Research Article

Easy mass spectrometry for metabolomics and quality control of vegetable and animal fats

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Easy ambient sonic spray ionization (EASI), a novel desorption/ionization technique for ambient mass spectrometry analysis, is shown to permit the characterization of different types of vegetable and animal fats. The taxonomic markers of the oils, mainly fatty acids and phenols, are extracted with methanol/water (1:1) solution and made alkaline with NH_4OH, then placed and dried on the surface of a glass tip and directly analyzed by EASI-MS in the negative-ion mode. EASI provides a supersonic cloud of charged droplets that causes efficient desorption and ionization of the oil markers directly from the surface of the glass tip. As proof-of-principle cases, EASI(−)-MS was applied to genuine samples of olive oil, hazelnut oil, soybean oil, grape seed oil, canola oil, butter, and lard. Characteristic metabolomics EASI(−)-MS profiles of fatty acids and eventually phenols were obtained.

Keywords: Ambient mass spectrometry / EASI-MS fingerprinting / Fatty acid / Vegetable oil

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1 Introduction

Recently, we reported a new desorption/ionization technique for ambient mass spectrometry analysis: easy ambient sonic spray ionization (EASI) [1]. This technique requires little or no sample preparation and can be performed directly on a variety of matrices. EASI uses supersonic spray [2] to create a supersonic cloud of charged droplets which bombard the surface, causing desorption and ionization of the analyte. EASI has therefore the advantages of requiring no pre-separation steps and no or little sample preparation, and its ionization softness often produces intact molecular species, which facilitates mixture analysis with less background noise from solvent ionization [1]. EASI-MS has been applied with success to the identification of components in commercial drug tablets [1], to quantify analytes on-line in environmental and body fluids using a membrane interface [3], to typify fabric softeners [4] and perfumes [5], and has been coupled to TLC [6] and then applied to biodiesel analysis [7].

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Adulteration of highly valuable vegetable oils with lower-priced oils is a common, widespread, and illegal practice. This practice is especially intense for olive oil, which is commonly adulterated either by hazelnut oil (owing to its similar composition) or soybean oil (owing to low price and availability) [8, 9]. As the authenticity of one particular type of oil is important for both health and commercial reasons, there is continued need for improved, rapid, and simple methods for oil typification and to control adulteration or counterfeits [10, 11].

Vegetable oils present characteristic compounds as triglycerides, free fatty acids, and phenols. These constituents constitute natural chemotaxonomic markers for vegetable oil metabolomics, classification, and purity control. These profiles are commonly analyzed by HPLC or GC, sometimes preceded by hydrolysis or derivatization methods [10–12]. Direct infusion ESI-MS and MALDI-MS have also been used for metabolic fingerprinting, typification, and quality control, with excellent results for oils [13–19].

We have recently demonstrated, for different types of vegetable oils, that direct-infusion ESI(–)-MS of a simple oil extract (with water/methanol plus 0.1% ammonium hydroxide solution) is useful to selectively extract, concentrate, and then provide typical ion profiles mainly from the ionization of free fatty acids and biphenols. We have also

demonstrated the ability of this technique to screen for olive oil adulteration by soybean oil [14]. Herein, we report that similar characteristic profiles [20] can be obtained for vegetable oils and animal fat in a faster and simplified procedure (in a matter of seconds) using EASI(–)-MS.

2 Materials and methods

2.1 Chemicals and samples

Ammonium hydroxide and methanol of HPLC grade were purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore) purification unit. A total of 15 samples of vegetable oils and animal fats were obtained from the most trustable suppliers, and their authenticity was also evidenced by characteristic EASI(+)-MS TAG profiles (not shown); these included olive (A) unrefined oil, and hazelnut (B), soybean (C), grape seed (D), canola (E), butter (F), and lard (G) refined oils and fats.

2.2 Sample preparation

The oils samples (100 μL) were homogenized in a flask with 200 μL of a solution of methanol/water (1:1) plus 0.1% ammonium hydroxide. The phases were allowed to separate and the water/alcohol mixture phase was removed. A homemade glass tip (Fig. 1) was dipped into the oil extract (water/alcohol mixture phase) and allowed to dry for a few seconds.

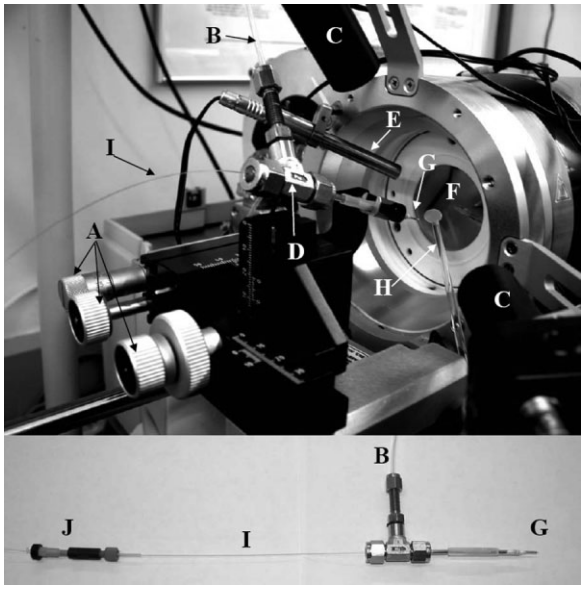

Figure 1. A picture of the EASI source used for oil fingerprinting: (A) position adjustment nods (3-D variation), (B) nebulizing gas tubing (N_2), (C) camera, (D) sonic spray device, (E) light source, (F) cone orifice, (G) stainless-steel sonic spray capillary, (H) glass sample tip, (I) fused-silica capillary, and (J) syringe connector.

The tip was then manually placed in the ionization/desorption region of the EASI source (Fig. 1) for the dried extract to be analyzed by EASI(–)-MS.

2.3 Instrumental conditions

Experiments were performed on a Q-Trap® triple-quadrupole mass spectrometer (Applied Biosystems) using a homemade EASI source (Figs. 1, 2) constructed using a commercial nano-ESI source as the basic platform. The mass spectrometer was operated in the negative-ion mode. EASI(–)-MS conditions were as follows: water/methanol (1:1) plus 0.1% ammonium hydroxide (100 mL) for EASI(–)-MS at a flow rate of 20 μL/min, nebulizing gas backpressure of about 30 bar, curtain gas pressure of 5 bar, declustering potential of 100 V, glass tip distances of about 2 mm, and capillary-tip-entrance angle of about 30°. Tandem mass spectrometric experiments (MS/MS) were performed using the product ion scan mode *via* Q1 selection of the desirable precursor ion, q2 CID with nitrogen, and mass analysis of the CID ionic fragments by Q3. The collision energy ranged from 10 to 40 eV, depending on the dissociation proclivity of the precursor ion.

3 Results and discussion

Figure 1 shows a picture with major details of the EASI source; Fig. 2 presents a schematic of the EASI-MS process whereas Fig. 3 shows EASI(–)-MS fingerprints in the 200–400 *m*/*z* range for the extracts of the vegetable oils investigated. Note that these spectra display characteristic profiles of fatty acids (and eventually biphenols) that allow prompt differentiation between the seven types of oils studied (Table 1).

The EASI(–)-MS fingerprints of all oil samples show characteristic distributions of mainly the following fatty acids: palmitic (P), linoleic (L), oleic (O), and stearic (S) detected as the deprotonated molecules of *m*/*z* 255, 279, 281, and 283, respectively. These ions have also been used as the markers to

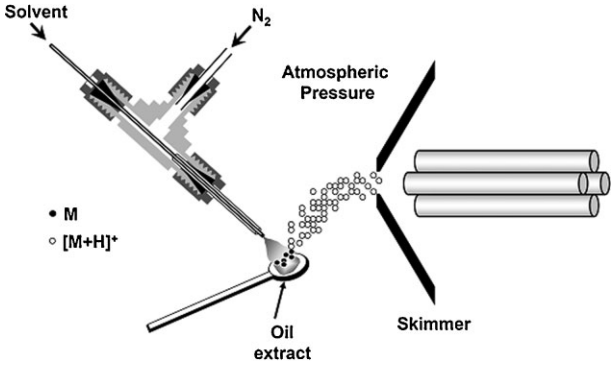

Figure 2. Schematics of the EASI(–)-MS analysis of the dry extracts of oils placed on glass sample tips.

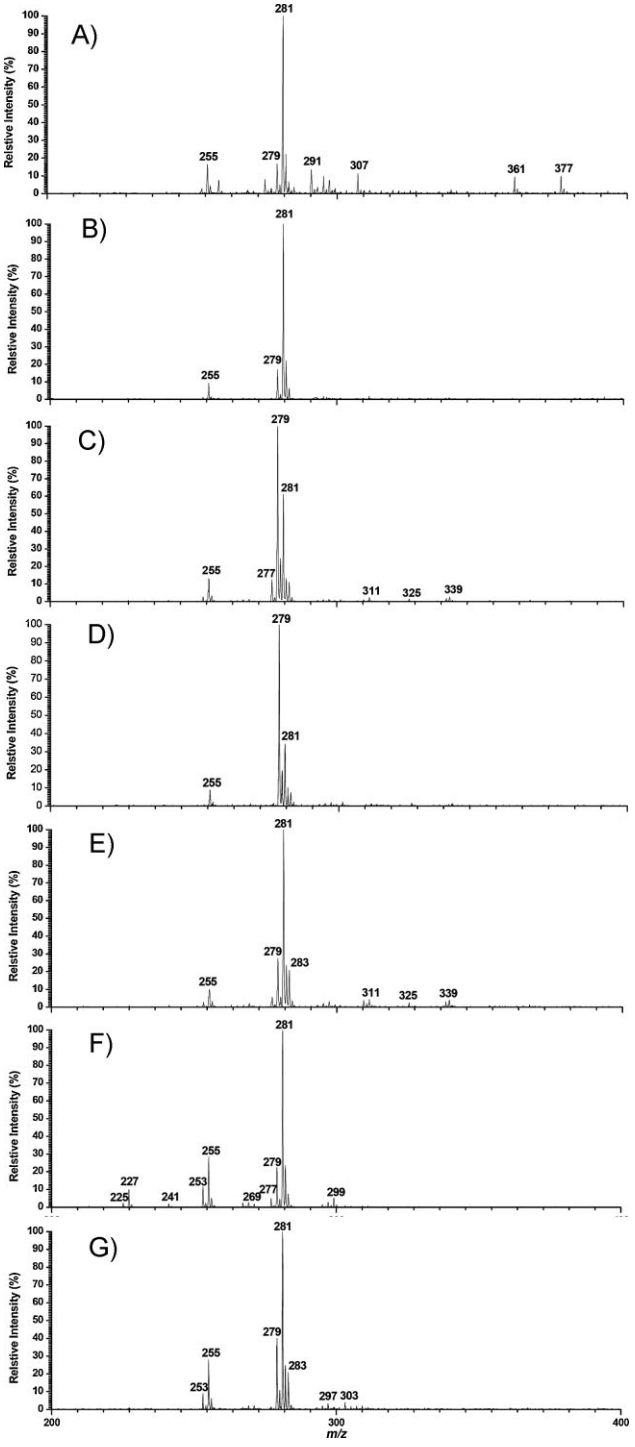

Figure 3. EASI(−)-MS fingerprints of dry extracts of (A) olive oil, (B) hazelnut oil, (C) soybean oil, (D) grape seed oil, (E) canola oil, (F) butter, and (G) lard.

characterize several oil samples analyzed by ESI-MS by Wu *et al.* [13] and Catharino *et al.* [14]. Olive oil is unique (Fig. 3A) since it is an unrefined vegetable oil and the only one to display in its ESI(−)-MS the unique marker ions of *m*/*z* 291, 307, 361, and 377 (see discussion below) [21]. Hazelnut oil (Fig. 3B) displays for its three most abundant ions of *m*/*z* 255 (P), *m*/*z* 279 (L), and *m*/*z* 281 (O) nearly the same ratio (*ca.* 3:4:20) as that of olive oil (Fig. 3A), but since hazelnut oil is a refined oil, biphenol ions are undetectable. This feature, unique for the EASI(−)-MS fingenprints of the oil extracts, permits the differentiation of hazelnut and olive oil, a challenging analytical task [8]. The EASI-MS fingerprint of soybean oil (Fig. 3C) is characterized by five major ions of *m*/*z* 255 (P), *m*/*z* 277 (Ln), *m*/*z* 279 (L), *m*/*z* 281 (O), and *m*/*z* 283 (S), in a ratio of *ca.* 3:2:20:12:2, but most particularly by the predominance of that of *m*/*z* 279 (L). Figure 3D shows that the EASI(−)-MS fingerprint of grape seed oil is characterized by an even greater abundance of the ion of *m*/*z* 279 (L) and very little of *m*/*z* 277 (Ln). These relative abundances of palmitic (P), linolenic (Ln), linoleic (L), oleic (O), and stearic (S) acids are known to be characteristic for these types of edible oils and have also been obtained by chromatographic analysis [22]. Canola oil (Fig. 3E) displays also an EASI(−)-MS with five marker ions of *m*/*z* 255 (P), *m*/*z* 277 (Ln), *m*/*z* 279 (L), *m*/*z* 281 (O), and *m*/*z* 283 (S) in a ratio of about 2:1:6:20:4, and its most peculiar feature is the relatively high abundant ion of *m*/*z* 283 (S). The EASI(−)-MS of the butter dry extract (Fig. 3F) is also unique; being an animal fat it produces a typical and rich spectrum displaying mainly the ions of *m*/*z* 255 (P), *m*/*z* 279 (L), *m*/*z* 281 (O), and *m*/*z* 283 (S) in a ratio of about 3:2:10:1, and the unique set of marker ions of *m*/*z* 225 (Mo), *m*/*z* 227 (M), *m*/*z* 241 (Pt), *m*/*z* 253 (Po), and *m*/*z* 269 (margaric acid). Lard (Fig. 3G), another typical animal fat, also produces a quite characteristic EASI(−)-MS showing the four common marker ions of *m*/*z* 255 (P), *m*/*z* 279 (L), *m*/*z* 281 (O), and *m*/*z* 283 (S) in a ratio of about 6:8:20:5 as well as that of *m*/*z* 253 (Po).

Tandem mass spectrometric experiments, *i.e.* EASI(−)-MS/MS, also permit the characterization of these marker ions *via* comparison with reported CID data and structurally diagnostic dissociations. For instance, the following biphenolic acids were characterized *via* EASI(–)-MS/MS in the olive oil dry extract: *o*-cumaric acid (*m*/*z* 307), ligstroside aglycon (*m*/*z* 361), and hydroxytyrosylenoate (*m*/*z* 377). These natural biphenolic acids are known as diagnostic markers for olive oils and are of prime importance for its superior biological antioxidant effects [21].

4 Conclusions

A simple and fast method for oil metabolomics and typification based on EASI(−)-MS fingerprinting was demonstrated. Sample preparation is minimal and oil identification is achieved *via* the detection of characteristic sets of ions arising from major chemotaxonomic oil markers. If needed or desired, MS/MS (EASI-MS/MS) can also be applied to add an additional MS dimension for increased selectivity. Based on the present data and our previous successful

Table 1. Main fatty acids identified *via* EASI-MS

Elemental composition	FA[a]	CN/DB[b]	$[M - H]^-$ *m/z*	Olive oil[c]	Hazelnut oil[c]	Soybean oil[c]	Grape seed oil[c]	Canola oil[c]	Butter[c]	Lard[c]
$C_{14}H_{26}O_2$	Mo	14:1	225	0	0	0	0	0	5	0
$C_{14}H_{28}O_2$	M	14:0	227	0	0	0	0	0	10	0
$C_{15}H_{30}O_2$	Pt	15:0	241	0	0	0	0	0	5	0
$C_{16}H_{30}O_2$	Po	16:1	253	5	0	5	0	0	10	10
$C_{16}H_{32}O_2$	P	16:0	255	20	15	15	10	10	30	30
$C_{18}H_{30}O_2$	Ln	18:3	277	8	0	10	0	5	5	0.5
$C_{18}H_{32}O_2$	L	18:2	279	20	20	100	100	30	20	40
$C_{18}H_{34}O_2$	O	18:1	281	100	100	60	30	100	100	100
$C_{18}H_{36}O_2$	S	18:0	283	5	0	10	5	20	10	25

[a]Fatty acids.
[b]Carbon number:number of double bonds.
[c]Relative abundances.
Mo, Myristoleic (14:1); M, myristic (14:0); Pt, pentadecylic (15:0); P, palmitic (16:0); Po, palmitoleic (16:1); Ln, linolenic (18:3); L, linoleic (18:2); O, oleic (18:1); S, stearic (18:0).

applications of direct-infusion ESI-MS for vegetable oil analysis, it is clear that EASI(−)-MS may be used not only to typify vegetable and animal oils in general, but also to detect adulteration, counterfeiting, and aging. These additional applications of EASI(−)-MS for oil analysis are under extensive evaluation in our laboratory, with successful results.

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