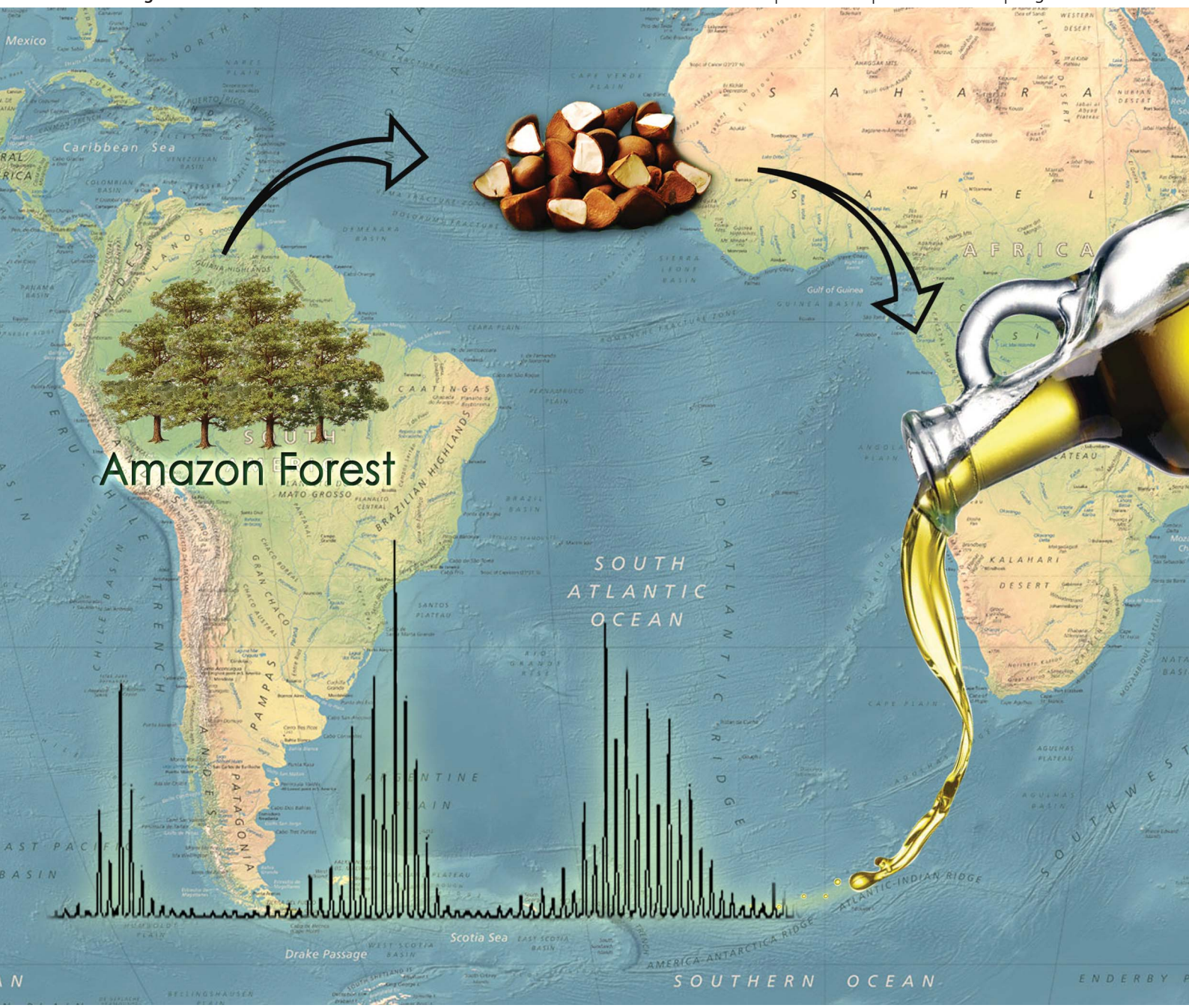
Analytical Methods

www.rsc.org/methods

Volume 5 | Number 6 | 21 March 2013 | Pages 1357–1630



ISSN 1759-9660

RSC Publishing

PAPER

Marcos N. Eberlin *et al.*

Typification and quality control of the Andiroba (*Carapa guianensis*) oil *via* mass spectrometry fingerprinting

1759-9660(2013)5:6;1-#

Analytical Methods

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, **5**, 1385

Typification and quality control of the Andiroba (*Carapa guianensis*) oil *via* mass spectrometry fingerprinting

Elaine C. Cabral,†[a] Georgiana F. da Cruz,[b] Rosineide C. Simas,[a] Gustavo B. Sanvido,[b] Lenise de V. Gonçalves,[c] Rodrigo V. P. Leal,[c] Regina C. F. da Silva,[c] Julio C. T. da Silva,[d] Lauro E. S. Barata,[d] Valnei S. da Cunha,[c] Luiz F. de França,[e] Romeu J. Daroda,[c] Gilberto F. de Sá[f] and Marcos N. Eberlin*[a]

Received 12th July 2012
Accepted 12th January 2013

DOI: 10.1039/c3ay25743f

www.rsc.org/methods

The oil from the seeds of the "Andiroba" tree, which is found throughout most tropical America, contains high levels of unsaturated triacylglycerols (TAG), which makes it attractive to the cosmetics industry. A significant amount of limonoids also confers to this Amazonian oil several pharmaceutical and medical properties. In addition, the oil is also a potential feedstock for biodiesel production, and its many uses have intensified its extractive exploitation in recent years. Herein we report on the characterization of the TAG, free fatty acids (FFA) and limonoid profiles of the Andiroba oil *via* mass spectrometry (MS) fingerprinting using direct electrospray ionization mass spectrometry (ESI-MS). An ambient desorption/ionization technique known as easy ambient sonic-spray ionization (EASI-MS) was also evaluated with similar results. ESI-MS was performed either for a methanolic solution of a few microliters of the fresh oil or from a simple aqueous extract whereas EASI-MS was applied directly to a droplet of the oil resting on a paper surface. The efficacy of these MS fingerprinting techniques requiring no pre-separation and no or very simple sample preparation protocols was investigated and compared for the typification and quality of this valuable Amazonian oil.

Introduction

Carapa guianensis Aublet is a famous Amazonian tree popularly known as Andiroba. It belongs to the Meliaceae family and is found throughout most tropical America.[1] The oil from the seeds of the Andiroba tree is famous for its many uses such as an insecticide added to candles used to control the Anopheles and *Aedes aegypti* mosquitoes that transmit malaria and dengue. It is also widely used in folk medicine as an analgesic, anti-inflammatory, anti-bacterial, anti-parasitic and as an anti-cancer agent.[2] The Andiroba oil is also attractive to the cosmetics industry since it is composed mostly of triacylglycerols with high levels of unsaturated fatty acids such as oleic (51.81%), palmitic (25.76%), stearic (9.08%), and linoleic acid (8.3%). Its unsaponifiable content varies from 2 to 5%,[2a] and is composed of triterpenes, steroids, coumarins, flavonoids and limonoids (Scheme 1), including 17β-hydroxyazadiradione (1), gedunin (2), 6α-acetoxy-gedunin (3), 7-deacetoxy-7-oxogedunin (4), 1,2-dihydro-3β-hydroxy-7-deacetoxy-7-oxogedunin (5), methyl angolensate (6), and xyloccensin k (7).[2a,3]

Additionally, the density, viscosity and calorific value of the Andiroba oil are quite similar to those of other vegetable oils extracted from traditional seeds, such as soybean and cotton, which has made it an alternative for biodiesel production.[4] Due to its diverse uses and properties, the extractive and sometimes illegal exploitation of Andiroba oil has greatly intensified in recent years. The controlled and sustainable use of this valuable Amazonian oil with unique properties would greatly benefit from its proper characterization and standardization. The proof of authenticity of a particular type of oil, most particularly those from tropical forests or endogenous species, calls for the development of simple, rapid and accurate methods to determine origin, quality and adulteration.

Traditionally, typification and purity of vegetable oils are based on the measurement of the composition of free fatty acids (FFA) as well as of mono- (MAG), di- (DAG) and triacylglycerides (TAG). FFA are obtained *via* TAG hydrolysis and are often derivatized by sterification before chromatography analysis. The intact TAG are also derivatized by esterification and both

[a]*ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas – UNICAMP, Campinas, SP, Brazil. E-mail: eberlin@iqm.unicamp.br; Fax: +55 19-35213073; Tel: +55 19-35213073*

[b]*Laboratory of Engineering and Petroleum Exploration, North Fluminense State University – LENEP/UENF, Macaé, RJ, Brazil*

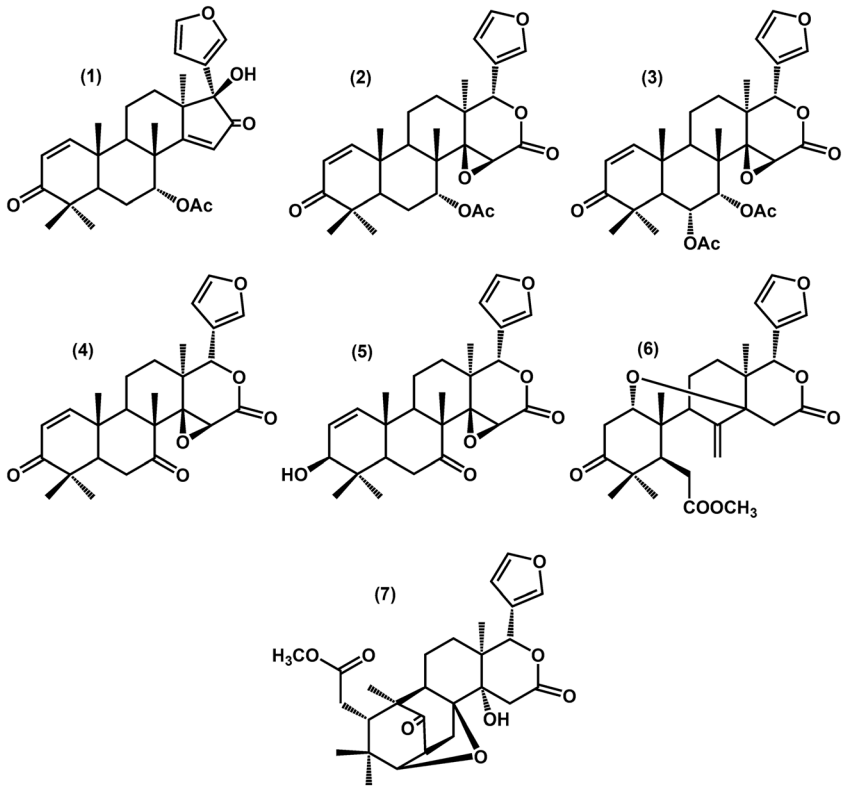
[c]*National Institute of Metrology, Quality and Technology – INMETRO, Division of Chemistry, Duque de Caxias, RJ, Brazil*

[d]*Laboratory of Natural Products, Institute of Chemistry, University of Campinas – UNICAMP, Campinas, SP, Brazil*

[e]*Instituto de Tecnologia – Universidade Federal do Pará – UFPA, Belém, PA, Brazil*

[f]*Department of Chemistry, Federal University of Pernambuco – UFPE, Recife, PE, Brazil*

† Present address: Department of Chemistry, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada.



Scheme 1 Limonoids found in *C. guianensis* species.

the derivatized FFA and TAG are subsequently analyzed by gas chromatography (GC) commonly with a flame ionization detector (FID)[5] or *via* GC coupled to mass spectrometry (GC-MS).[6] Intact TAG are also analyzed most commonly by high performance liquid chromatography (HPLC).[7] But these methods based on derivatization and separation techniques are considerably demanding in terms of time and reagent/solvent consumption. New methodologies that require no or minimal sample preparation offering higher simplicity and speed of analysis with comparable accuracy in the determination of TAG contents would simplify and speed up the oil analysis.

Mass spectrometry (MS) has become a major separation plus characterization technique for the analysis of complex chemical mixtures mainly due to its unmatched ability to separate by mass and charge (m/z), detect, count and characterize molecules of many types, compositions and sizes.[8] Direct MS analysis using soft ionization techniques performed under atmospheric conditions, such as electrospray ionization (ESI-MS), of complex mixtures has been shown to provide fast and reliable fingerprint characterization of complex mixtures *via* distinctive chemical profiles.[9] We have successfully used the MS fingerprinting approach with direct infusion electrospray ionization (ESI) applied for sample solutions to characterize numerous samples with reliable qualitative distinction.[10] Recently, MS fingerprinting has been further simplified with the development of a series of new desorption/ionization techniques applied to samples in their natural matrices or placed on auxiliary surfaces and a new field known collectively as ambient mass spectrometry[11] has been established. We have introduced one of such techniques termed easy ambient sonic-spray ionization (EASI).[12] Being based on sonic spray ionization,[13] EASI is assisted only by compressed nitrogen (or air) and creates, *via* sonic spraying of a polar solvent, a bipolar stream of minute charged droplets that bombard the sample surface.[14] Compared with desorption electrospray ionization (DESI), desorption and ionization by sonic-spray ionization in EASI is advantageous since it uses neither heating nor high voltages at the spray

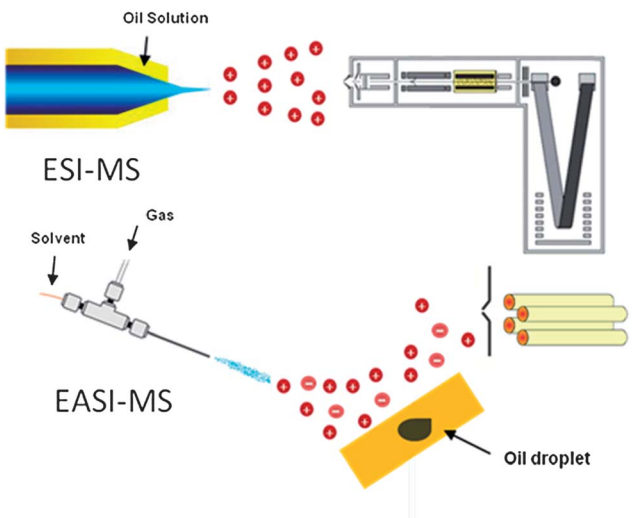


Fig. 1 Schematic of ESI(±)-MS and EASI(±)-MS fingerprinting tested for the typification and quality control of the Andiroba oil.

capillary,[12] as well as its very simple apparatus.[15] Another advantage of EASI is its freedom from electrical, discharge, thermal or oxidation interferences and its improved signal-to-noise ratios.[16] We have applied EASI to the analysis of vegetable oils,[17] lipids,[18] fuels[19] and biodiesel.[20]

This study aimed to investigate and compare the suitability of two direct mass spectrometry techniques, direct infusion ESI-MS of sample solutions and extracts and EASI-MS with direct desorption and ionization of a droplet of the sample resting on an auxiliary paper surface (Fig. 1) to characterize the Andiroba oil *via* typical profiles of its natural markers. The goal was to establish fast, simple and accurate techniques for use in the typification, standardization and quality control of the Andiroba oil, a valuable and environmentally important Amazonian product.

Materials and methods

Plant material

Fresh Andiroba oils were extracted from seeds collected between 25 and 30 April 2008 at twelve different locations (islands) of São Sebastião da Boa Vista – Marajó/PA, Brazil. Five samples of commercial soybean oil and one sample of refined Andiroba oil were also analyzed for comparison and adulteration screening.

Andiroba oil extraction

The fresh seeds were previously peeled and stored at −18 °C. Approximately 70 g of sample were weighed, cut into small pieces and crushed into uniform size. The resulting material was then primed in an extraction cartridge, and placed in cold extraction coupled to a condenser at the top and bottom of a flask of 1000 mL containing porcelain beads and 600 mL of hexane. The balloon was heated by a heater blanket for *ca.* 4 h of continuous extraction. After completion of extraction, the solvent was distilled out and the oil was obtained and filtered after addition of sodium sulfate. The average extraction yield for the oil was 49.1 ± 3.2%.

Sample preparation

Aqueous extracts. 200 μL of oil was diluted in 1 mL of a mixture of methanol–H_2O (1 : 1) with 0.1% of an additive: formic acid for ESI(+) or ammonium hydroxide for ESI(−). This solution was subjected to agitation in a vortex for 1 min and then centrifuged for an additional 1 min. A 10 mL aliquot of the aqueous phase was diluted in 1 mL of methanol with 0.1% of additive.

Direct oil analysis. 20 μL of the oil was diluted with 1 mL of toluene and the solution was subjected to agitation in a vortex for 1 min. A 10 mL aliquot was diluted in 1 mL of methanol with 0.1% of additive. For ESI(+)-MS, 10 μL of a NaCl saturated aqueous solution was added.

ESI-MS. The analyte solutions were directly infused to the ESI source, and the total time for acquisition of each spectrum was set at 1 min. The ESI-MS and ESI-MS/MS were acquired in the negative or positive ion mode using a QTof (Micromass, Manchester, UK) mass spectrometer. The operation conditions were: 3.0–4.0 kV capillary voltage, 100 °C source temperature, desolvation temperature of 100 °C and cone voltage of 20–40 V. Diluted samples were injected by using an automatic injection pump (Harvard Apparatus) with a continuous flow of 10 μL min^{-1}. The full scan ESI-MS were acquired in the range of *m*/*z* 50 to 1500. The ESI-MS/MS were acquired from *m*/*z* 50 to a value slightly above the ion under study and with collision energies varying from 10 to 30 eV. Argon was used as the collision gas. Spectra were processed using the MassLynx 4.0 software (Waters, Manchester, UK). From each spectrum (TGA profile), after the exclusion of isotopic peaks, the twenty most intense ions were considered and included in the principal component analysis (PCA), which was performed using Pirouette v.3.11 (Infometrix Inc., Woodinville, WA, USA).

EASI-MS. These spectra were collected in the positive ion mode using a unit mass resolution compact single-quadrupole mass spectrometer (Shimadzu LCMS 2010, Japan) equipped with a homemade EASI source assisted only by compressed nitrogen and electrical pumping for the spray solvent, which has been described in detail elsewhere.[12] A tiny droplet of the sample (2 μL) was dipped directly onto a paper surface (brown Kraft envelope paper). The solvent (methanol) flow rate was 20 μL min^{-1}, N_2 was used as a nebulizing gas at 3 L min^{-1}, and the paper-entrance angle was ~30°. Mass spectra were accumulated over 60 s and scanned over the *m*/*z* 50–1200 range.

Oxidative stability

This stability was evaluated by measuring the oxidation induction period (IP) with the use of Rancimat apparatus (Metrohm 873) as described by EN14112 (36). Andiroba and soybean oil samples (3 g) were heated to 110 °C, air was then passed through the samples at a flow rate of 20 L h^{-1}, and then through a trap containing a water solution. The kinetics of oxidation was followed by the sudden increase in conductivity of the water as a result of the formation of volatile acidic organic compounds. All determinations were performed in duplicate and the mean value is reported. The IP of oil samples was also determined. The analysis of variance (ANOVA) and *t*-test were used to verify whether Rancimat results were significantly different and it was performed using Assistat v.7.5 (DEAG-CTRN-UFCG, Campina Grande/PA, Brazil) (37).

Results and discussion

To properly search for and characterize biomarker profiles that would indeed represent most typical natural components, samples of the Andiroba oil were extracted directly from fresh Andiroba seeds. Analysis was therefore performed for twelve samples of authentic fresh Andiroba oils, one sample of commercial refined Andiroba oil and five samples of commercial soybean oil.

ESI(+)-MS of the oil solution in methanol was found to favor ionization *via* both $[M + H]^+$ and $[M + Na]^+$. This "dual" ionization for each single analyte compromises reproducibility in MS fingerprinting hence solutions were spiked with traces of

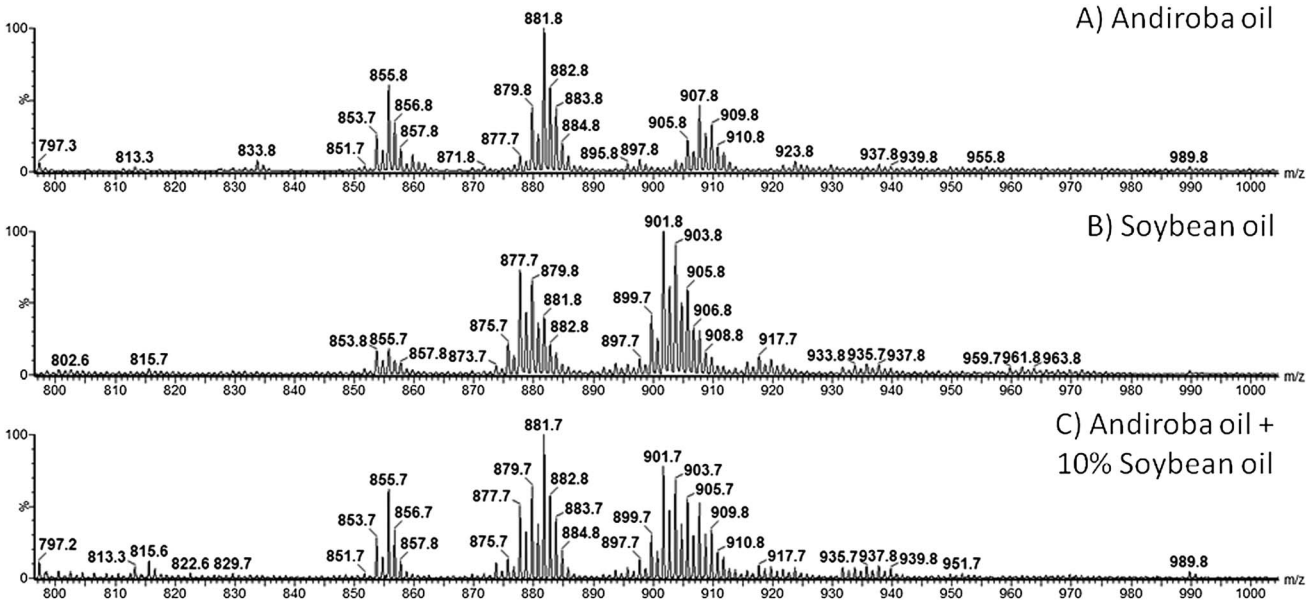


Fig. 2 ESI(+)-MS of methanol–water solutions of Andiroba and soybean crude oils and of an admixture of fresh Andiroba oil with 10% (w/w) of soybean oil.

NaCl to favor detection predominantly *via* $[M + Na]^+$. As Fig. 2 illustrates, characteristic and quite contrasting profiles of $[TAG + Na]^+$ ions were obtained for the spiked solutions of the authentic Andiroba and soybean oils. Similar TAG profiles for the fresh and refined oils were also obtained, as expected. The TAG profile of the Andiroba oil is dominated by TAG composed of palmitic, oleic and linoleic acids, that is, by a set of $[TAG + Na]^+$ ions: PPL of *m*/*z* 853, PPO of *m*/*z* 855, PLO of *m*/*z* 879, POO of *m*/*z* 881, POS of *m*/*z* 883, OOL or LLS of *m*/*z* 905, OOO or SOL of *m*/*z* 907 and OOS or SSL of *m*/*z* 909. For soybean oil, ESI(+)-MS detects mainly the following $[TAG + Na]^+$ ions: PLL of *m*/*z* 877, PLO of *m*/*z* 879, LLLn of *m*/*z* 899, LLL or OLLn of *m*/*z* 901, LLO or OOLn of *m*/*z* 903 and OOL of *m*/*z* 905.

The TAG profiles were also very reproducible. All 14 samples of the authentic fresh Andiroba oil, as well as the refined sample, displayed a similar ESI(+)-MS profile. These characteristics allow the use of ESI(+)-MS to detect adulteration of the Andiroba oil (*C. guianensis*) with as low as 10% of soybean oil (Fig. 2C). TGA attributions were done based on ESI-MS/MS experiments (not shown) and comparison with reported data.[4,10d] Table 1 summarizes the main TAG identified from the ESI(+)-MS profiles.[1,17b]

Fig. 3 displays a statistical evaluation *via* PCA[21] of the ESI(+)-MS fingerprinting data to test its ability to characterize Andiroba oil and soybean oil as well as adulteration at a level as low as 10%. Note that both types of oils were very well resolved with proper grouping, as well as the adulterated samples. This confirms the ability of ESI(+)-MS fingerprinting to provide proper standardization and quality control for the Andiroba oil which, in Brazil, is commonly subject to adulteration *via* admixtures with the cheaper soybean oil.

ESI(−)-MS of the aqueous extracts (diluted in methanol) of the Andiroba oil was also obtained to test the ability of the technique to provide characterization *via* a more comprehensive set of natural markers. Indeed, ESI(−) was found to provide a quite rich, reproducible and characteristic profile of marker ions for the aqueous extract of the fresh Andiroba oil (Fig. 4A) in which deprotonated molecules $[M - H]^-$ of free fatty acid (FFA) as well as a diverse set of natural products, mainly limonoids, predominate. Also characteristic was the ESI(−)-MS profile of the refined oil (Fig. 4B) in which the

Table 1 Assignment and relative percentages of the main $[TAG + Na]^+$ ions detected by ESI(+)-MS of methanol–water solutions of Andiroba and soybean oils spiked with NaCl

$[M + Na]^+$ *m*/*z*	Formula	TAG[a]	CN/DB[b]	Andiroba[c] (%)	Soybean[c] (%)
851	$C_{53}H_{96}O_6$	PPLn	50 : 03 : 00	1.0	0.6
853	$C_{53}H_{98}O_6$	PPL	50 : 02 : 00	4.6	3.2
855	$C_{53}H_{100}O_6$	PPO	50 : 01 : 00	14.5	5.1
857	$C_{53}H_{102}O_6$	PPS	50 : 00 : 00	2.2	0.9
873	$C_{55}H_{94}O_6$	LnLnP	52 : 06 : 00	0.0	0.8
877	$C_{55}H_{98}O_6$	PLL	52 : 04 : 00	0.0	13.9
879	$C_{55}H_{100}O_6$	PLO	52 : 03 : 00	8.8	12.5
881	$C_{55}H_{102}O_6$	POO	52 : 02 : 00	25.0	0.6
883	$C_{55}H_{104}O_6$	POS	52 : 01 : 00	11.2	3.6
885	$C_{55}H_{106}O_6$	PSS	52 : 00 : 00	5.3	1.8
899	$C_{57}H_{96}O_6$	LLLn	54 : 07 : 00	0.0	6.2
901	$C_{57}H_{98}O_6$	LLL	54 : 06 : 00	0.0	15.5
903	$C_{57}H_{100}O_6$	LLO, OOLn	54 : 05 : 00	0.7	15.1
905	$C_{57}H_{102}O_6$	OOL, LLS	54 : 04 : 00	4.2	11.7
907	$C_{57}H_{104}O_6$	OOO, SOL	54 : 03 : 00	11.0	5.5
909	$C_{57}H_{106}O_6$	OOS, SSL	54 : 02 : 00	8.0	2.7
911	$C_{57}H_{108}O_6$	SSO	54 : 01 : 00	3.5	0.4

[a] Abbreviations of fatty acids: P, palmitic acid; O, oleic acid; S, stearic acid; L, linoleic acid; Ln, linolenic acid. [b] Carbon number/number of double bonds of the three fatty acid moieties. [c] Relative intensity comes from the comparison to the most intense peak normalized to 100%.

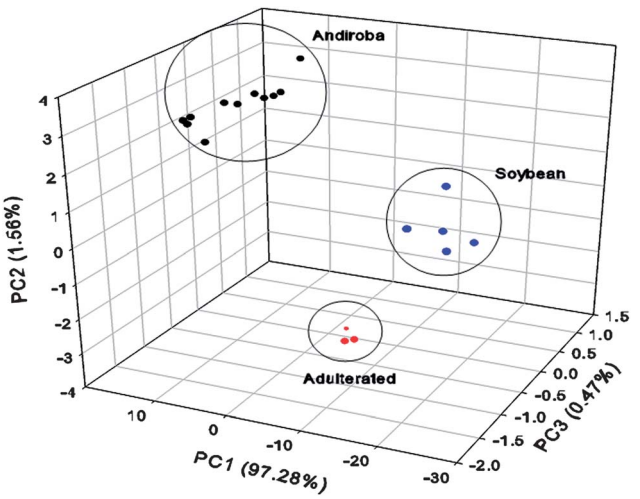


Fig. 3 PCA for the ESI(+)-MS data of fresh Andiroba oil, soybean oil and Andiroba oil adulterated with 10% of soybean oil.

FFA ions again predominate, but due to oil refining, the set of limonoid ions are no longer detected.

Note in Fig. 4A and B that the ions of *m*/*z* 253, 255, 269, 279, 281 and 283 are, respectively, the deprotonated molecules $[M - H]^-$ of palmitoleic, palmitic, margaric, linoleic, oleic and stearic acids. The ions of *m*/*z* 511, 537 and 563 are dimeric $[2M - H]^-$ species.

ESI(+)-MS fingerprints were also collected in the *m*/*z* 200–600 range for the aqueous extracts of both the crude and refined Andiroba oils (Fig. 4C and D). In these spectra, the limonoids are again detected now as their protonated molecules $[M + H]^+$, with greater abundance for the fresh oil. The ion of *m*/*z* 439 was assigned to 7-oxogedunin (4), *m*/*z* 483 to gedunin (2) and *m*/*z* 541 to 6α-acetogedunin (3). Ions of *m*/*z* 457, 467, 469, 471 and 489 could not be unambiguously assigned, but they are likely due to limonoids since their ESI(+)-MS/MS displayed similar dissociation patterns as those of the protonated molecules of 2–4. Fig. 5A shows a typical ESI(+)-MS/MS for protonated 7-oxogedunin (4). Note the great similarity to the spectrum of standard 4 isolated from the Andiroba oil.[2a,3]

Samples of the fresh and refined Andiroba oil and commercial refined soybean oil were also subjected to the oxidation test. The induction periods of fresh Andiroba oil (13.2[a]), refined Andiroba oil (3.3[c]) and refine soybean oil (9.5[b]) were significantly different. The contrasting differences in oxidation stability between the fresh and refined Andiroba oils are most likely due to the presence (or absence) of limonoids (Fig. 4) which are natural antioxidants for this valuable Amazonian oil.

Fig. 6 shows a typical EASI(+)-MS fingerprint for a droplet of fresh and oxidized Andiroba oil obtained in the lower resolution but more compact and less expensive monoquadrupole mass spectrometer. The purpose here was to evaluate the ability of the simpler and more direct EASI(±)-MS fingerprinting technique to characterize and to control the quality of the oil from a droplet dipped on a paper surface comparing it with the more conventional direct infusion ESI(±)-MS fingerprinting of sample or extract solutions.[19] The EASI(+)-MS profiles from the

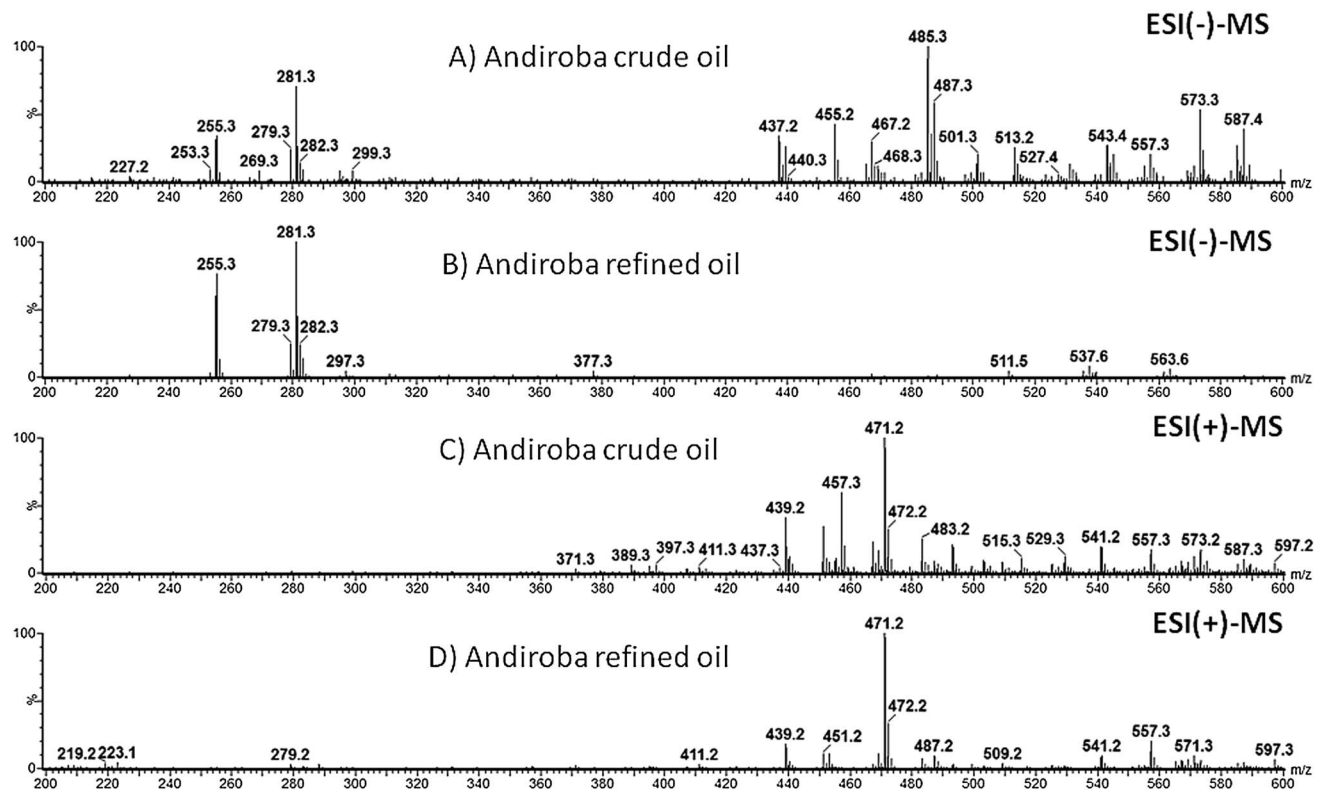


Fig. 4 ESI(−)-MS and ESI(+)-MS of aqueous extracts (diluted in methanol) of crude and refined Andiroba oil.

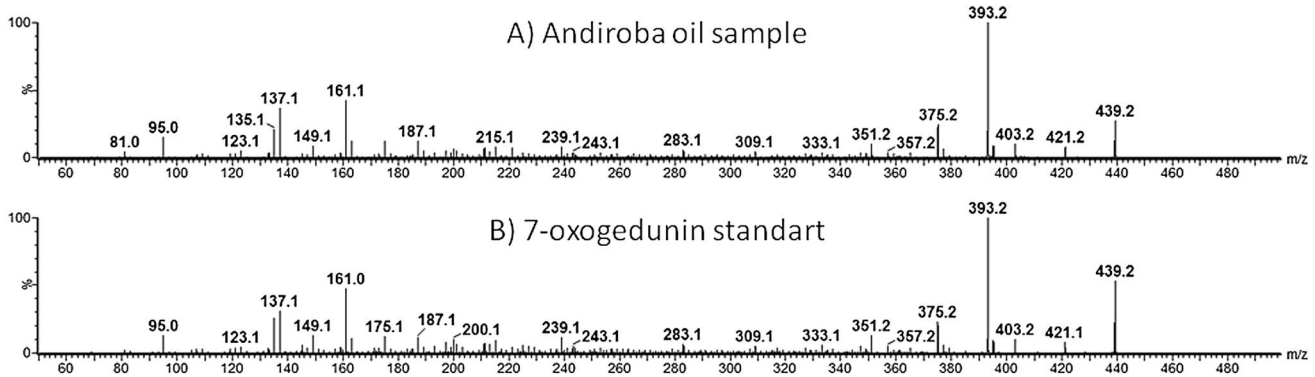


Fig. 5 ESI(+)-MS/MS of (A) the ion of *m/z* 439 from the Andiroba oil and (B) from the 7-oxogedunin standard isolated directly from the fresh Andiroba oil.

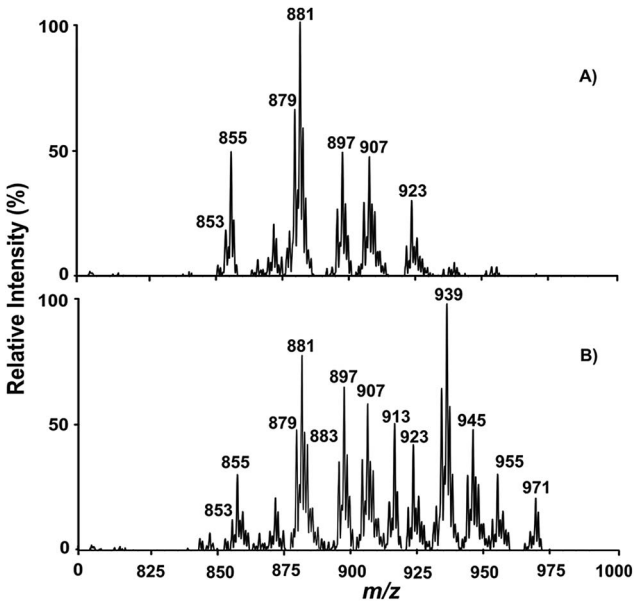


Fig. 6 EASI(+)-MS obtained directly from a droplet of (A) fresh and (B) oxidized Andiroba oil placed on a paper surface.

fresh Andiroba oil (Fig. 6A) were found to be dominated mainly by $[TAG + Na]^+$ ions hence there was no need for NaCl spiking. The TAG profiles obtained *via* EASI(+)-MS fingerprinting were also found to be quite reproducible and similar to those provided by ESI(+)-MS (Fig. 2A) of the NaCl spiked solutions. Hence, due to this similarity, a quite similar PCA plot for the EASI-MS data (not shown) was obtained. Adulteration of the Andiroba oil with soybean oil (spectra not shown) as well as oxidation (Fig. 6B) were also monitored by EASI(+)-MS with quite similar spectra and results. The spectra obtained directly from the oil droplet in the negative ion mode, *i.e. via* EASI(−)-MS were, however, very noisy with some ions associated with FFA and limonoids, but with poor reproducibility.

Conclusions

The direct infusion ESI(+)-MS technique applied to a diluted methanolic solution of the Andiroba oil provides characteristic TAG profiles, and can therefore be used as a fast and reproducible MS fingerprinting approach for the typification and quality control of this valuable Amazonian oil. Sets of additional FFA and limonoid biomarkers can also be screened by ESI(−)-MS of a simple aqueous extract of the oil. Alternatively, the recently described Venturi EASI(−)-MS technique could be used in its liquid mode (V_L-EASI-MS).[22] For the refined oil, as expected, the limonoids are no longer detected but the unique TAG and FFA profiles still can be used to characterize the sample as a refined Andiroba oil. The simpler ambient desorption/ionization EASI(+)-MS technique can also be applied directly to a droplet of the crude sample placed on a paper surface for typification and quality control, but the technique in the negative ion, *viz.* EASI(−)-MS, fails to provide proper profiles of FFA and limonoids which are therefore better obtained *via* ESI(−)-MS. Both techniques in the positive ion mode could detect adulteration of the Andiroba oil with soybean oil at levels as low as 10%. Likely therefore, adulteration with other types of vegetable oils could also be detected. Degradation by oxidation causes major alteration in the TAG profile of the Andiroba oil and could also be readily detected by both ESI(+)-MS and EASI(+)-MS fingerprinting.

Acknowledgements

We thank the State of São Paulo Research Foundation (FAPESP), the Brazilian National Council for Scientific and Technological Development (CNPq) and the Financing Agency of Studies and Projects (FINEP) for financial assistance.

Notes and references

1 C. Penido, K. A. Costa, R. J. Pennaforte, M. F. S. Costa, J. F. G. Pereira, A. C. Siani and M. G. M. O. Henriques, *Inflammation Res.*, 2005, **54**, 295.

2 (*a*) A. R. P. Ambrozin, A. C. Leite, F. C. Bueno, P. C. Vieira, J. B. Fernandes, O. C. Bueno, M. F. d. G. F. Da Silva, F. C. Pagnocca, M. J. A. Hebling and M. B. J. Junior, *J. Braz. Chem. Soc.*, 2006, **17**(3), 542–547; (*b*) J. Bickii, N. Njifutie, J. A. Foyere, L. K. Basco and P. J. Ringwald, *J. Ethnopharmacol.*, 2000, **69**, 27; (*c*) B. Gilbert, D. F. Teixeira, E. S. Carvalho, A. E. S. Paula, J. F. G. Pereira,

J. L. P. Ferreira, M. B. S. Alameida, R. S. Machado and V. Cascon, *An. Acad. Bras. Cienc.*, 1999, **71**, 265; (*d*) S. MacKinnon, T. Durst and J. T. Arnason, *J. Nat. Prod.*, 1997, **60**, 336; (*e*) M. D. Moura, J. Souza-Silva, R. A. G. De Oliveira, M. F. F. M. Diniz and J. M. Barbosa-Filho, *Acta Farm. Bonaerense*, 2002, **27**, 61.

3 (*a*) D. Lavie, E. C. Levy and R. Zelnik, *Bioorg. Chem.*, 1972, **2**, 59; (*b*) B. G. Marcelle and B. S. Moto, *Phytochemistry*, 1975, **14**, 2717; (*c*) W. D. Ollis, A. D. Ward, H. M. De Oliveira and R. Zelnik, *Tetrahedron*, 1970, **26**, 1637; (*d*) W. D. Ollis, A. D. Ward and R. Zelnik, *Tetrahedron Lett.*, 1964, **37**, 2607; (*e*) S. H. Qi, D. G. Wu, Y. B. Ma and X. D. Luo, *Acta Bot. Sin.*, 2003, **45**, 1129; (*f*) S. H. Qi, D. G. Wu, S. Zhang and X. D. Luo, *Pharmazie*, 2004, **59**, 488.

4 D. Firestone, *Physical and chemical characteristics of oils, fats and waxes*, AOCS Press, Washington, D.C, 1999.

5 B. B. Mlayah, D. Bereau, J. Banoub and R. J. Bravo, *J. Am. Oil Chem. Soc.*, 2003, **80**, 49.

6 J. Gamazo-Vázquez, M. S. Garcia-Falcón and J. Simal-Gándara, *Food Control*, 2003, **14**, 463.

7 (*a*) E. Christopolou, M. Lazaraki, Y. Komatis and K. Kaselimis, *Food Chem.*, 2004, **84**, 463; (*b*) A. Romani, N. Mulinacci, C. Galardi, F. F. Vincieri, L. Liberatore and A. Cichelli, *Chromatographia*, 2001, **53**, 279.

8 (*a*) A. G. Marshall and R. P. Rodgers, *Acc. Chem. Res.*, 2004, **37**, 53; (*b*) Y. E. Corilo, B. G. Vaz, R. C. Simas, H. D. L. Nascimento, C. F. Klitzke, R. C. L. Pereira, W. L. Bastos, E. V. Santos Neto, R. P. Rodgers and M. N. Eberlin, *Anal. Chem.*, 2010, **82**, 3990.

9 (*a*) R. Flamini, F. Agnolin, R. Seraglia, M. De Rosso, A. Panighel, F. De Marchi, A. D. Vedova and P. Traldi, *Rapid Commun. Mass Spectrom.*, 2012, **26**, 355; (*b*) L. Mattoli, F. Cangi, C. Ghiara, M. Burico, A. Maidecchi, E. Bianchi, E. Ragazzi, L. Bellotto, R. Seraglia and P. Traldi, *Metabolomics*, 2011, **7**, 437; (*c*) J. C. F. Mendonça, A. S. Franca, L. S. Oliveira and M. Nunes, *Food Chem.*, 2008, **111**, 490; (*d*) M. J. Lerma-García, G. Ramis-Ramos, J. M. Herrero-Martínez and E. F. Simó-Alfonso, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 973.

10 (*a*) R. R. Catharino, H. M. S. Milagre, S. Saraiva, C. M. Garcia, U. Schuchardt, R. Augusti, R. A. Cardoso, M. Guimarães, G. F. de Sá, J. M. Rodrigues, V. de Souza and M. N. Eberlin, *Energy Fuels*, 2007, **21**, 3698; (*b*) A. S. Araújo, L. L. Rocha, D. M. Tomazela, A. C. H. F. Sawaya, R. R. Almeida, R. R. Catharino and M. N. Eberlin, *Analyst*, 2005, **130**, 884; (*c*) R. Garrett, B. G. Vaz, A. M. C. Hovell, M. N. Eberlin and C. M. Rezende, *J. Agric. Food Chem.*, 2010, **60**, 4253; (*d*) S. A. Saraiva, E. C. Cabral, M. N. Eberlin and R. R. Catharino, *J. Agric. Food Chem.*, 2009, **57**, 4030.

11 (*a*) R. M. Alberici, R. C. Simas, G. S. Sanvido, W. Romão, P. M. Lalli, M. Benassi, I. B. S. Cunha and M. N. Eberlin, *Anal. Bioanal. Chem.*, 2010, **398**, 2654; (*b*) G. A. Harris, L. Nyadong and F. M. Fernandez, *Analyst*, 2008, **133**, 1297; (*c*) D. R. Ifa, C. P. Wu, Z. Ouyang and R. G. Cooks, *Analyst*, 2010, **135**, 669; (*d*) G. A. Harris, A. S. Galhena and F. M. Fernandez, *Anal. Chem.*, 2011, **83**, 4508.

12 (*a*) R. Haddad, R. Sparrapan and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2901; (*b*) R. Haddad, R. Sparrapan, T. Kotiaho and M. N. Eberlin, *Anal. Chem.*, 2007, **80**, 898.

13 A. Hirabayashi, M. Sakairi and H. Koizumi, *Anal. Chem.*, 1994, **66**, 4557.

14 R. Haddad, R. R. Catharino, L. A. Marques and M. N. Eberlin, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 3662.

15 N. V. Schwab, A. M. Porcari, M. B. Coelho, E. M. Schmidt, J. L. Jara, J. V. Visentainer and M. N. Eberlin, *Analyst*, 2012, **137**, 2537.

16 (*a*) M. B. Sorensen, P. Aaslo, H. Egsgaard and T. Lund, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 455; (*b*) S. P. Pasilis, V. Kertesz and G. J. Van Berkel, *Anal. Chem.*, 2008, **80**, 1208.

17 (*a*) M. F. Riccio, S. A. Saraiva, L. A. Marques, R. Alberici, R. Haddad, J. C. Moller, M. N. Eberlin and R. R. Catharino, *Eur. J. Lipid Sci. Technol.*, 2010, **112**, 434; (*b*) R. C. Simas, R. R. Catharino, I. B. S. Cunha, E. C. Cabral, D. Barrera-Arellano, M. N. Eberlin and R. M. Alberici, *Analyst*, 2010, **135**, 738.

18 (*a*) L. C. Alberici, H. C. F. Oliveira, R. R. Catharino, A. E. Vercesi, M. N. Eberlin and R. M. Alberici, *Anal. Bioanal. Chem.*, 2011, **401**, 1651; (*b*) K. Schäfer, J. Balog, T. Szaniszló, D. Szalay, G. Mezey, J. Dénes, L. Bognár, M. Oertel and Z. Takáts, *Anal. Chem.*, 2011, **83**, 7729.

19 (*a*) A. M. A. P. Fernandes, D. U. Tega, J. L. P. Jara, I. B. S. Cunha, G. F. de Sá, R. J. Daroda, M. N. Eberlin and R. M. Alberici, *Energy Fuels*, 2012, **26**, 3042; (*b*) R. M. Alberici, R. C. Simas, V. de Souza, G. F. de Sá, R. J. Daroda and M. N. Eberlin, *Anal. Chim. Acta*, 2010, **659**, 15.

20 (*a*) L. S. Eberlin, P. V. Abdelnur, A. Passero, G. F. de Sá, R. J. Daroda, V. de Souza and M. N. Eberlin, *Analyst*, 2009, **134**, 1652; (*b*) P. V. Abdelnur, L. S. Eberlin, G. F. de Sá, V. de Souza and Marcos. N. Eberlin, *Anal. Chem.*, 2008, **80**, 7882.

21 (*a*) K. Gorzolka, M. Lissel, N. Kessler, S. Loch-Ahring and K. Niehaus, *J. Biotechnol.*, 2012, **159**, 177; (*b*) A. Farag Mohamed and A. Wessjohann Ludger, *Planta Med.*, 2012, **78**, 488.

22 V. G. Santos, T. Regiani, F. F. G. Dias, W. Romão, C. F. Klitzke, F. Coelho and M. N. Eberlin, *Anal. Chem.*, 2011, **83**, 1375.