Analytical Methods

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, **5**, 5944

Coffee origin discrimination by paper spray mass spectrometry and direct coffee spray analysis

Rafael Garrett,[ab] Claudia M. Rezende[b] and Demian R. Ifa*[a]

Received 24th July 2013
Accepted 30th August 2013

DOI: 10.1039/c3ay41247d

www.rsc.org/methods

Here we explore the application of the ambient ionization technique paper spray mass spectrometry (PS-MS) combined with the multivariate statistical tools principal component analysis and hierarchical cluster analysis as a fast and simple method to discriminate green arabica coffee beans from three different origins in Brazil. PS-MS does not require nebulizing gas or complex protocols for sample preparation and by the analysis of 5 μL of whole coffee bean extracts it was possible to discriminate the coffee origin. Furthermore, we report the direct analysis of a single coffee bean using a bean slice as the sample and also the substrate for electrospray ionization.

Introduction

Coffee is an extremely important commodity for many developing countries, including Brazil, the largest coffee producer and exporter.[1] It is appreciated everyday by millions of coffee consumers. Its quality is generally related to good agriculture and handling practices as well as to the geographic origin and climate conditions, species and cultivar.[2,3]

Among the various coffee species, *Coffea arabica* L. (arabica coffee) and *C. canephora* var. *robusta* (robusta coffee) are the only two species commercialized for coffee brew consumption. Arabica coffee provides a high-quality brew compared to robusta and thus, its price is about 2–3 times higher.[4,5] After harvesting, arabica coffee fruits can basically be processed by three ways: (a) direct sun or machine drying followed by pulp removal (dry process/natural coffee); (b) pulp removal followed by drying (semi-dry process/pulped natural coffee); or (c) pulp followed by mucilage removal in water fermentation tanks, and then drying (wet process/washed coffee). Each of these processes generates coffees with differences in body, acidity, sweetness, and flavor and also influences its final market price and consumer choice.[6,7]

The geographic origin also plays an important role in coffee quality and discussions about origin certification and *terroir* are very common nowadays.[8] Therefore, the development of methods for analysis, certification of authenticity, and quality control of coffees is an urgent analytical task. Although methods based on liquid or gas chromatography/mass spectrometry,[9–11] direct-infusion electrospray ionization mass spectrometry,[12–14] infrared spectroscopy,[15] and carbon nuclear magnetic resonance[16] have been successfully applied to discriminate coffee origin or process using fingerprinting analysis and multivariate statistical approaches, the need for simple and fast methods avoiding elaborate or time-consuming protocols for sample preparation still persists.

The idea of using paper as a substrate to perform electrospray ionization was considered by John Fenn over a decade ago.[17] He showed that after supplying liquid to wicking elements, such as unwaxed nylon dental floss, cotton or glass fibers, paper strips and thin layer chromatography plates, and by applying a strong electrical field to these wetted materials it was possible to generate a fine spray of charged droplets at their apexes where ejection and evaporation of the liquid led to gaseous ions that could be mass analyzed.[17]

Paper spray mass spectrometry (PS-MS) was introduced three years ago by Wang *et al.*[18] and it is done by directly loading the sample onto a triangular piece of paper and applying a high voltage at its base after wetting the paper with a solvent. The spray of charged droplets is formed placing the paper tip in front of the mass spectrometer inlet, and the desolvation occurs without any sheath gas.[18] PS-MS has been widely explored in the last few years to directly analyze complex samples such as illicit compounds in raw urine,[19] pharmaceuticals in whole blood,[20] biological tissue,[21] contaminants in foodstuffs,[22] and Sudan dyes in chili pepper.[22,23] Its application in quality assessment and control of herbal tea using fingerprinting analysis and chemometrics was also demonstrated recently.[24]

Furthermore, other wicking elements such as microporous polymers,[25] wooden toothpicks,[26] plant leaves (leaf spray) or other vegetable materials were also employed as the sample and substrate for electrospray.[27–29]

Here we report the application of a simple method based on PS-MS and multivariate statistical analysis to discriminate arabica coffees from different origins and postharvest

[a]*Centre for Research in Mass Spectrometry, York University, Toronto, Ontario, M3J 1P3 Canada. E-mail: ifadr@yorku.ca; Fax: +1 416 736-5936; Tel: +1 416 736-2100 ext. 33555*

[b]*Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, 21945-970 Brazil. E-mail: rafael_garrett@iq.ufrj.br; Fax: +55 21 2562 7106; Tel: +55 21 2562-7370*

processes, and also a direct "coffee spray" analysis using the coffee bean as both the sample and ionization source.

Experimental

Materials and reagents

Nine samples of green arabica coffee beans from 2011/12 crop were obtained from coffee producers of Bahia (BA), Rio de Janeiro (RJ), and Paraná (PR) states (northeast, southeast, and south region of Brazil, respectively). Each producer processed the coffee fruits using three types of postharvest methods: dry method using the whole coffee fruit, semi-dry method using the pulped fruit, and the wet method. Samples were kept at −4 °C in a freezer until analysis and the defective beans were manually removed before extraction to avoid other sources of variations. Methanol and water (LC/MS grade) were purchased from Fisher Scientific (Whitby, Canada), ultrasonic cleaner from Branson Ultrasonic Corporation (Danbury, USA), MX35 premier microtome blade from Thermo Scientific (Whitby, Canada), and qualitative filter paper no. 1 (180 μm thickness) was purchased from Whatman International Ltd (Maidstone, England).

Paper spray and coffee spray mass spectrometry

For paper spray ionization, papers were cut into a triangular shape (12 mm height and 7 mm of base) and held by a metal alligator clip at a distance of 5–7 mm from the mass spectrometry inlet. Around 20 intact coffee beans (3 g) were directly extracted in triplicate with 10 mL of methanol–water solution (9 : 1, v/v) for 10 min in an ultrasonic bath and 5 μL of the extract was spotted onto the paper without further sample treatment. After drying, a high voltage (6 kV) was applied to the paper that was wetted again with 5 μL of methanol to generate the MS spectra.

Coffee spray experiment was performed by wetting a cross-sectional coffee slice of approximately 1 mm thickness with 20 μL of methanol and applying a voltage of 6 kV at its base. The distance between the coffee slice and the MS inlet was 5 mm.

All mass spectra and MS/MS experiments were performed using a Thermo LTQ mass spectrometer (Thermo Fisher Scientific, USA). The spray voltage was set to 6 kV, capillary temperature to 275 °C, tube lens to 65 V, and capillary voltage to 30 V. For PS-MS, full scan mass spectra were acquired in the positive ion mode over the range of *m*/*z* 170–800 and for coffee spray positive and negative ion modes were used over the range of *m*/*z* 100–1000. Tandem mass spectrometry was performed using collision-induced dissociation with collision energy of 15–30% (manufacturer's unit).

Multivariate statistical analysis

Lists containing the rounded *m*/*z* values and relative intensities of the thirty most abundant ions from each sample were exported from the Xcalibur software and saved as .csv files to be uploaded into the MetaboAnalyst web server for multivariate analyses (http://www.metaboanalyst.ca).[30] Data were aligned, treated by Pareto scaling to reduce the differences between large and small peaks, and submitted to principal component analysis (PCA) and hierarchical cluster analysis (HCA). Pearson's correlation was used as the similarity measure and Ward's linkage was used as the clustering method in HCA analysis.

Results and discussion

Most of the signals detected in the positive ion mode PS-MS were from sugar compounds (Fig. 1). Carbohydrates are present in high amounts in green coffee beans, accounting for approximately 50% of its dry base.[31] Besides, it seems that amino acids, sugars and other low mass compounds are the most common signals detected in vegetable samples using positive ion mode PS-MS and leaf spray.[24,27,28] The ions of *m*/*z* 203 and 219 were identified by literature comparison and MS/MS experiments as $[M + Na]^+$ and $[M + K]^+$ adducts of glucose, respectively.[27] The same pattern was observed for sucrose (*m*/*z* 365 $[M + Na]^+$ and *m*/*z* 381 $[M + K]^+$). Other ions identified were the H^+ adducts of caffeine (*m*/*z* 195) and arginine (*m*/*z* 175).

Negative ion mode resulted mostly in high background peaks and poor signal spectra compared to positive. As a result, only the positive ion mode was used for PS-MS analysis.

As shown in Fig. 1, basically the same set of compounds was observed by PS-MS of coffees from different origins. All coffee beans analyzed belong to the arabica species, therefore, differences in the chemical composition among these samples using fingerprinting and direct mass spectrometry analysis were expected to be small. However, coffee origin discrimination by PCA and HCA could be achieved by reproducible differences in ion intensities. This is a common approach employed for direct-infusion electrospray ionization data treatment[12–14] and has also been applied recently for PS-MS analysis.[24]

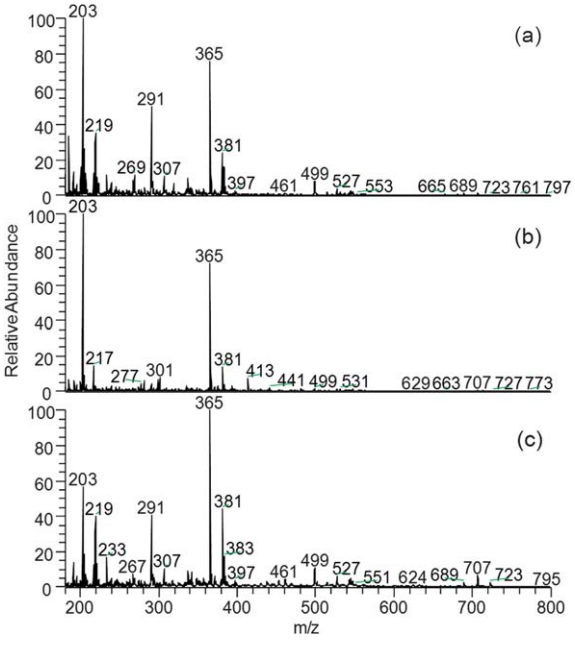

Fig. 1 Positive ion paper spray mass spectrum of green arabica coffee extracts from (a) Bahia, (b) Paraná, and (c) Rio de Janeiro states in Brazil.

Fig. 2 shows the PCA and HCA statistical analysis of coffees from different origins and postharvest methods. For PCA, five PCs were used and it explained 83.6% of the variance. The formation of 3 clusters related to the different coffee origins – Paraná (PR), Rio de Janeiro (RJ), and Bahia (BA) state – is clear even with each of them being processed by different ways, pointing to the reliability of combining PS-MS with chemometric analysis. BA coffees were clustered and distinguished from RJ and PR by the first two PCs, while PR coffees could be discriminated the most by PC1. The dashed lines forming the clusters in Fig. 2a represent the 95% confidence level. The variables with *m*/*z* values of 291, 173, 219, 307 on PC1, and 365, 203, 185, 381 on PC2 showed the highest absolute loading values in the loading plot (not shown), being mainly responsible for the separations achieved in the PCA analysis. Postharvest discrimination among coffees with the same origin was also observed and this can be better seen in the HCA plot in Fig. 2b.

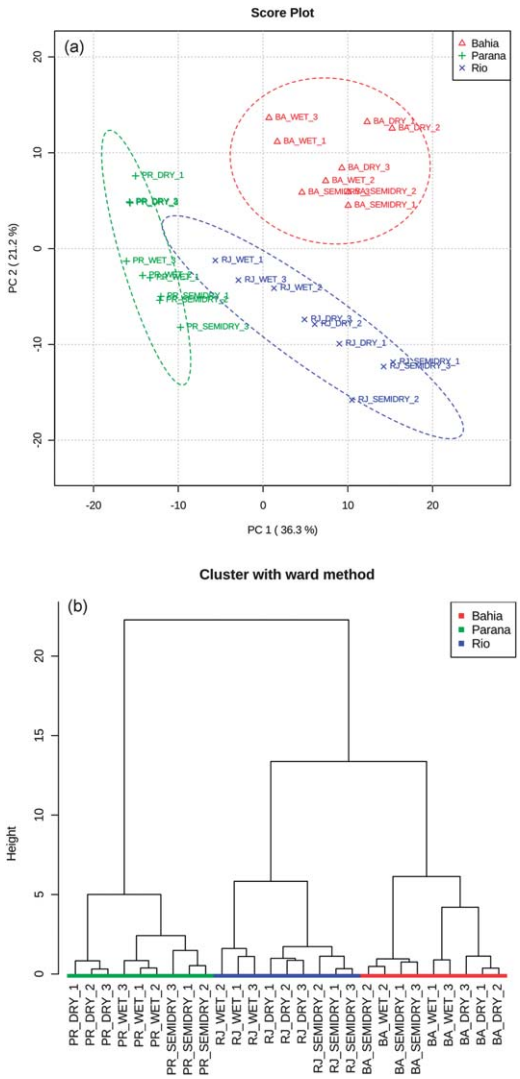

Fig. 2 (a) Principal component analysis score plot and (b) hierarchical cluster analysis dendrogram showing the discrimination of coffee origins by the formation of three clusters: Bahia (BA), Paraná (PR) and Rio de Janeiro (RJ) states in Brazil. Dry, semi-dry and wet are the coffee postharvest methods employed.

In the direct coffee spray analysis, coffee slices were held in front of the MS inlet by an alligator clip and an optimized voltage of 6 kV was applied after wetting the slices with 20 μL of pure methanol (Fig. 3a). The process of wetting the sample with a spray solvent was necessary due to the low water content of the coffee bean. A 5 mm distance between the coffee slice and the MS inlet was chosen, because a shorter distance could lead to electrical discharge while a longer one led to a decrease in ion intensity. Both negative and positive ion modes were successfully employed, and a stable spray with an average of 2 min duration was obtained using a flat cut slice. This time could also be increased by adding more solvent onto the sample. It was not necessary to cut the slice into a triangular shape to create the electrospray because of the natural sharpness of its tip. Indeed, it was shown in the literature for a leaf spray experiment that a better quality of data was obtained using the uncut leaf itself rather than a leaf cut in a triangular shape.[29] Fig. 3b and c show the coffee spray MS spectrum in negative and positive ion modes, respectively.

Different mass spectra profiles were observed comparing the positive direct coffee spray and positive PS-MS. The PS-MS analysis was done using untreated extracts from the surface of intact coffee beans, and most of the signals detected were below the *m*/*z* value of 400. Background peaks from paper were also detected. On the other hand, coffee spray spectra resulted from direct extraction of compounds located internally in the bean, because of the cross-sectional slice used in the analysis, and more intense peaks of high *m*/*z* were observed in addition to those of low *m*/*z*.

The negative coffee spray spectrum was very similar to previous electrospray MS spectrum obtained by our group using methanol extraction of ground green coffee beans,[12,13] in which chlorogenic acids were the major compounds detected. The ions of *m*/*z* 191, 337, 353, 367, 515 and 695 were identified as deprotonated quinic acid, coumaroylquinic acid, caffeoylquinic acid, feruloylquinic acid, dicaffeoylquinic acid, and a possible derivative of caffeoylquinic acid, respectively.[12–14] These deprotonated molecules usually generate key fragments *via* MS^n experiments that allow their identification. For example, fragmentation of dicaffeoylquinic acid (*m*/*z* 515) led to formation of product ions related to caffeoylquinic acid and quinic acid (*m*/*z* 353 and 191, respectively).[32] Furthermore, chlorogenic acids are known as the major group of phenolic compounds in coffee. They are responsible for its antioxidant activity and bitter taste, and they are also commonly used as chemical markers to differentiate arabica from robusta species.

In the positive ion mode, peaks with *m*/*z* of 138 and 176 were identified as the coffee flavor precursor trigonelline ($[M + H]^+$ and $[M + K]^+$, respectively). Other ions identified were choline $[M + H]^+$ and sucrose $[M + K]^+$ (*m*/*z* 104 and 381, respectively).

Coffee spray analysis allowed endogenous compounds to be directly extracted and ionized from the coffee bean tissue with little sample preparation. This could be very useful to help understand changes in chemical composition that may occur during its development in the coffee tree or when it is damaged by insect or fungal attack.

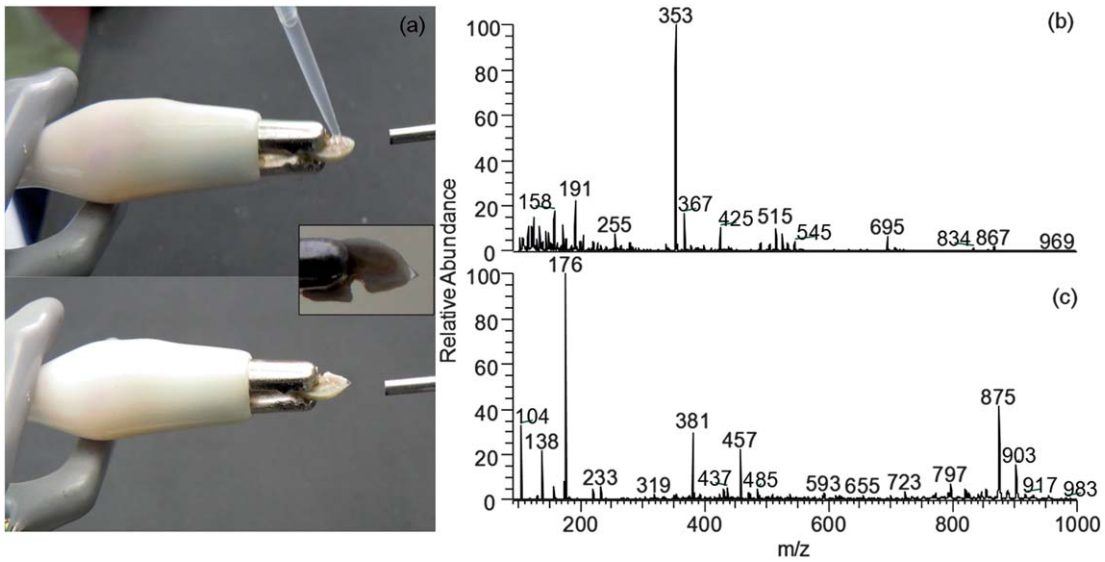

Fig. 3 (a) Coffee spray ionization photograph. A slice of green arabica coffee bean was held by an alligator clip in front of the mass spectrometer inlet and the mass spectrum was acquired after applying a high voltage (6 kV) to the slice wetted by 20 μL of MeOH. (b) Coffee spray spectrum acquired in negative and (c) positive ion mode.

Conclusions

Paper spray and direct coffee spray are simple, fast and open air ionization techniques that can be used either for high throughput analysis or investigation of a single coffee bean. They do not require sheath gas, expensive materials for sample analysis or complex sample preparation. Besides, with the improvements of portable mass spectrometers,[33] they have the potential to be used for field studies in coffee plantations or coffee farmers' cooperatives to monitor the origin certification and the quality of the processes.

Acknowledgements

The authors thank Dr Luiz Filipe P. Pereira from IAPAR for providing access to coffee samples, and the Natural Sciences and Engineering Research Council of Canada (NSERC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

Notes and references

1 International Coffee Organization (IOC), Monthly coffee market report: March 2013, http://dev.ico.org/documents/cy2012-13/cmr-0313-e.pdf, accessed 20 May 2013.
2 P. Vaast, B. Bertrand, J.-J. Perriot, B. Guyot and M. Génard, *J. Sci. Food Agric.*, 2006, **86**, 197–204.
3 T. Joët, A. Laffargue, F. Descroix, S. Doulbeau, B. Bertrand, A. de Kochko and S. Dussert, *Food Chem.*, 2010, **118**, 693–701.
4 F. Anzueto, T. W. Baumann, G. Graziosi, C. R. Piccin, M. R. Söndahl and H. A. M. van der Vossen, in *Espresso coffee: the science of quality*, ed. A. Illy and R. Viani, Elsevier, London, 2nd edn, 2005, ch. 2, pp. 21–86.
5 P. Lashermes and F. Anthony, in *Technical Crops: Genome Mapping and Molecular Breeding in Plants*, ed. C. Kole, Springer, Berlin, 2007, ch. 5, pp. 109–118.
6 S. Knopp, G. Bytof and D. Selmar, *Eur. Food Res. Technol.*, 2006, **223**, 195–201.
7 G. Bytof, D. Selmar and P. Schieberle, *J. Appl. Bot.*, 2000, **74**, 131–136.
8 R. Teuber, *J. Int. Food Agribus. Mark.*, 2010, **22**, 277–298.
9 M. Y. Choi, W. Choi, J. H. Park, J. Lim and S. W. Kwon, *Food Chem.*, 2010, **121**, 1260–1268.
10 B. Bertrand, D. Villarreal, A. Laffargue, H. Posada, P. Lashermes and S. Dussert, *J. Agric. Food Chem.*, 2008, **56**, 2273–2280.
11 R. M. Alonso-Salces, F. Serra, F. Reniero and K. H. Berger, *J. Agric. Food Chem.*, 2009, **57**, 4224–4235.
12 A. C. L. Amorim, A. M. C. Hovell, A. C. Pinto, M. N. Eberlin, N. P. Arruda, E. J. Pereira, H. R. Bizzo, R. R. Catharino, Z. B. Morais-Filho and C. M. Rezende, *J. Braz. Chem. Soc.*, 2009, **20**, 313–321.
13 R. Garrett, E. M. Schmidt, L. F. P. Pereira, C. S. G. Kitzberger, M. B. S. Scholz, M. N. Eberlin and C. M. Rezende, *LWT–Food Sci. Technol.*, 2013, **50**, 496–502.
14 R. Garrett, B. G. Vaz, A. M. C. Hovell, M. N. Eberlin and C. M. Rezende, *J. Agric. Food Chem.*, 2012, **60**, 4253–4258.
15 N. Wang, Y. Fu and L. T. Lim, *J. Agric. Food Chem.*, 2011, **59**, 3220–3226.
16 F. Wei, K. Furihata, M. Koda, F. Hu, R. Kato, T. Miyakawa and M. Tanokura, *J. Agric. Food Chem.*, 2012, **60**, 10118–10125.
17 J. B. Fenn, *US Pat.* 6 297 499, 2001.
18 H. Wang, J. J. Liu, R. G. Cooks and Z. Ouyang, *Angew. Chem., Int. Ed.*, 2010, **49**, 877–880.
19 J. J. Liu, H. Wang, N. E. Manicke, J. M. Lin, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2010, **82**, 2463–2471.

20 N. E. Manicke, Q. Yang, H. Wang, S. Oradu, Z. Ouyang and R. G. Cooks, *Int. J. Mass Spectrom.*, 2011, **300**, 123–129.
21 H. Wang, N. E. Manicke, Q. Yang, L. Zheng, R. Shi, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2011, **83**, 1197–1201.
22 Z. Zhang, R. G. Cooks and Z. Ouyang, *Analyst*, 2012, **137**, 2556–2558.
23 D. Taverna, L. Di Donna, F. Mazzotti, B. Policicchio and G. Sindona, *J. Mass Spectrom.*, 2013, **48**, 544–547.
24 J. Deng and Y. Yang, *Anal. Chim. Acta*, 2013, **785**, 82–90.
25 G. Tepper and R. Kessick, *Appl. Phys. Lett.*, 2009, **94**, 084106.
26 B. Hu, P. K. So, H. Chen and Z. P. Yao, *Anal. Chem.*, 2011, **83**, 8201–8207.
27 J. Liu, H. Wang, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2011, **83**, 7608–7613.
28 N. Malaj, Z. Ouyang, G. Sindona and R. G. Cooks, *Anal. Methods*, 2012, **4**, 1913–1919.
29 D. Sarkar, A. Srimany and T. Pradeep, *Analyst*, 2012, **137**, 4559–4563.
30 J. Xia, R. Mandal, I. V. Sinelnikov, D. Broadhurst and D. S. Wishart, *Nucleic Acids Res.*, 2012, **40**, W127–W133.
31 R. Redgwell and M. Fischer, *Braz. J. Plant Physiol.*, 2006, **18**, 165–174.
32 M. N. Clifford, K. L. Johnston, S. Knight and N. Kuhnert, *J. Agric. Food Chem.*, 2003, **51**, 2900–2911.
33 E. Sokol, R. J. Noll, R. G. Cooks, L. W. Beegle, H. I. Kim and I. Kanik, *Int. J. Mass Spectrom.*, 2011, **306**, 187–195.

