

Brazil Nut Oil: Quality Control via Triacylglycerol Profiles Provided by Easy Ambient Sonic-Spray Ionization Mass Spectrometry

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ABSTRACT: Brazil nut oil is one of the important Amazonian natural products in the global market. Despite its health benefits and applications in food and cosmetic industries, authentication and quality control of the oil are far from satisfactory. Several samples of Amazonian Brazil nut oil (authentic oils of different geographic origins, commercial oils, and oils adulterated with soybean oil) were evaluated by easy ambient sonic-spray ionization mass spectrometry (EASI-MS). The samples were characterized on the basis of triacylglycerol profiles, and their major ions were subjected to chemometric treatment (principal component analysis). The ambient mass spectrometry analysis and the set of major ions allowed the characterization of authentic oils, commercial oils, and adulterated oils (5% soybean oil) in a few minutes and without sample preparation. Therefore, it is shown that quality control of Amazonian vegetable oils can be readily accomplished by EASI-MS.

KEYWORDS: ambient mass spectrometry, sonic-spray ionization, Amazonian Brazil nut oil, triacylglycerol

■ INTRODUCTION

Brazil nut (*Bertholletia excelsa* Bonpl., Lecythidaceae) is a large tree native to the Amazon region, reaching more than 30 m in height. Its fruits, because of the characteristic and pleasant flavor and nutritional value, are not only consumed by local people but have also found industrial uses, both nationally and internationally.¹ Because the tree requires natural pollinators (specific bees) for fruit production, the Brazil nut is collected exclusively from natural forests.² Similarly to other Amazonian fruit trees or palm species, such as andiroba, cupuaçu, and buriti, the Brazil nut also provides oil that is highly valued in food and cosmetic markets due to its unique composition and health benefits.³ However, the achievement of reliable compositional quality of these oils is difficult because of thermal oxidation or inconsistent handling during extraction, transportation, and storage. Variation in the oil's composition is also associated with seasonality, age of the plants, and soil characteristics.⁴ For these reasons and to increase the reliability of Amazonian products, it is therefore important to guarantee the quality of the Brazil nut oil and authenticate its geographic origin and purity.⁵

Mass spectrometry (MS) has proven to be a powerful tool for characterizing vegetable oils.^{6–8} Preseparation is normally performed, but direct MS analysis such as via electrospray ionization mass spectrometry (ESI-MS) has been used for classification of vegetable oils and determination of levels of adulteration and aging using water/methanol (1:1) extracts. These extracts are rich in free fatty acids (FFA) and phenolic compounds.⁹ Unique triacylglycerol (TAG) profiles of oils (Brazil nut, buriti, andiroba, and passion fruit) and fats

(murumuru, ucuúba, and cupuaçu) have been determined by matrix-assisted laser ionization mass spectrometry (MALDI-MS).¹⁰

Recently, direct MS analysis has been further simplified with the development of a series of new desorption/ionization techniques for MS analysis, and a new field known collectively as ambient MS^{11–13} has been established. We have introduced one of such techniques termed easy ambient sonic-spray ionization (EASI).^{14,15} EASI is based on sonic-spray ionization,¹⁶ and is therefore assisted only by compressed nitrogen (or air). It creates a bipolar stream of charged droplets (cations and anions) that bombard the sample surface via sonic spraying of a polar solvent, most typically methanol.¹⁵ The main advantages of EASI are its very simple apparatus and the use of no heating, radiation, or voltages, hence its freedom from electrical, discharge, thermal, or oxidation interferences and much reduced solvent noise with improved signal-to-noise ratios.¹⁴ EASI-MS requires no sample preparation; the analyses are therefore performed directly for a small droplet of the crude, untreated sample dipped on a paper surface. EASI is one of the simplest, gentlest, and most easily implemented ionization sources for ambient MS, and numerous applications are described elsewhere,¹¹ mainly in vegetable oils and animal fats analysis.^{17–24} In this work, we tested the applicability of

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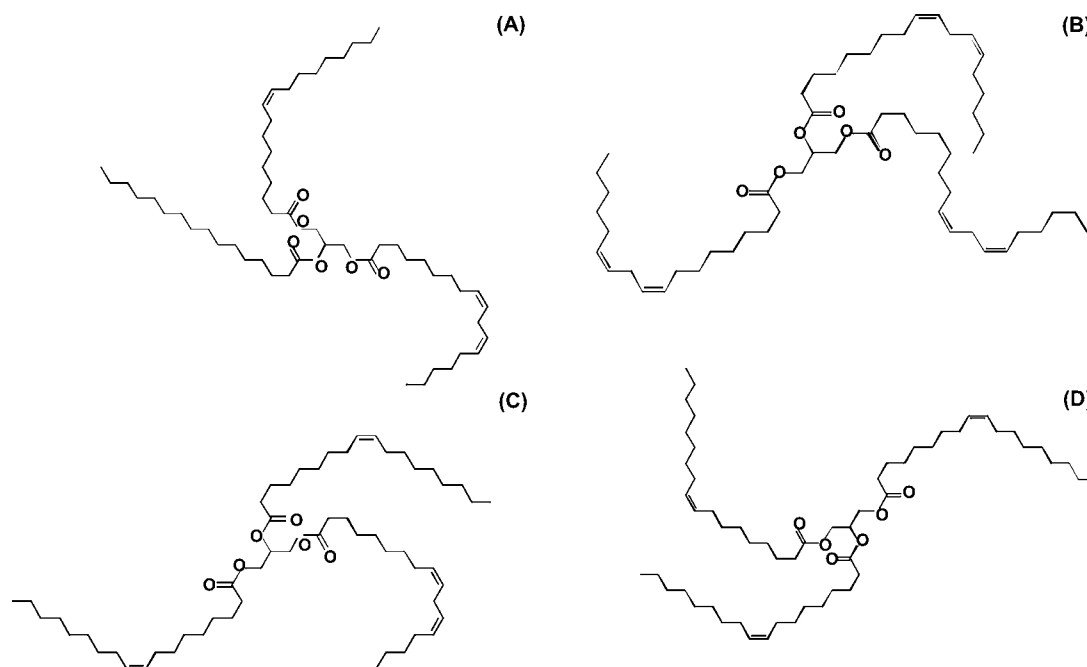
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Table 1. Main TAG in the Pure Brazil Nut Oil and Adulterated with Different Amounts of Soybean Oil Obtained by EASI(+)-MS

[TAG + Na] ⁺ <i>m/z</i>	relative abundance (%)							
	0% ^a	2%	5%	10%	20%	30%	50%	soybean
877	26	32	42	44	62	50	53	29
879	80	53	49	72	87	90	47	14
881	61	59	49	37	77	62	34	0
899		8	9	13	15	22	25	19
901	16	12	39	40	58	89	100	100
903	55	46	54	47	60	100	90	77
905	94	77	60	72	100	89	57	39
907	100	100	100	100	82	90	35	9
909	24	24	11	36	32	34	16	0
901/907	0.16	0.12	0.39	0.40	0.71	0.99	2.86	11.07

^aBrazil nut oil from São João da Baliza-RR.**Table 2.** Main TAG in the Authentic Oils of Different Geographic Origin and Commercial Oils of Brazil Nut Oil Obtained by EASI(+)-MS

[TAG + Na] ⁺ <i>m/z</i>	TAG ^a	ACN/DBN ^b	relative abundance (%)									
			Rio Preto da Eva	Manicoré (1)	Manicoré (2)	São João da Baliza	Caroebe	IND A	IND B	IND C	IND D	IND E
877	PLL	52:4	6	43	32	26	17	42	32	28	33	32
879	PLO	52:3	35	56	47	80	40	51	53	54	39	41
881	POO	52:2	55	44	36	61	48	20	24	18	12	14
899	LLLn/ OLnLn	54:7									4	4
901	LLL/OLLn	54:6	25	27	16	16	14	59	54	56	40	40
903	OLL/OOLn	54:5	39	59	79	55	26	100	100	100	96	100
905	OOL/LLS	54:4	97	100	100	94	100	96	96	95	100	98
907	OOO/OLS	54:3	100	69	54	100	31	59	40	43	44	48
909	OOS/SSL	54:2	22	21	30	24	15	19	25	25	14	16

^aFA abbreviations: P = palmitic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid, and S = stearic acid. ^bAcyl carbon number/double bonds number of the three FA moieties.**Figure 1.** Representative structures of TAG identified by EASI-MS in the Brazil nut oil: (A) *m/z* 881 (PLO), (B) *m/z* 901 (LLL), (C) *m/z* 905 OOL, and (D) *m/z* 907 (OOO).

EASI-MS for the characterization and quality control of Brazil nut oils.

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC)-grade methanol and hexane were purchased from Merck SA and used without further purification.

Plant Material and Oil Extraction. Five authentic oils were prepared by extraction of Brazil nuts from different geographic origins in the Brazilian Amazon [i.e., Rio Preto da Eva-AM, Manicoré-AM (two samples), São João da Baliza-RR, and Caroebe-RR] according to the following procedure. The samples were dried in a ventilated stove at 40 °C. The samples were milled and extracted with 1 L of hexane using a Soxhlet apparatus. Two hundred grams of triturated sample was extracted using hexane in the proportion of 1:5 m/v, at 5 h and 69 °C. All samples were extracted in triplicate. There were a total of five commercial oil samples, four of which were acquired from different oleochemical suppliers: Pharmakos D'Amazônia (Manaus, Brazil), Beraca (two samples, São Paulo, Brazil), and Covema (Manicoré, Brazil), and one was obtained from the local market (Alta Floresta, Brazil). Adulterated samples were prepared by adding different amounts of commercial soybean oil (2, 5, 10, 20, 30, and 50%) to the authentic Brazil nut oil from São João da Baliza-RR.

EASI-MS Analysis. Experiments were performed on a single-quadrupole mass spectrometer LCMS-2010 (Shimadzu) with an in-house-generated EASI source^{14,15} operated in the positive ion mode. Nebulizing gas (N₂) 3 L/min and a methanol flow rate of 20 μL/min were used to form the sonic spray. The surface-entrance angle was ca. 30°. Oils (2 μL) without any sample preparation were dropped on a paper surface (brown Kraft envelope paper), and mass spectra were collected for about 30 s, initially scanning over the range of *m/z* 50–1000. As EASI is a soft ionization source, no TAG fragments were observed in the spectra, and TAG were observed only at the range of *m/z* 800–1000. The results were obtained from triplicate measurement. The average coefficient of variation (ACV) of the EASI(+)-MS analysis is ca. 10%. For classification of oil samples, the Pirouette v.4.0 program (Infometrix, Seattle, WA) was utilized to perform principal component analysis (PCA) of the data. Seventeen oil samples (objects) and nine *m/z* ions (variables) were considered (Tables 1 and 2). Figure 1 shows representative structures of the main TAG identified in this study by EASI-MS.

RESULTS AND DISCUSSION

Oils extracted from Brazil nuts of different geographic origin, Brazil nut oils supplied by oleochemical industries, and Brazil nut oils adulterated with soybean oil were analyzed. Initially, samples of authentic Brazil nut oil were analyzed. Figure 2B shows a representative EASI(+) mass spectrum of a sample from the city of São João da Baliza in the state of Roraima in Brazil. The ions observed in the *m/z* 800–1000 range correspond to TAG, which were detected mainly in the form of sodium adducts [TAG + Na]⁺. Potassium adducts [TAG + K]⁺ were also detected with minor abundances. This characteristic and rich EASI(+)-MS TAG profile is very similar to that obtained by MALDI-MS.¹⁰ The main [TAG + Na]⁺ ions are assigned as follows: *m/z* 877 [palmitodilinolein (PLL), C52:4]; *m/z* 879 [palmitolinoleo-olein (PLO), C52:3]; *m/z* 881 [dioleopalmitin (POO), C52:2]; *m/z* 901 [trilinolein (LLL) or oleolinoleolinolenin (OLLn), C54:6]; *m/z* 903 [oleodilinolein (OLL) or dioleolinolenin (OOLn), C54:5]; *m/z* 905 [dioleolinolein (OOL) or dilinoleinstearin (LLS), C54:4]; *m/z* 907 [triolein (OOO) or oleolinoleostearin (OLS), C54:3]; and *m/z* 909 [dioleostearin (OOS) or distereolinolein (SSL), C54:2]. [TAG + K]⁺ were of *m/z* 895 (PLO, C52:3); *m/z* 919 (OLL or OOLn, C54:5); *m/z* 921 (OOL or LLS, C54:4); and *m/z* 923 (OOO or OLS, C54:3). The major FA

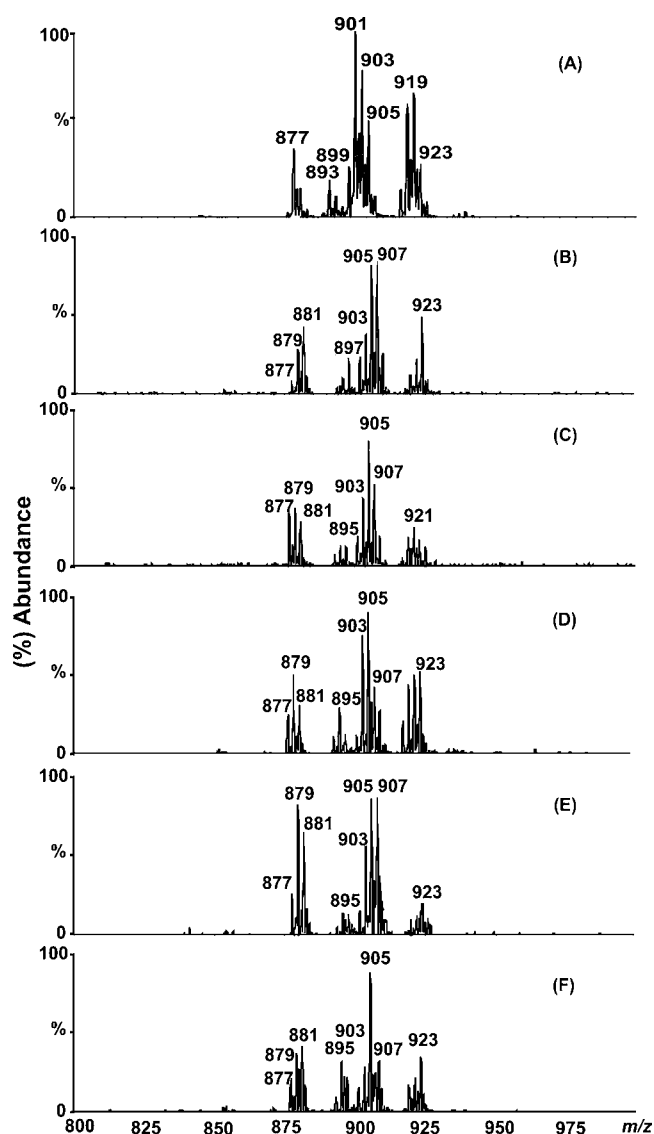


Figure 2. Representative EASI(+)-MS of (A) soybean oil. Brazil nut oil of (B) Rio Preto da Eva-AM, (C) Manicoré (1)-AM, (D) Manicoré (2)-AM, (E) São João da Baliza-RR, and (F) Caroebe-RR.

constituting TAG were oleic, linoleic, and palmitic acids. This result is in accordance with gas chromatography analysis of the total FA content of Brazil nut oil, which found a composition of oleic acid (31–56%), linoleic acid (26–46%), and palmitic acid (14–16%).²⁵ As an illustrative example of the uniqueness of the TAG profile of this Amazonian oil, Figure 2A shows EASI(+)-MS of soybean oil, which was chosen here since it represents the cheapest and most widely commercialized vegetable oil in Brazil. [TAG + Na]⁺ ions are common in both spectra, but very significant differences in the relative abundance of the several ions such as those of *m/z* 879, 881, 901, 905, and 907 were observed. Note, for instance, the ion of *m/z* 899 [dilinoleinolenin (LLLn) or oleodilinolenin (OLLn), C54:7], which was observed only in soybean oil, can be used as a marker of adulteration of Brazil nut oil with soybean oil. For this goal, oil mixtures prepared from Brazil nut oil and different amounts of soybean oil (2, 5, 10, 20, 30, and 50%) were analyzed by EASI(+)-MS (Table 1). As expected from Figure 2, the relative abundance of the marker ion of *m/z* 899 increases steadily with increasing adulteration, from 0 to 19%. Accordingly, the ratio of

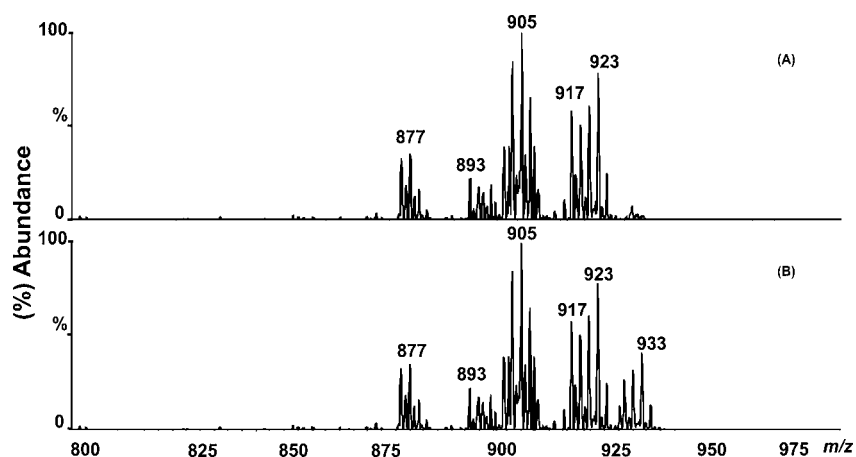


Figure 3. Representative EASI(+)-MS of commercial Brazil nut oil from industry: (A) fresh sample and (B) oxidized sample.

the abundances of the ions of m/z 901 and 907 also increased proportionally, most so at 5% of adulteration oil. Consequently, using these marker ions, adulteration of the Brazil nut oil with soybean oil at a level of 5% or higher can be easily detected.

Figures 2B–F show the main $[\text{TAG} + \text{Na}]^+$ ions observed in the authentic Brazil nut oils from different geographic origins within the Amazonian region, that is, from the cities of Rio Preto da Eva (B) and Manicoré (C and D) in the state of Amazonas (AM) state and São João da Baliza (E) and Caroebe (F) from the state of Roraima (RR). These samples from different geographical origins showed consistent TAG profiles characteristics of the Brazil nut oil, but some fine features seem to be the markers of their geographical origins. The most intense and detectable variations in the relative abundances are within the set of the ions of m/z 903, 905, and 907 as well as those of m/z 877, 879, and 881.

To test the use of the technique for quality control, EASI(+)-MS analyses were also performed on commercial Brazil nut oils acquired from industry and at a local market. Major $[\text{TAG} + \text{Na}]^+$ ions were the same as those of the authentic oils (Table 1), but some marked differences in the relative intensity were observed. Table 2 summarizes the ions observed for the authentic and commercial oils (IND A–E). Sample IND D also shows ions of m/z 905 (OOL or LLS) as the most abundant ion, and in samples IND A, IND B, IND C, and IND E, the ions of m/z 903 (OLL or OOLn) predominate. Furthermore, among the samples analyzed, eight ions were consistently represented (m/z 877, 879, 881, 901, 903, 905, 907, and 909), whereas the ions of m/z 899 (LLLn and OLnLn) characteristic of soybean adulteration were detected with 4% relative abundance in samples IND D and IND E. This low percentage, however, is below the level of detection for such adulteration (Table 1) and may not be directly related to illegal addition of soybean oil.

Small amounts of oxidation products via the ions of m/z 933, 935, and 937, which correspond to monohydroperoxides from LLL/OOLn, LLO/OOLn, and OOL/LLS, respectively, were observed in samples IND B–E (Figure 3). These ions were not observed in the authentic oils, but the occurrence of them in rancid soybean and olive oils was detected in our previous work.^{17,21} Brazil nut oil is rich in unsaturated FA, primarily linoleic and linolenic acids, which are sensitive to oxidation. Therefore, EASI-MS may also be used to monitor the oxidation level in Brazil nut oil, an important parameter of quality.

To test the performance of EASI(+)-MS for the characterization of Brazil nut oil, PCA statistical treatment of the relative abundances of the main ions listed in Tables 1 and 2 was performed. Figure 4 presents a graph of scores obtained from

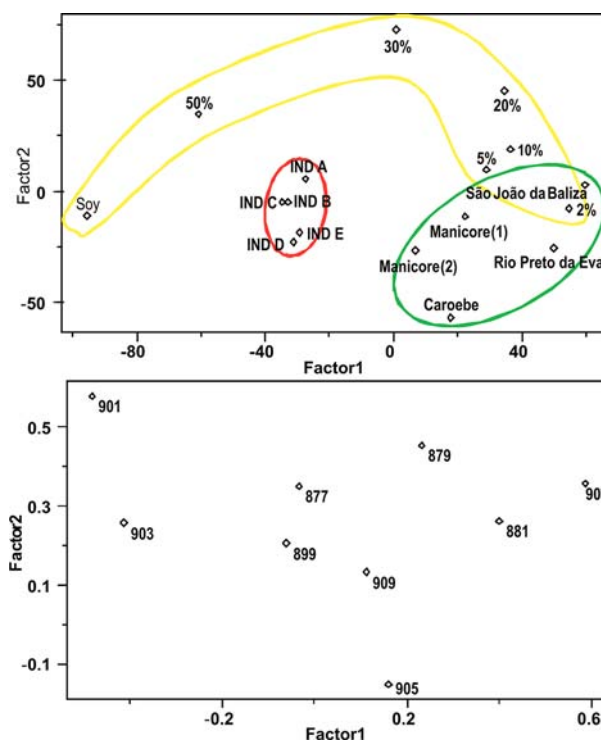


Figure 4. PCA score plot and PCA loading plot of Brazil nut oils [Rio Preto da Eva-AM, Manicoré-AM (1), Manicoré-AM (2), São João da Baliza-RR, and Caroebe-RR], commercial oils (IND A–E), and Brazil nut oil (São João da Baliza-RR) adulterated with different amount of soybean oil (2, 5, 10, 20, 30, and 50%).

PC1 and PC2 evaluations of EASI(+)-MS of 10 Brazil nut oil samples (five authentic and five commercial oil), six samples having different levels of adulteration with soybean oil (2, 5, 10, 20, 30, and 50%), and soybean oil. Note the grouping of all of the five authentic samples, the five commercial samples, and the adulterated samples (admixtures of Brazil nut oil of São João da Baliza-RR and soybean oil). The consistent differences but close proximity in Figure 3 between the authentic oils extracted in the laboratory and the commercial oils seem here to be due

to refinement process used during industrial processing and does not suggest adulteration.

In conclusion, direct EASI(+)-MS has been shown to be a powerful technique to characterize Brazil nut oils and to monitor its quality, adulteration, and oxidation. These analyses are fast and simple, being performed in a few minutes without sample preparation.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Mori, S. A.; Prance, G. T. Taxonomy, ecology and economic botany of the Brazil nut (*Bertholletia excelsa* Humb. and Bonpl.: Lecythidaceae). *Adv. Econ. Bot.* **1990**, *8*, 130–150.
- (2) Freitas-Silva, O.; Venâncio, A. Brazil nuts: Benefits and risks associated with contamination by fungi and mycotoxins. *Food Res. Int.* **2011**, *44*, 1434–1440.
- (3) Yang, J. Brazil nuts and associated health benefits: A review. *LWT—Food Sci. Technol.* **2009**, *42*, 1573–1580.
- (4) Gobbo-Neto, L.; Lopes, N. P. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. *Quim. Nova* **2007**, *30*, 374–381.
- (5) Veiga, V. F., Jr.; Pinto, A. C.; Maciel, M. A. M. Medicinal plants: Safe cure? *Quim. Nova* **2005**, *28*, 519–528.
- (6) Wu, Z.; Rodgers, R. P.; Marshall, A. G. Characterization of vegetable oils: Detailed compositional fingerprints derived from electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 5322–5328.
- (7) Jakab, A.; Nagy, K.; Héberger, K.; Vékey, K.; Forgács, E. Differential of vegetable oils by mass spectrometry combined with statistical analysis. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2291–2297.
- (8) Lay, J. O., Jr.; Liyanage, R.; Durham, B.; Brooks, J. Rapid characterization of edible oils by direct matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis using triacylglycerols. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 952–958.
- (9) Catharino, R. R.; Haddad, R.; Cabrini, L. G.; Cunha, I. B. S.; Sawaya, A. C. H. F.; Eberlin, M. N. Characterization of vegetable oils by ESI mass spectrometry fingerprint: Classification, quality, adulteration and aging. *Anal. Chem.* **2005**, *77*, 7429–7433.
- (10) Saraiva, S. A.; Cabral, E. C.; Eberlin, M. N.; Catharino, R. R. Amazonian vegetable oils and fats: Fast typification and quality control via triacylglycerol (TAG) profiles from dry matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry fingerprinting. *J. Agric. Food Chem.* **2009**, *57*, 4030–4034.
- (11) Alberici, R. M.; Simas, R. C.; Sanvido, G. B.; Romão, W.; Lalli, P. M.; Benassi, M.; Cunha, I. B. S.; Eberlin, M. N. Ambient mass spectrometry bringing MS into the real world. *Anal. Bioanal. Chem.* **2010**, *398*, 265–294.

(12) Harris, G. A.; Galhena, A. S.; Fernández, F. M. Ambient sampling/ionization mass spectrometry: application and current trends. *Anal. Chem.* **2011**, *83*, 4508–4538.

(13) Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G. Desorption electrospray ionization and other ambient ionization methods: Current progress and preview. *Analyst* **2010**, *135*, 669–681.

(14) Haddad, R.; Sparrapan, R.; Eberlin, M. N. Desorption sonic spray ionization for (high) voltage-free ambient mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2901–2905.

(15) Haddad, R.; Milagres, H. M. S.; Catharino, R. R.; Eberlin, M. N. Easy ambient sonic-spray ionization mass spectrometry combined with thin-layer chromatography. *Anal. Chem.* **2008**, *80*, 2744–2750.

(16) Hirabayashi, A.; Sakairi, M.; Koizumi, H. Sonic spray ionization method for atmospheric pressure ionization mass spectrometry. *Anal. Chem.* **1994**, *66*, 4557–4559.

(17) Simas, R. C.; Catharino, R. R.; Cunha, I. B. S.; Cabral, E. C.; Barrera-Arellano, D.; Eberlin, M. N.; Alberici, R. M. Instantaneous characterization of vegetable oils via TAG and FFA profiles by easy ambient sonic-spray ionization mass spectrometry. *Analyst* **2010**, *135*, 738–744.

(18) Riccio, M. F.; Saraiva, S. A.; Marques, L. A.; Alberici, R.; Haddad, R.; Moller, J. C.; Eberlin, M. N.; Catharino, R. R. Easy mass spectrometry for metabolomics and quality control of vegetable and animal fats. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 434–438.

(19) Riccio, M. F.; Sawaya, A. C. H. F.; Abdelnur, P. V.; Saraiva, S. A.; Haddad, R.; Eberlin, M. N.; Catharino, R. R. Easy ambient sonic-spray ionization mass spectrometric of olive oils: Quality control and certification of geographical origin. *Anal. Lett.* **2011**, *44*, 1489–1497.

(20) Cardoso, K. C.; Da Silva, M. J.; Grimaldi, R.; Stahl, M.; Simas, R. C.; Cunha, I. B. S.; Eberlin, M. N.; Alberici, R. M. TAG profiles of *Jatropha curcas* L. seed oil by easy ambient sonic-spray ionization mass spectrometry. *J. Am. Oil Chem. Soc.* **2012**, *89*, 67–71.

(21) Simas, R. C.; Catharino, R. R.; Eberlin, M. N.; Barrera-Arellano, D.; Souza, V.; Alberici, R. M. Triacylglycerols oxidation in oils and fats monitored by easy ambient sonic-spray ionization mass spectrometry. *J. Am. Oil Chem. Soc.* **2012**, *89*, 1193–1200.

(22) Alberici, L. C.; Oliveira, H. C. F.; Catharino, R. R.; Vercesi, A. E.; Eberlin, M. N.; Alberici, R. M. Distinct hepatic lipid profile of hypertriglyceridemic mice determined by easy ambient sonic-spray ionization mass spectrometry. *Anal. Bioanal. Chem.* **2011**, *401*, 1651–1659.

(23) Porcari, A. M.; Schwab, N. V.; Alberici, R. M.; Cabral, E. C.; Moraes, D. R.; Montanher, P. F.; Ferreira, C. R.; Eberlin, M. N.; Visentainer, V. Intact triacylglycerol profiles of fats and meats via thermal imprinting easy ambient sonic-spray ionization mass spectrometry. *Anal. Methods* **2012**, *4*, 3551–3557.

(24) Fernandes, G. D.; Alberici, R. M.; Pereira, G. G.; Cabral, E. C.; Eberlin, M. N.; Barrera-Arellano, D. Direct characterization of commercial lecithins by easy ambient sonic-spray ionization mass spectrometry. *Food Chem.* **2012**, *135*, 1855–1860.

(25) Firestone, D. *Physical and Chemical Characteristics of Oils, Fats and Waxes*; AOCS Press: Washington, DC, 2006.