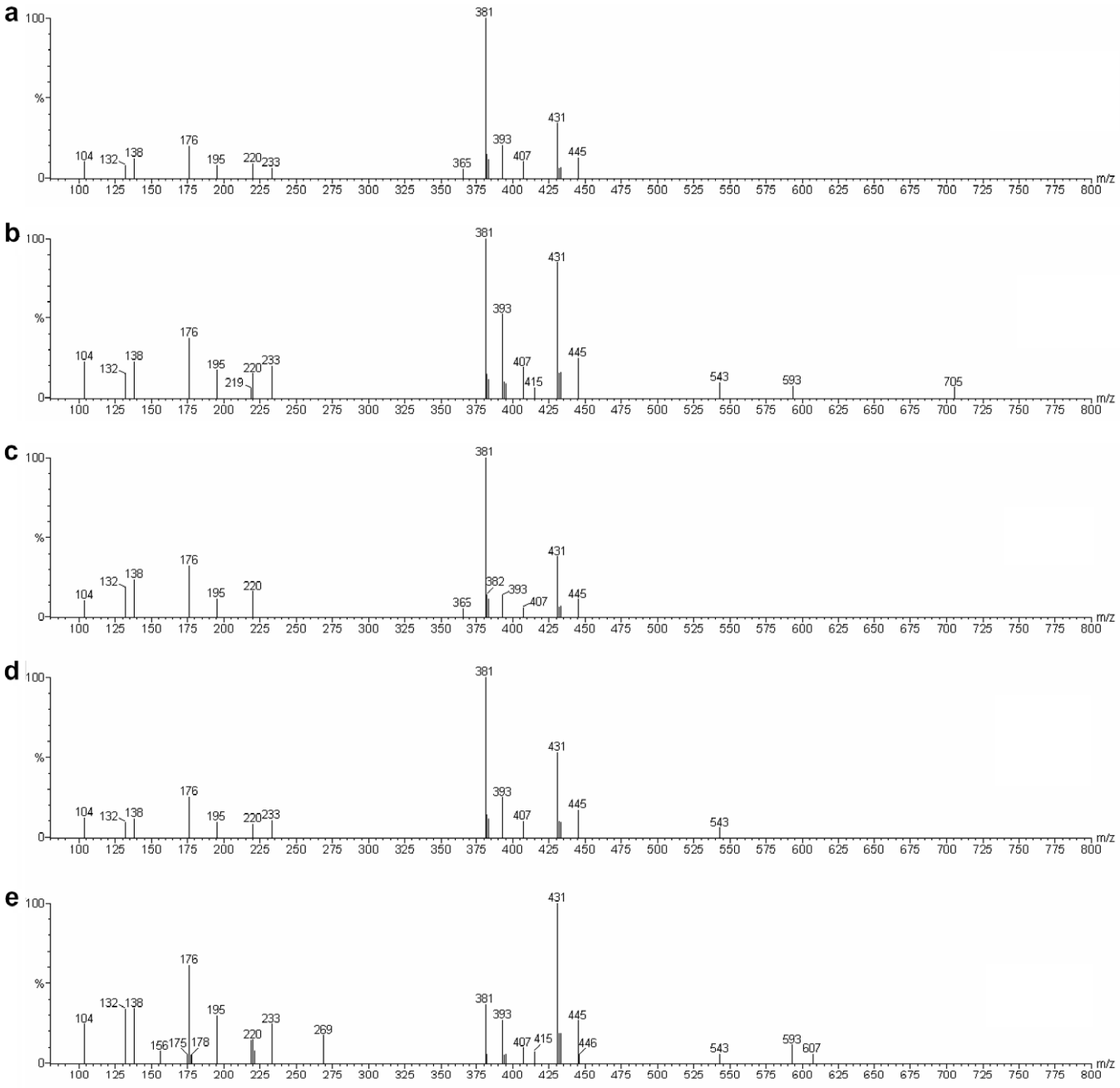

Fig. 2. ESI(+)-MS of Robusta (R) coffee samples: (a) non-defective (ND); (b) Immature (IM); (c) Light Sour (LS); (d) Dark Sour (DS); (e) Black (BL).

times of 1, 2 and 3 h. A tendency for increasing standard deviations was observed with decreasing extraction time, and thus extraction time was maintained at 3 h. Therefore, aqueous extraction of the defatted solid was performed employing an ultrasonic bath, according to the methodology proposed by Mecozzi et al. (1999) – 3 h extraction in deionized water at 25 °C. A Q-TOF mass spectrometer (Micromass, Manchester,UK) was used for fingerprinting ESI-MS analysis. ESI-MS was performed by direct infusion of the aqueous extract, in both the negative and positive modes. The general conditions were: source temperature of 80 °C, capillary voltage of 2.5 kV and cone voltage of 40 V. Mass spectra were acquired and accumulated over 60 s and spectra were scanned in the range between 100 and 800 *m*/*z*.

2.3. ESI-MS data handling and statistical treatment

All mass spectra were handled using MassLynx 4.0 software (Waters, Manchester, UK). To discard noise signals, only the ions with a relative abundance higher than 5% were included in the final data matrix. For principal component analysis (PCA) and hierarchical cluster analysis (HCA), data matrices were assembled so that each row corresponded to a coffee sample and each column represented the *m*/*z* ratios and intensities of detected ions. Final matrices of 20 objects (samples) and 85/21 variables (*m*/*z* ratios and corresponding intensities of detected ions) resulted for the positive and negative ionization modes, respectively. Complete linkage method and Euclidean distance were used to generate the dendrograms in the HCA method.

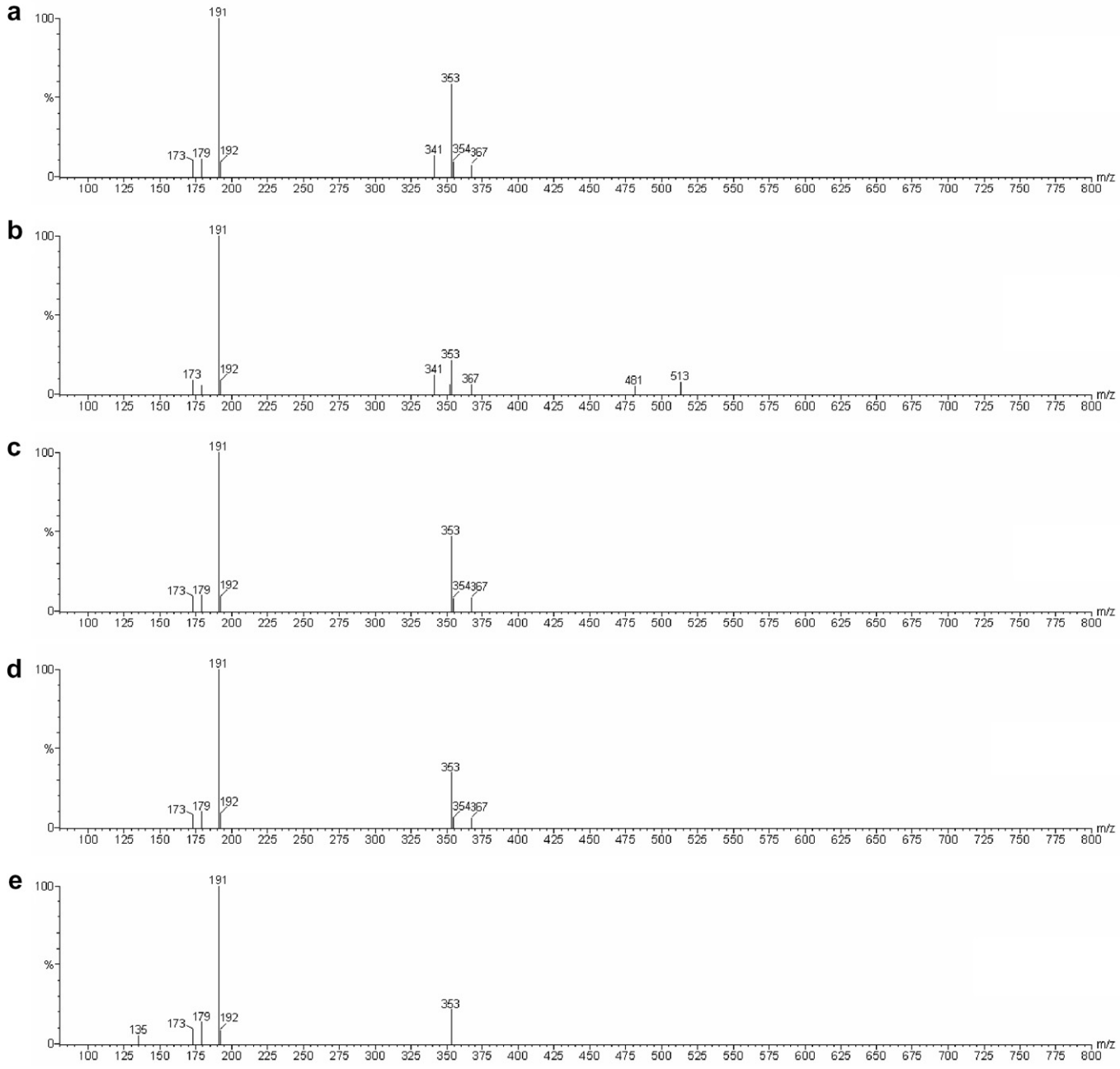

Fig. 3. ESI(−)-MS of Arabica (A) coffee samples: (a) non-defective (ND); (b) immature (IM); (c) light sour (LS); (d) dark sour (DS); (e) black (BL).

3. Results and discussion

Figs. 1 and 2 show typical ESI(+)-MS fingerprints of non-defective and defective coffee beans of the Arabica and Robusta species, respectively. A comparison between Figs. 1 and 2 indicate that, for defective coffee beans, the amount of protonated substances was higher for Arabica coffees in comparison to Robusta. It can also be observed that, for Arabica, both the number and intensity of protonated substances are higher for the samples that underwent fermentation (Fig. 1c–e) in comparison to the ones that did not (Fig. 1a and b) and cations in the *m*/*z* range of 195–369 were only detected in the defective beans. Non-defective, immature and light sour beans of both species are mainly characterized by the abundant ion of *m*/*z* 381, whereas the main diagnostic cation for black beans is that of *m*/*z* 431. A comparison of the spectra for light and dark sour, and black beans for both Arabica and Robusta shows an increase in intensity of the ion *m*/*z* 431, as fermentation becomes more intense (dark sour and black). It can also be observed that amount and intensity of the cations of low m/z (<270) seem to be associated to fermentation, mainly for Arabicas. Typical ESI(−)-MS fingerprints of non-defective and defective coffee beans are displayed in Figs. 3 and 4, for Arabica and Robusta species, respectively. The number of anions detected in the negative mode was quite small in comparison to the number of cations detected in the positive mode (see Figs. 1 and 2). Also, the amount of diagnostic anions was slightly smaller for Arabica samples in comparison to Robusta. A comparison of Figs. 3 and 4 show that, in this case, the spectra are quite similar, with the anion *m*/*z* 191 being the most abundant for all samples. The selection and tentative identification of the most important cations and anions based on the

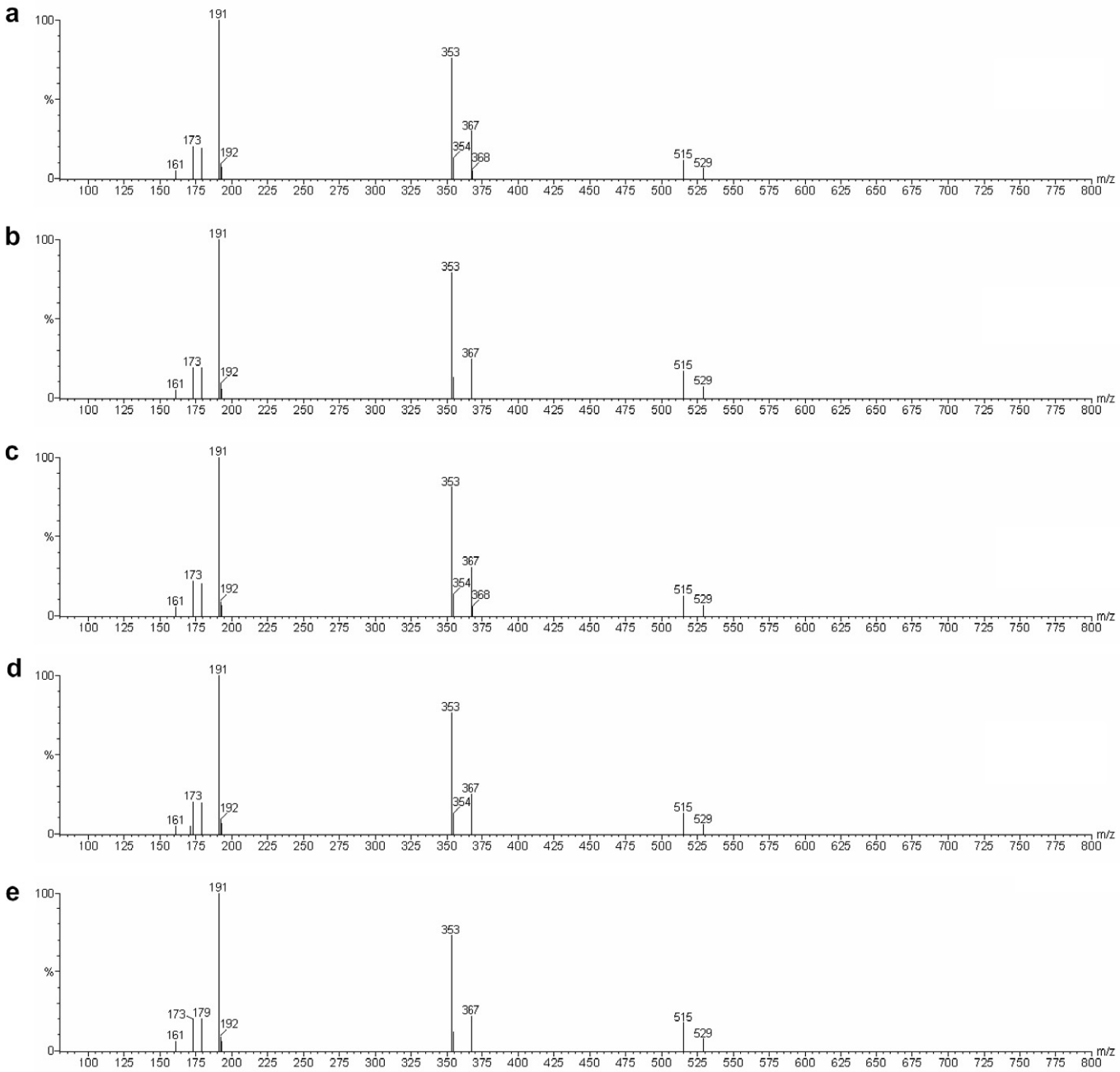

Fig. 4. ESI(−)-MS of Robusta (R) coffee samples: (a) non-defective (ND); (b) immature (IM); (c) light sour (LS); (d) dark sour (DS); (e) black (BL).

loadings of principal components will be discussed after presentation of the PCA and clusters results.

Multivariate statistical analysis (PCA and clusters) was performed in order to verify the possibility of discrimination between Arabica/Robusta and defective/non-defective coffees based on EI-MS profiles. The biplot of the Arabica and Robusta coffees ESI(+)-MS profiles is shown in Fig. 5a. The first two principal components (PCs) explained 79% and 13% of the data variance, respectively. A clear separation between Arabica and Robusta samples can be observed, based on the second component, which presented positive and negative values for Arabica and Robusta samples, respectively. The first component allowed for separation between defective and non-defective samples for both Arabica and Robusta (see dashed line in Fig. 5a). This can be confirmed by HCA analysis (Fig. 5b), since non-defective Arabica and Robusta coffee samples were well separated from the defective ones (0% similarity). These results indicate that the ESI(+)-MS profiles provide good separation between defective and non-defective coffees.

The substance that presented the highest influence on PC1 values based on the projection of score plot (not presented here), allowing for separation between defective and non-defective coffees, was the ion at *m*/*z* 381, which can be inferred as being a potassium adduct of sucrose (Chen & Hu, 1999). Although protonated molecular ions are the most typical ion formation in electrospray ionization, it is not uncommon to observe sodium and potassium ion adducts particularly when large numbers of sodium and potassium ions are present in the sample solution (Chen & Hu, 1999). In our study, this is attributed to the high concentration of potassium in comparison to other minerals in coffee beans (Custódio, Magalhães, Mansur, Franca, & Oliveira, 2005). Lower sucrose levels for defective coffees in comparison to non-defective ones have been previously reported by other studies employing Arabica coffee samples (Mazzafera, 1999; Vasconcelos et al., 2007). In the case of immature coffees, low sucrose levels can be associated to the bean maturity state whereas for black and sour beans, sucrose reduction is probably due to fermentation. The results presented here indicate that this also holds true for Robusta coffees. The ions that presented the highest influence on PC2 values based on the projection of score plot (not presented here) were the ones at *m*/*z* 219 and 431, both presenting similar levels, but the first one (219) contributing for positive PC2 and the second one (431) for negative PC2. The cation *m*/*z* 219 has been previously identified as a fragment of the potassium adduct of sucrose (Chen & Hu, 1999). The results obtained in this study indicate that this ion is the main responsible for the separation between Arabica and Robusta coffees based on the ESI(+)-MS fingerprints. This can be attributed to the higher sucrose contents of Arabica coffees in comparison to Robusta (Bradbury, 2001).

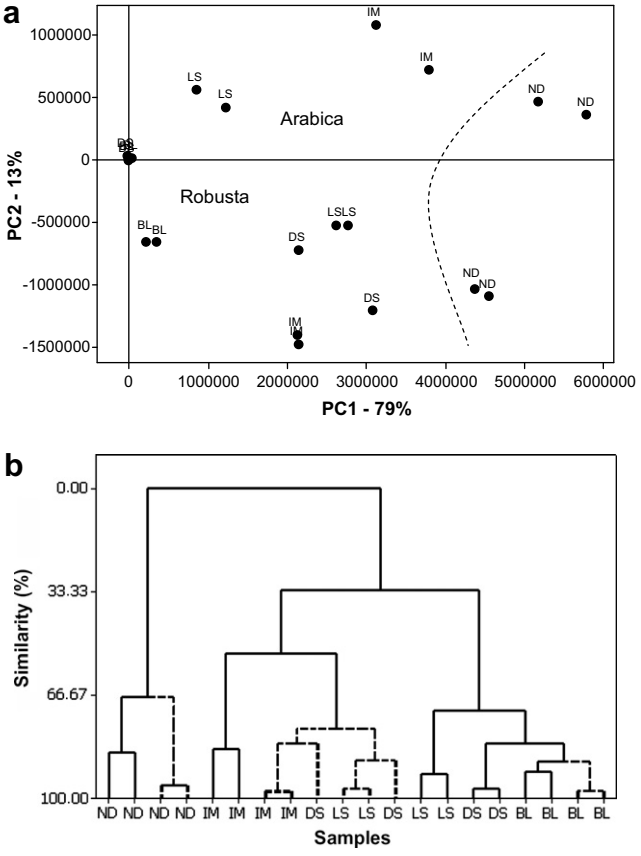

Fig. 5. (a) PCA scores scatter plot and (b) cluster analysis of ion abundance values obtained from the data of ESI(+)-MS fingerprints of Arabica and Robusta coffee samples. Solid and dashed lines correspond to Arabica and Robusta species, respectively. ND, non-defective; IM, immature; LS, light sour; DS, dark sour; BL, black.

The cation at *m*/*z* 431 has been previously reported in the reviewed literature as Kaempferol-3-*O*-α-L-rhamnoside-7-*O*-α-L-rhamnopyranoside, Kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside and Kaempferol-3-*O*-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranosyl-(1 → 2)-α-D-rhamnopyranoside (Kuçukislamoglu, Yayli, Senturk, & Genç, 2000). Given (i) the natural occurence of flavonoids (phenolic compounds) in green coffee (Farah & Donangelo, 2006; Hertog, Hollman, & Van De Putte, 1993), (ii) the presence of both rhamnose and glucose in green coffee (Oosterveld, Voragem, & Schols, 2003), and (iii) the fact that flavonoids generally occur in plants as *O*-glycosides (Hertog et al., 1993), possible structures for the molecular ions that could originate the cation *m*/*z* 431 are the ones presented by Kuçukislamoglu et al. (2000). This hypothesis is reinforced by the fact that this cation is associated with Robusta coffees, which are known to present higher quantities of phenolic compounds in comparison to Arabica (Clifford, 1985; Grosch, 2001). Further studies are needed in order to validate this hypothesis if identification of chemical markers is of primary relevance.

The biplot for the Arabica and Robusta coffees ESI(−)-MS profiles is shown in Fig. 6a. The first two principal components (PCs) explained 89% and 8% of the data variance, respectively. A clear separation between Arabica and Robusta samples can be observed, based on both the first and second components. This can be confirmed by HCA analysis (Fig. 6b), since Arabica and Robusta coffee samples were well separated into two distinct clusters. These results indicate that the ESI(−)-MS profiles provide good separation between Arabica and Robusta coffees. It can also be observed that immature and black coffees were grouped together. Such type of clustering was also reported in reference to the volatile profiles of black and immature Arabica coffee beans (Mancha Agresti et al., 2008), thus reinforcing the hypothesis that black beans could be associated to fermentation of immature ones.

The anions that presented the highest influence on PC1 and PC2 values, based on the projection of score plot (not presented here), providing for separation between Arabica and Robusta coffees were those of *m*/*z* 353, 191, 367, 515, 173, 179 and 341, listed from the highest to the lowest effect. Such compounds correspond to a series of esters known as chlorogenic acids, generally associated with esterification between the quinic and caffeic acids (Clifford, Knight, Surucu, & Kuhnert, 2006; Clifford, Kirkpatrick, Kuhnert, Roozendaal, & Salgado, 2008). The major groups of chlorogenic acids found in green (crude) coffee include caffeoylquinic, dicaffeoylquinic, feruloylquinic, *p*-coumaroylquinic and caffeoyl feruloylquinic acids (Clifford, 1985). The anion *m*/*z* 353 corresponds to the following chlorogenic acids: 3-*O*-Caffeoylquinic acid, 4-*O*-Caffeoylquinic acid, 5-*O*-Caffeoylquinic acid (Bravo, Goya, & Lecumberri, 2007) and is the

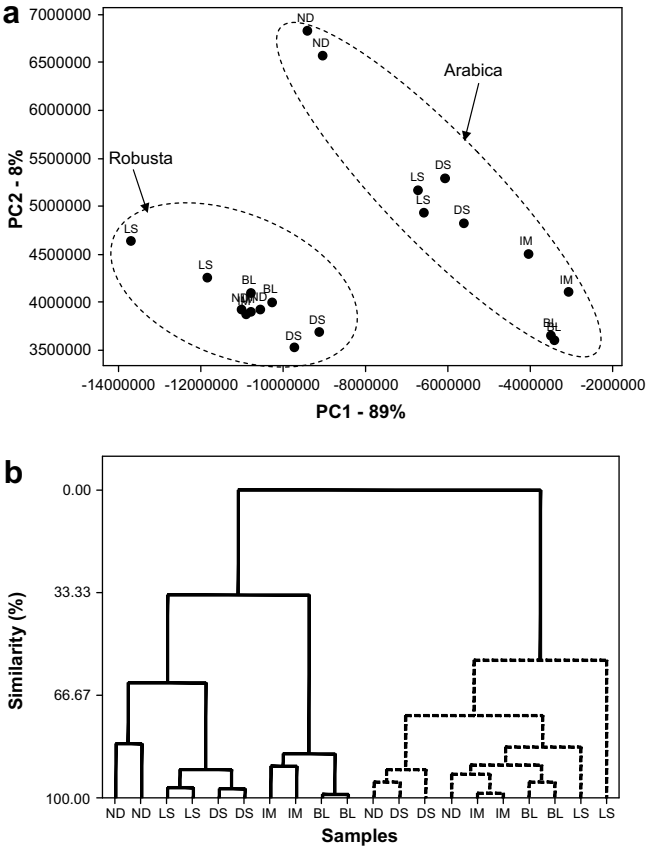

Fig. 6. (a) PCA scores scatter plot and (b) cluster analysis of ion abundance values obtained from the data of ESI(−)-MS fingerprints of Arabica and Robusta coffee samples. Solid and dashed lines correspond to Arabica and Robusta species, respectively. ND, non-defective; IM, immature; LS, light sour; DS, dark sour; BL, black.

main anion characterizing Robusta coffees. According to Farah and Donangelo (2006), the isomer 5-*O*-Caffeoylquinic acid represents 56–62% of the total chlorogenic acids. The fact that such substances allow for discrimination between Arabica and Robusta coffees is in agreement with the literature reports on higher levels of chlorogenic acids in Robusta (7–12% d.b.) in comparison to Arabica (5–9% d.b.) (Clifford, 1985; Farah & Donangelo, 2006; Ky et al., 2001). The remaining anions that were characteristic of Robusta coffees presented *m*/*z* 367, 515, 173 and 179, being respectively associated to the following acids: feruloylquinic acid, 3,4-dicaffeoylquinic acid, dehydrated quinic acid and caffeic acid (Bravo et al., 2007; Roesler, Catharino, Malta, Eberlin, & Pastore, 2007). The anions *m*/*z* 191 and 341 were characteristic of non-defective and defective Arabica coffees, respectively. The first one (*m*/*z* 191) corresponds to the deprotonated form of quinic acid (Roesler et al., 2007). According to the reviewed literature, free quinic acid occurs only in small quantities in green coffee beans, but a greater quantity of quinic acids occurs as a series of esters (chlorogenic acids) (Clifford, 1985). The study by Rodrigues et al., 2007 presents results of quinic acid determination in several samples of brewed coffee from both the Arabica and Robusta species. Quinic acid was detected only in one of the Robusta samples from India and was not present in the Arabica samples. Such information, in association with the fact that this anion was the most abundant in all samples, Arabica or Robusta, indicates that it probably resulted from the chlorogenic acids and further studies are necessary in order to properly determine its origin. The anion *m*/*z* 341 has been previously reported as a dimeric adduct of the dicaffeic acid (Bravo et al., 2007), or caffeoyl glucose (Roesler et al., 2007).

4. Conclusions

A comparative evaluation of ESI-MS profiles of green coffees of the Arabica and Robusta species was performed, for both good quality (non-defective) and low quality (defective) coffees. ESI(+)-MS profiles allowed for separation between defective and non-defective coffees, as a result of high sucrose levels of non-defective coffees as opposed to the low sucrose levels of defective beans that either did not attain the desired maturity levels (immature) or underwent fermentation (black and sour). ESI(−)-MS profiles provided separation between Arabica and Robusta coffees, in association with the higher content of phenolic compounds, mainly chlorogenic acids, of Robusta coffees in comparison to Arabica. Also, clustering of immature and black defects was observed for both species, confirming previous studies reporting that black beans could be associated to fermentation of immature ones.

Acknowledgements

The authors acknowledge financial support from the following Brazilian Government Agencies: CAPES, CNPq and FAPEMIG. The authors would like to thank Profs. Adriano Pimenta (Departamento de Bioquímica e Imunologia/UFMG) and Rodinei Augusti (Departamento de Química/UFMG) for helping with the ESI-MS analysis.

References

Araújo, A. S., Rocha, L. L., Tomazela, D. M., Sawaya, A. C. H. F., Almeida, R. R., Catharino, R. R., et al. (2005). Electrospray ionization mass spectrometry fingerprinting of beer. *Analyst, 130*, 884–889.

Bradbury, A. G. W. (2001). Carbohydrates. In R. J. Clarke & O. Z. Vitzthum (Eds.), *Coffee recent developments* (pp. 1–7). Blackwell Science: Oxford.

Bravo, L., Goya, L. E., & Lecumberri, E. (2007). LC/MS characterization of phenolic constituents of mate (Ilex paraguariensis, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Research International, 40*(3), 393–405.

Chen, Y.-C., & Hu, A. (1999). Simultaneous determination of trace benzodiazepines from drinks by using direct electrospray probe/mass spectrometry (DEP/MS). *Forensic Science International, 103*(2), 79–88.

Clifford, M. N. (1985). Chlorogenic acids. In R. J. Clarke & R. Macrae (Eds.), *Coffee volume 1: Chemistry* (pp. 153–202). London: Elsevier Applied Science.

Clifford, M. N., Knight, S., Surucu, N., & Kuhnert, N. (2006). Characterization by LC–MS of four new classes of chlorogenic acids in coffee beans: Dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and ferulloyl-dimethoxycinnamoylquinic acids. *Journal of Agricultural and Food Chemistry, 54*(6), 1957–1969.

Clifford, M. N., Kirkpatrick, J., Kuhnert, N., Roozendaal, H., & Salgado, P. R. (2008). LC–MS^n analysis of the cis isomers of chlorogenic acids. *Food Chemistry, 106*(1), 379–385.

Cooper, H. J., & Marshall, A. G. (2001). Electrospray ionization Fourier transform mass spectrometric analysis of wine. *Journal of Agricultural and Food Chemistry, 49*, 5710–5718.

Custódio, B. M., Magalhães, F. S., Mansur, M. B., Franca, A. S., Oliveira, L. S. (2005). Elemental analysis of defective and non-defective beans in green and roasted coffee. ASAE Paper no. 056085, St. Joseph: Michigan, ASAE.

De Souza, P. P., Siebald, H. G. L., Augusti, D. V., Neto, W. B., Amorim, V. M., Catharino, R. R., et al. (2007). Electrospray ionization mass spectrometry fingerprinting of Brazilian artisan cachaça aged in different wood casks. *Journal of Agricultural and Food Chemistry, 55*, 2094–2102.

Farah, A., Monteiro, M. C., Calado, V., Franca, A. S., & Trugo, L. C. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chemistry, 98*(2), 373–380.

Farah, A., & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology, 18*(1), 23–36.

Franca, A. S., Oliveira, L. S., Mendonça, J. C. F., & Silva, X. A. (2005a). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry, 90*(1–2), 89–94.

Franca, A. S., Mendonça, J. C. F., & Oliveira, S. D. (2005b). Composition of green and roasted coffees of different cup qualities. *LWT – Food Science and Technology, 38*(7), 709–715.

Goodacre, R., York, E. V., Heald, J. K., & Scott, I. M. (2003). Chemometric discrimination of unfractionated plants extracts analyzed by electrospray mass spectrometry. *Phytochemistry, 62*, 859–863.

Grosch, W. (2001). Chemistry III: Volatile compounds. In R. J. Clarke & O. Z. Vitzthum (Eds.), *Coffee: Recent developments* (pp. 68–89). Oxford: Blackwell.

Hertog, M. G. L., Hollman, P. C. H., & Van De Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *Journal of Agricultural and Food Chemistry, 41*(8), 1242–1246.

Kuçukislamoglu, M., Yayli, N., Senturk, H. B., & Genç, H. (2000). Flavonol glycosides from Consolida armeniaca. *Turkish Journal of Chemistry, 24*, 191–197.

Ky, C.-L., Louarn, J., Dussert, S., Guyot, B., Hamon, S., & Noirot, M. (2001). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica L.* and *C. canephora P.* accessions. *Food Chemistry, 75*(2), 223–230.

Liu, A.-H., Lin, Y.-H., Yang, M., Guo, H., Guan, S.-H., Sun, J.-H., et al. (2007). Development of the fingerprints for the quality of the roots of Salvia miltiorrhiza and its related preparations by HPLC-DAD and LC–MS^n. *Journal of Chromatography B, 846*(1–2), 32–41.

Mancha Agresti, P. C. M., Franca, A. S., Oliveira, L. S., & Augusti, R. (2008). Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. *Food Chemistry, 106*(2), 787–796.

Mazzafera, P. (1999). Chemical composition of defective coffee beans. *Food Chemistry, 64*(4), 547–554.

Mecozzi, M., Amici, M., Pietrantonio, E., & Acquistucci, R. (1999). Ultrasound-assisted analysis of total carbohydrates in environmental and food samples. *Ultrasonic Sonochemistry, 6*(3), 133–139.

Oliveira, L. S., Franca, A. S., Mendonça, J. C. F., & Barros-Júnior, M. C. (2006). Proximate composition and fatty acids profile of green and roasted defective coffee beans. *LWT – Food Science and Technology, 39*, 235–239.

Oosterveld, A., Voragem, A. G. J., & Schols, H. A. (2003). Effect of roasting on the carbohydrate composition of Coffea Arabica beans. *Carbohydrate Polymers, 54*(2), 183–192.

Ramalakshmi, K., Kubra, I. R., & Rao, L. J. M. (2007). Physicochemical characteristics of green coffee: Comparison of graded and defective beans. *Journal of Food Science, 72*, S333–S337.

Rodrigues, C. I., Marta, L., Maia, R., Miranda, M., Ribeirinho, M., & Maguas, C. (2007). Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC. *Journal of Food Composition and Analysis, 20*(5), 440–448.

Roesler, R., Catharino, R. R., Malta, L. G., Eberlin, M. N., & Pastore, G. (2007). Antioxidant activity of Annona crassiflora: Characterization of major components by electrospray ionization mass spectrometry. *Food Chemistry, 104*(3), 1048–1054.

Santos, L. S., Catharino, R. R., Aguiar, C. L., Tsai, S. M., & Eberlin, M. N. (2006). Chemotaxonomic markers of organic, natural, and genetically modified soybeans detected by direct infusion electrospray ionization mass spectrometry. *Journal of Radioanalytical and Nuclear Chemistry, 269*(2), 505–509.

Vasconcelos, A. L. S., Franca, A. S., Glória, M. B. A., & Mendonça, J. C. F. (2007). A comparative study of chemical attributes and levels of amines in defective green and roasted coffee beans. *Food Chemistry, 101*(1), 26–32.