Instantaneous characterization of vegetable oils *via* TAG and FFA profiles by easy ambient sonic-spray ionization mass spectrometry†

Rosineide C. Simas,[a] Rodrigo R. Catharino,[b] Ildenize B. S. Cunha,[a] Elaine C. Cabral,[c] Daniel Barrera-Arellano,[d] Marcos N. Eberlin[a] and Rosana M. Alberici*[a]

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A fast and reliable method is presented for the analysis of vegetable oils. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is shown to efficiently desorb and ionize the main oil constituents from an inert surface under ambient conditions and to provide comprehensive triacylglyceride (TAG) and free fatty acid (FFA) profiles detected mainly as either $[TAG + Na]^+$ or $[FFA - H]^-$ ions. EASI(±)-MS analysis is simple, easily implemented, requires just a tiny droplet of the oil and is performed without any pre-separation or chemical manipulation. It also causes no fragmentation of TAG ions hence diacylglyceride (DAG) and monoacylglyceride (MAG) profiles and contents can also be measured. The EASI(±)-MS profiles of TAG and FFA permit authentication and quality control and can be used, for instance, to access levels of adulteration, acidity, oxidation or hydrolysis of vegetable oils in general.

Introduction

Vegetable oils[1] are complex chemical mixtures containing a wide range of compounds in which triacylglycerides (TAG) predominate with small amounts of diacylglycerides (DAG), free fatty acids (FFA), phospholipids, and other minor polar components such as tocopherols, sterols, alcohols, aldehydes and esters. The profiles of such constituents, particularly TAG and FFA, constitute chemical signatures for each type of vegetable oil, varying according to the biochemistry of the feedstock. TAG are tri-esters of glycerol with variable compositions of long-chain fatty acids (FA). The variable physico-chemical properties of TAG are essential for life and are finely tuned by the total carbon number of the FA tails, the degree of unsaturation and the exact position and *cis*-configuration of the double bonds in the FA tails. Most common vegetable oils have relatively simple TAG profiles in which C18:1 (oleic) and C18:2 (linoleic) fatty acids are predominant (C*x*:*y* where *x* is the total number of carbons and *y* is the number of double bonds). TAG of olive oil contains, for instance, *ca.* 70% of oleic acid, whereas those of soybean oil and corn oil contain 50–60% of linoleic acid. But the FA composition in TAG may vary dramatically as, for instance, in castor oil, a unique oil that contains up to 90% of ricinoleic acid (C18:1 with an 9-OH substituent). The so-called tropical oils, such as palm oil, are also typical since they contain higher amounts of saturated (C*x*:0) fatty acids. Oils extracted from Amazonian native plants also have a very diverse TAG composition.[2] These 'exotic' oils are attracting increasing interest for the production of pharmaceuticals and cosmetics, causing a rapid expansion of their worldwide market. Brazil nut (*Bertholletia excelsa*), andiroba (*Carapa guianensis*), babaçu (*Orbignya* spp.), urucum (*Bixa orellana* L.), tucumã (*Astrocaryum aculeatum* P.), açaí (*Euterpe oleracea* P.), buriti (*Mauritia flexuosa*) and passion fruit (*Passiflora* spp.) oils are representative examples of the many Amazonian vegetal oils of economic importance.

Obtaining precise TAG profiles of oils at the molecular level has, however, provided a challenging analytical task. Many combinations of FA in the glycerol template are possible leading to a large number of individual TAG species with many isomers and isobars. Traditionally, TAG analysis has relied on hydrolysis followed by FA derivatization *via* methylation which forms a mixture of fatty acid methyl esters (FAME) which are then separated and detected mainly by gas chromatography (GC) with flame ionization detection (FID)[3] or GC coupled to mass spectrometry (GC-MS).[4] This indirect procedure reduces chemical information, providing no TAG profiles but only the total percentage of their FA composition. The sample workup steps are also time-consuming and hard to automate. Enzymatic methods[5] have also been employed to analyze TAG in oils; they are excellent for measuring total TAG concentration but also fail to provide TAG profiles. High performance liquid chromatography (HPLC)[6] is capable of separating intact TAG in vegetable oils but HPLC relies on retention time (measured for standards) for separation and identification and peaks are sometimes only partially resolved.[7]

Mass spectrometry alone, owing to its unmatched combination of speed, sensitivity and selectivity, has also provided a powerful technique to characterize vegetable oils. Soft ionization methods such as electrospray ionization mass spectrometry (ESI-MS)[8] and matrix-assisted laser desorption ionization MS

[a]*ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, UNICAMP, 13083-970 Campinas, SP, Brazil*
[b]*Department of Clinical Pathology, School of Medical Sciences, University of Campinas, UNICAMP, 13083-887 Campinas, SP, Brazil*
[c]*Institute of Chemistry, University of São Paulo, USP, 05513-970 São Paulo, SP, Brazil*
[d]*Fats and Oils Laboratory, Food and Technology Department, Faculty of Food Engeneering, University of Campinas, UNICAMP, 13083-970 Campinas, SP, Brazil*
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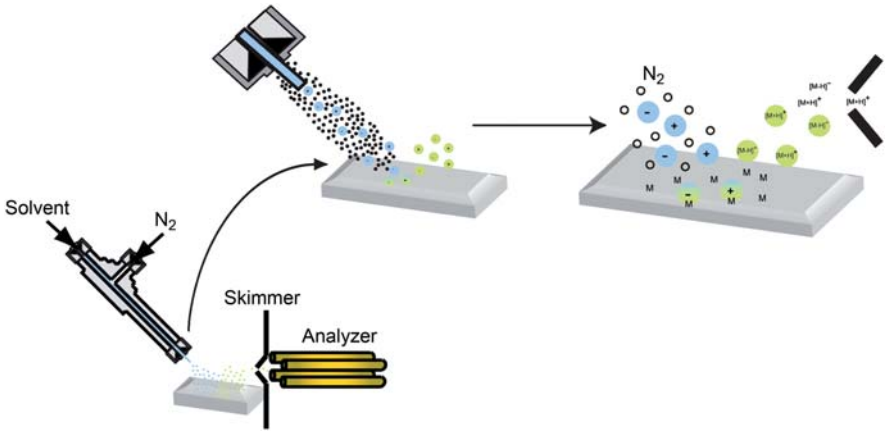

Fig. 1 Illustration of the mechanism of EASI(±)-MS. The analyte molecules (M) rest on their original surface or are placed on an auxiliary inert surface under ambient conditions. Through a process assisted only by compressed nitrogen gas (or even air), a super-sonic stream of very minute bipolar charged droplets of a proper solvent is produced and bombards the surface. M are desorbed and detected by EASI(+)-MS mainly as $[M + H]^+$ or in cationized forms such as $[M + Na]^+$ and $[M + K]^+$ for samples with relatively high salt contents. Detection can also performed by EASI(−)-MS, mainly *via* $[M - H]^-$ ions due to the negatively charged droplets concomitantly produced during SSI. The mounting of an EASI source requires only a Swagelok T-element, simple ferrules and gas tubing, and a fused-silica capillary.

(MALDI-MS)[9] have been shown to provide structural information on biomolecules in complex mixtures with no pre-separation methods. Direct infusion ESI-MS, for instance, has been used to analyze vegetable oils after dilution of the sample with chloroform or dichloromethane, and has provided TAG and FA profiles but with substantial carry-over effects.[10,11] ESI-MS has also been used to detect adulteration of vegetable oils after selective solvent extraction with methanol–water (1:1).[12] These diluted extracts are enriched in the more polar and acidic constituents, mostly FFA and phenols, and were directly subjected to ESI(−)-MS with much reduced carry-over effects.[12] MALDI has also been used for the quantitative and qualitative determination of TAG profiles of crude vegetable oils after co-crystallization with a suitable organic matrix.[13] Amazonian vegetable oils have also been characterized *via* unique TAG profiles provided by MALDI-MS.[14] But ESI-MS and MALDI-MS still operate under the normal vacuum conditions of MS, requiring some sorts of sample preparation step.

Recently, sample workup has been fully eliminated or substantially reduced with the introduction of a variety of new desorption/ionization MS techniques performed under ambient conditions directly from samples placed on surfaces or on their natural matrices.[15–18] Among these techniques, easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is one of the simplest, softest and most easily implemented.[19] EASI forms ions in an unprecedented way since it uses no voltage, no UV light, no laser beams, no corona or glow discharges, and no heating. An EASI source can also be constructed and installed in a few minutes from a few simple MS laboratory parts and is assisted only by compressed N_2 or even air (no substantial oxidation occurs). EASI is based on super-sonic-spray ionization (SSI)[20] that creates droplets of solvent (*e.g.* acidified methanol) which end up being charged (both positively and negatively at the same time) due to the statistically unbalanced distribution of cations and anions in these very minute droplets with limited charge-carrying capability (Fig. 1). The dense stream of the minute super-sonic (bipolar) charged droplets promotes analyte pick up from the surface, concomitant ionization as either cations or anions, and then transfers the analyte ions to the gas phase. EASI-MS has been applied with success to the analysis of different analytes and matrices such as fuels,[18] drug tablets,[19] perfumes,[21] surfactants,[22] biodiesel,[23,24] and inks[25] and has been coupled to membrane introduction mass spectrometry,[26] thin layer chromatography[27] and used together for improved selectivity with an active surface containing molecularly imprinted polymers that function as selective analyte sequesters.[28]

We report herein our investigation of the suitability of EASI-MS in both positive and negative ion modes for direct and nearly instantaneous characterization of vegetable oils from different feedstocks *via* TAG and FFA profiles using a tiny droplet of the oil placed on an inert surface under ambient conditions. In related work, the ambient MS techniques extractive ESI (EESI),[29] direct analysis in real time (DART)[30] and low-temperature plasma (LTP)[31] ionization have been used to characterize some constituents of vegetable oils, but these reports have mainly focused on olive oil or not on TAG but on their compositions of FFA or most volatile constituents.

Experimental

Chemicals

HPLC-grade methanol and ammonium hydroxide were purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. A total 20 of samples of vegetable oils were obtained from reliable producers, including olive (*Olea europaea*), soybean (*Glycine max*), palm (*Elaeis guineensis*), castor (*Ricinus communis*), grape seed (*Vitis vinifera*), hazelnut (*Corylus avellana*), sunflower (*Helianthus annus*), sesame (*Sesamum indicum*), linseed (*Linum usitatissimum*), canola (*Brassica napus* L.), cotton (*Gossypium hirsutum*), avocado (*Persea americana*), jatropha (*Jatropha curcas*), urucum (*Bixa orellana*), andiroba (*Carapa guianensis*), Brazil nut (*Bertholletia excelsa*), açaí (*Euterpe oleracea*), buriti (*Mauritia flexuosa*), passion fruit

(*Passiflora* spp.), and cannon ball tree (*Couroupita guianensis*) and no sample treatment was used. Their authenticities were also evidenced by characteristic EASI(+)-MS TAG profiles.

EASI-MS

Spectra were acquired either in the positive or negative ion mode using a single-quadrupole mass spectrometer (Shimadzu LCMS 2010) equipped with a homemade EASI source, which is described in detail elsewhere.[19,21] Typical EASI-MS conditions were as follow: N_2 nebulizing gas pressure of 100 psi, surface angle of *ca.* 30°, and methanol flow rate of 20 μL min^{-1}. Ammonium hydroxide (0.1% v/v) was added to the methanol to help induce ionization for EASI(−)-MS. A tiny droplet of the oil samples (2 μL) was placed directly onto a paper surface (brown Kraft envelope paper) and mass spectra were accumulated over 60 s and scanned over the 50–1000 *m/z* range. EASI-MS needs no polarity switching in going from the negative to positive ion mode, or *vice-versa*, since it employs no voltage to create both negatively and positively charged droplets. Modern mass spectrometers such as the one used in this work can be instantaneously and automatically switched from the positive to the negative ion mode.

Results and discussion

To assess the reliability of the TAG profiles provided by EASI-MS, as determined by its ability to desorb and ionize the many TAG in the oil mixture, and to compare EASI with the two most common ionization techniques applied for vegetable oil analysis, spectra using MALDI-MS, ESI-MS and EASI-MS for a sample of soybean oil (a common vegetable oil) were recorded (Fig. 2). Note that the resolution of the EASI-MS is lower than those observed for the MALDI-MS and ESI-MS since a compact and robust but low resolution quadrupole mass spectrometer was used for EASI whereas high resolution TOF analyzers were used for MALDI and ESI. Note that quite similar spectra were obtained with characteristic soybean oil TAG profiles[1] detected as $[TAG + Na]^+$ ions and attributed to PPL (C50:2, *m/z* 853), PPO (C50:1, *m/z* 855), PLL (C52:4, *m/z* 877), PLO (C52:3, *m/z* 879), POO (C52:2, *m/z* 881), LLLn or OLnLn (C54:7, *m/z* 899), LLL or OLLn (C54:6, *m/z* 901), OLL or OOLn (C54:5, *m/z* 903), OOL (C54:4, *m/z* 905) and OOO (C54:3, *m/z* 907) where: O = oleic acid, L = linoleic acid, Ln = linolenic acid and P = palmitic acid. Minor $[TAG + K]^+$ ions were also detected:[32] PLL (*m/z* 893), PLO (*m/z* 895), LLLn or OLnLn (*m/z* 915), LLL or OLLn (*m/z* 917), OLL or OOLn (*m/z* 919) and OOL (*m/z* 921). In all 3 spectra, the most abundant $[TAG + Na]^+$ ion is that of *m/z* 901 corresponding either to a trilinolein or oleyllinoleyllinolenin (C54:6), or a mixture of both. No substantial TAG discrimination during EASI desorption and ionization seems therefore to occur, and this uniform ionization efficiency is expected since TAG display similar structures with close physico-chemical properties. EASI-MS data are likely therefore to closely reflect the relative TAG concentrations in the original sample,[10] and this close match is indeed observed since the TAG composition measured by EASI-MS corresponds to the known composition of fatty acids of soybean oil: linoleic (49.7–56.9%), oleic (17.7–26.0%), palmitic (9.9–12.2%), linolenic (5.5–9.5%) and stearic (3.0–5.4%) acids.[1]

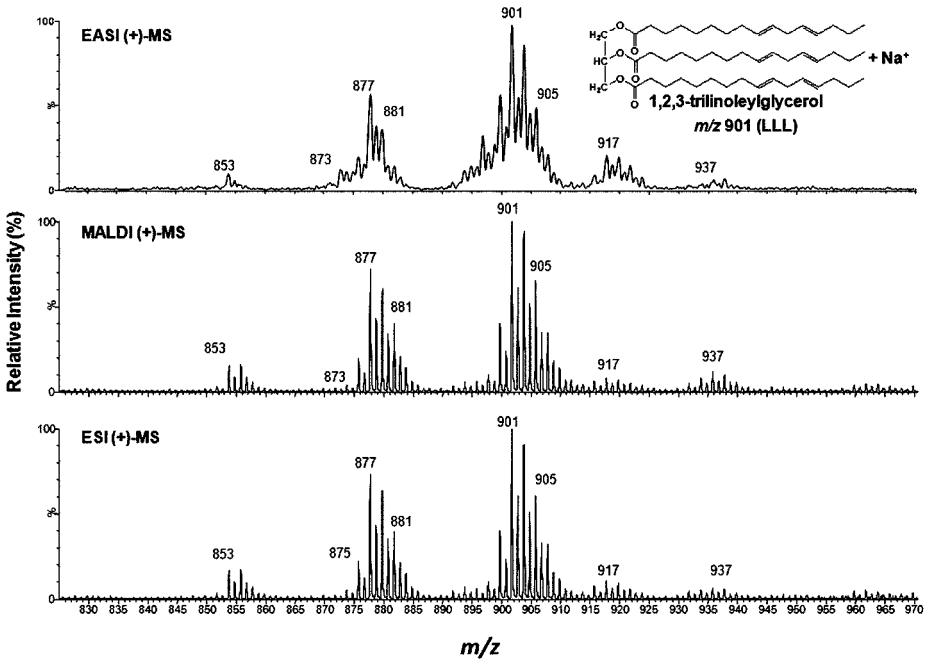

Fig. 2 TAG fingerprints of soybean oil obtained by three different ionization techniques. EASI(+)-MS was obtained in a simple and robust but low resolution mono-quadrupole mass spectrometer, see the Experimental section for details. ESI(+)-MS was obtained on a Q-TOF mass spectrometer (Micromass, UK) with *ca.* 5 K resolution using the following main conditions: source temperature of 100 °C, desolvation temperature of 100 °C, capillary voltage of 3.0 kV and cone voltage of 40 V. MALDI(+)-MS was obtained on a MALDI-TOF mass spectrometer (Micromass, UK) using the reflection mode with *ca.* 7 K resolution and the following main conditions: UV laser of 337 nm, pulse voltage of 2.5 kV, reflectron voltage of 2 kV and source voltage of 15 kV.

TAG profiles provided by EASI-MS seem therefore to match those obtained by ESI-MS and MALDI-MS, but the EASI-MS data are obtained in an easier and faster way (instantaneously in practical terms) directly from a tiny droplet of the crude sample at ambient conditions with no sample manipulation at all. MALDI-MS for oil analysis suffers from an inconvenience since a quite intense set of ions is also detected in the *m/z* 550–650 range (not shown in Fig. 2) due to fragments of the $[TAG + Na]^+$ ions associated with the loss of a fatty acid moiety.[12,33,34] This fragmentation has also been observed to a considerable extent during DART-MS of olive oil.[30] No detectable TAG fragmentation has been observed, however, during all EASI-MS of the vegetable oils investigated herein probably because EASI, based on SSI, is one of the softest ionization methods. EASI-MS can therefore be used to measure DAG contents in oils without the disturbing interference from TAG fragmentation. The percentage of DAG in oils as well as the corresponding FFA is a measure of oil hydrolysis and an important parameter of oil quality control.

Fig. 3 illustrates six EASI(+)-MS fingerprints of common vegetable oils. Note the characteristic TAG profiles in the 800–1000 *m/z* range detected mainly as $[TAG + Na]^+$ ions. The closest TAG profiles are seen for the soybean and grape seed oils (Fig. 3a and 3e) but their *m/z* 901/903 ratios are quite distinct. Palm oil (Fig. 3b) is known to be rich in palmitic acid (*ca.* 42%) and oleic acid (*ca.* 40%), hence the EASI-MS TAG profile of palm oil displays abundant ions of *m/z* 855 (C50:1, PPO) and 881 (C52:2, POO). Olive oil is commercialized worldwide and highly appreciated due to its improved organoleptic properties and health benefits, and is therefore commonly adulterated with lower priced oils such as soybean oil and hazelnut oil.[28] When Fig. 3c (olive oil) is compared with Fig. 3a (soybean oil) and 3d (hazelnut oil), characteristic TAG profiles are observed which provide secure identification and a reliable screening method for olive oil adulteration. We are currently analyzing a variety of olive oil blends with most common oils used for adulteration to access the figures of merit of EASI-MS adulteration detection in olive oils. Castor oil (Fig. 3f), as expected, provides the most characteristic TAG profile with a unique EASI-MS displaying a very intense ion of *m/z* 955 due to TAG formed by three 12-OH ricinoleic acids (C18:1-OH) (*ca.* 90% of the castor oil composition).[1]

Fig. 4 compares the EASI-MS fingerprints of four Amazonian oils. Each of these 'exotic' oils displays a unique TAG profile that allows their rapid typification. TAG profiles from andiroba oil

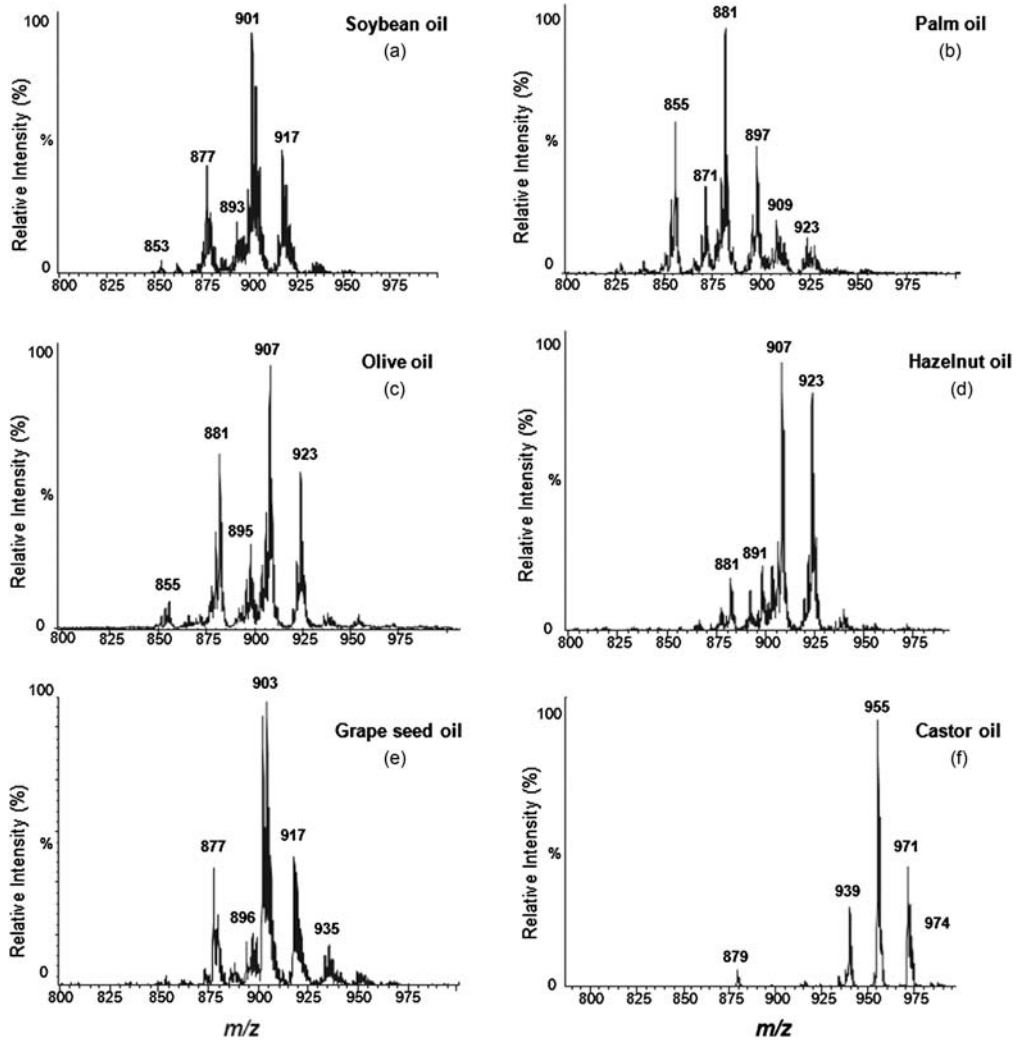

Fig. 3 TAG fingerprints obtained by EASI(+)-MS of typical vegetable oils.

(Fig. 4a) and açaí oil (Fig. 4b) are quite diverse and dominated by palmitic, oleic and linoleic acids: $[TAG + Na]^+$ ions of *m*/*z* 853 (C50:2, PPL), 855 (C50:1 PPO), 879 (C52:3, PLO), 881 (C52:2, POO), 883 (C52:1, POS), 905 (C54:4, OOL or LLS), 907 (C54:3, OOO or SOL) and 909 (C54:2, OOS or SSL), where S = stearic acid. The most distinguishing feature of these TAG profiles is the higher percentage of palmitic acid for the andiroba oil, as shown by the higher intensity of the TAG ion of *m*/*z* 855. Açaí oil is known to be rich in oleic acid, hence its TAG ion of *m*/*z* 907 is more abundant. The EASI-MS fingerprint of the urucum oil (Fig. 4c) displays TAG ions derived mainly from oleic and linoleic acids, and the most intense are those of *m*/*z* 901 (C54:6, LLL or OLLn), 903 (C54:5, LLO or OOLn) and 905 (C54:4, OOL or LLS). Oleic acid is known to be the main FA of buriti oil (73–78%), hence its EASI-MS (Fig. 4d) is dominated the $[TAG + Na]^+$ ions of *m*/*z* 881 (C52:2, POO) and 907 (C54:2, OOO or SOL).

FFA content is a major quality criterion for vegetable oils.[1] Fig. 5 illustrates typical EASI(−)-MS of andiroba oil and Brazil nut oil. Note that these fingerprints not only reveal the presence (and amount, see below) of FFA, but also their unique profiles. EASI(−)-MS displays mainly ions corresponding to the deprotonated molecules $[FFA - H]^-$ from palmitic (*m*/*z* 255), linolenic (*m*/*z* 277), linoleic (*m*/*z* 279), oleic (*m*/*z* 281) and stearic (*m*/*z* 283) acids. As expected from its known FA composition,[1] andiroba oil is characterized by a considerably more abundant ion of *m*/*z* 281 whereas the ion of *m*/*z* 279 predominates in the EASI(−)-MS of the Brazil nut oil.

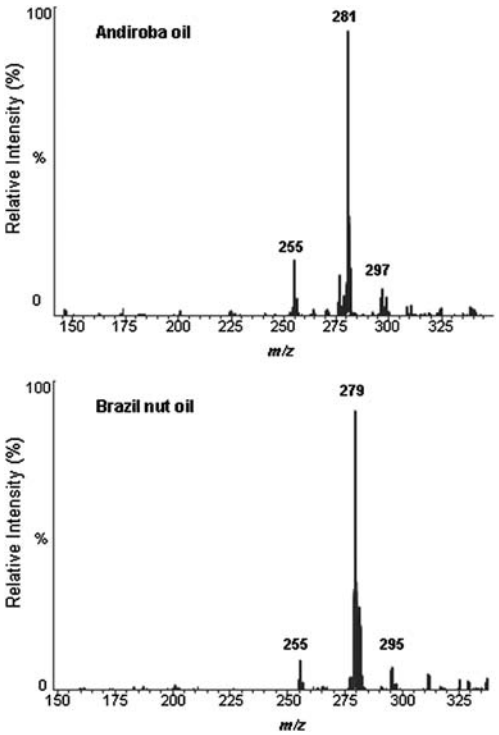

Fig. 5 FFA fingerprints obtained by EASI(−)-MS of andiroba and Brazil nut oils.

We have also evaluated the ability of EASI(−)-MS to quantify FFA levels in vegetable oils. For that we use, as a model case, a sample of olive oil which was spiked with pure oleic acid. Fig. 6 shows that a rather linear analytical curve was obtained indicating that indeed reasonably accurate quantitation of FFA in vegetable oils can also be performed instantaneously by direct EASI(−)-MS analysis using, for instance, spiking with an unnatural FA internal standard.

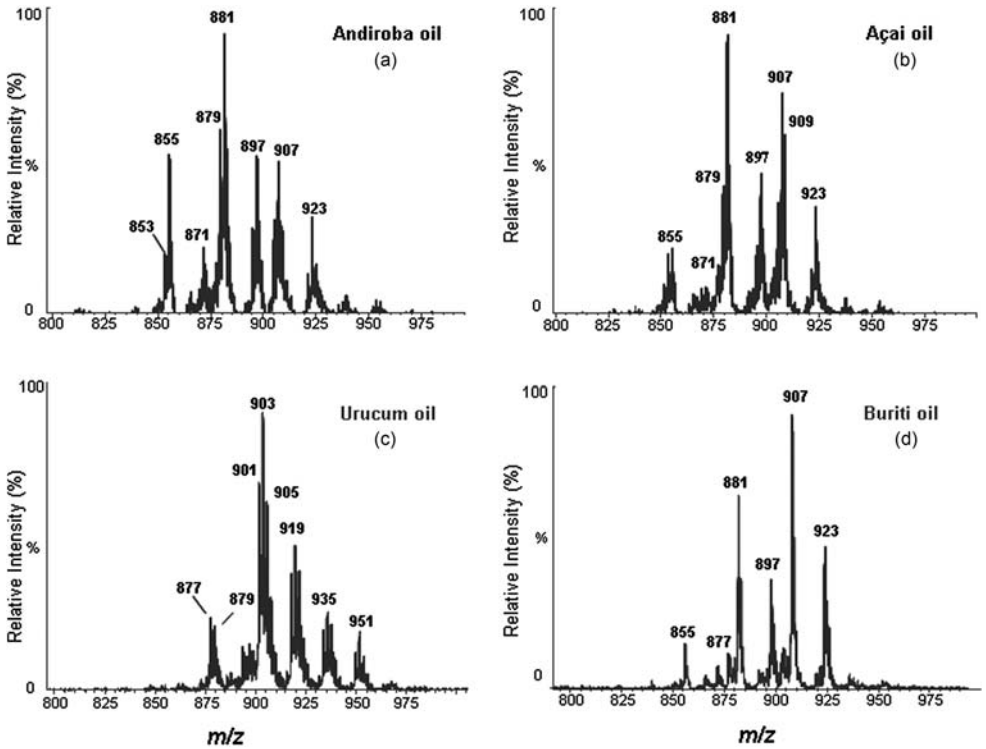

Fig. 4 TAG fingerprints obtained by EASI(+)-MS of typical Amazonian vegetable oils.

The level of oxidation is also a major quality parameter for vegetable oils.[1] Oxidation is the major cause of deterioration in oil quality, and produces rancid and unpleasant flavors and taste, spoiling the organoleptic characteristics of the oil. O_2 reacts primarily with unsaturated FA radicals formed at the TAG tails to form hydroperoxides. As Fig. 7 illustrates, EASI(+)-MS can also be used to determine the level of oil oxidation. Fig. 7a/b and 7c/d compare TAG profiles from EASI(+)-MS for fresh and oxidized soybean oil and olive oil, respectively. In Fig. 7b, for instance, note the instantaneous detection of oxidization *via* $[TAG + 2O + Na]^+$ ions of *m/z* 909, 913, 931, 933 and 935; $[TAG + 4O + Na]^+$ ions of *m/z* 963, 965, 967, and 971 and $[TAG + 6O + Na]^+$ ions of *m/z* 995, 997, 999 and 1003.

Conclusion

EASI(±)-MS performed on a tiny single droplet of the sample placed on an inert surface at ambient conditions allows direct and instantaneous analysis of vegetable oils *via* characteristic profiles of both TAG and FFA. These profiles permit oil typification and quality control as related to total carbon number and levels of unsaturation, oxidation, adulteration and FFA. The EASI(±)-MS technique is one of the softest and simplest ambient MS techniques and can perform oil analysis at the molecular level with no sample manipulation, no pre-separation and no chemical derivatization. DAG and MAG levels can also be detected with confidence since no substantial fragmentation of TAG ions occurs during EASI(+)-MS. EASI(±)-MS provides therefore a simple and fast method for the comprehensive analysis of vegetable oils.

Acknowledgements

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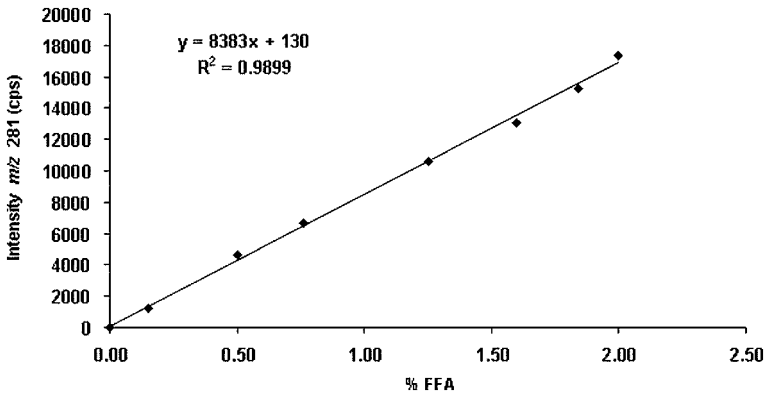

Fig. 6 Analytical curve for EASI(−)-MS quantitation of FFA in olive oil. Sample was spiked with known amounts of an oleic acid standard reaching FFA levels up to 2.0% w/w. FFA levels in the spiked samples were measured independently *via* a classical procedure as described in ref. 35.

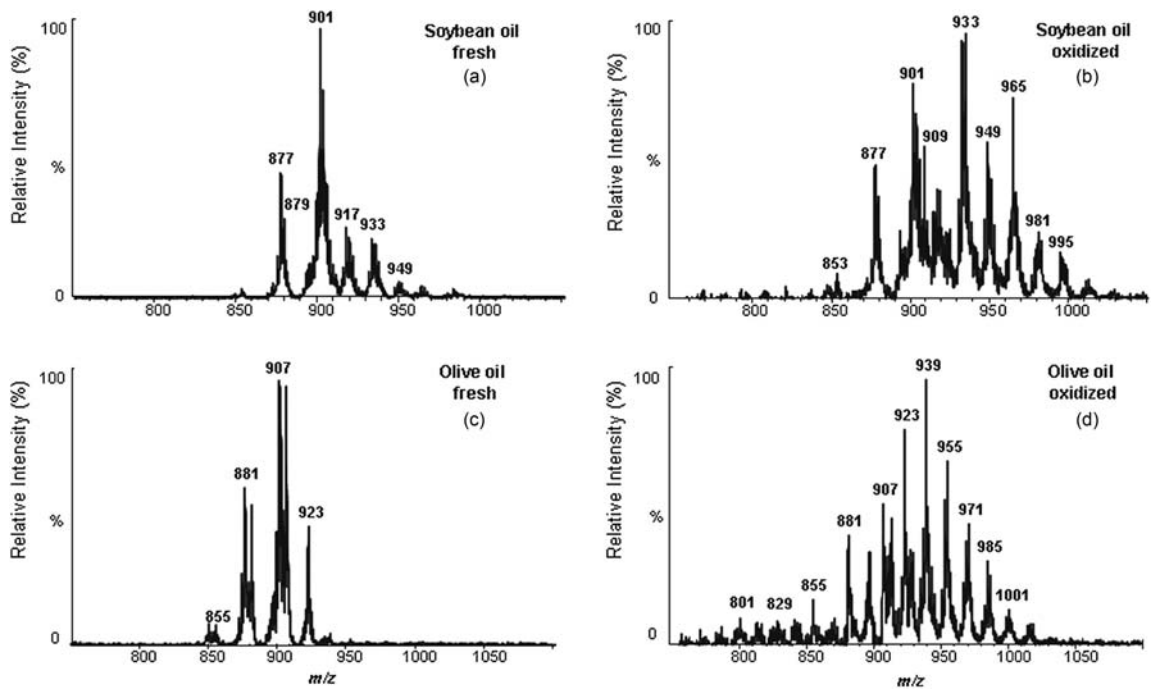

Fig. 7 TAG fingerprints obtained by EASI(+)-MS of two samples of fresh and oxidized soybean and olive oils.

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