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In situ analysis of soybeans and nuts by probe electrospray ionization mass spectrometry

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The probe electrospray ionization (PESI) is an ESI-based ionization technique that generates electrospray from the tip of a solid metal needle. In the present work, we describe the PESI mass spectra obtained by *in situ* measurement of soybeans and several nuts (peanuts, walnuts, cashew nuts, macadamia nuts and almonds) using different solid needles as sampling probes. It was found that PESI-MS is a valuable approach for *in situ* lipid analysis of these seeds. The phospholipid and triacylglycerol PESI spectra of different nuts and soybean were compared by principal component analysis (PCA). PCA shows significant differences among the data of each family of seeds. Methanolic extracts of nuts and soybean were exposed to air and sunlight for several days. PESI mass spectra were recorded before and after the treatment. Along the aging of the oil (rancidification), the formation of oxidated species with variable number of hydroperoxide groups could be observed in the PESI spectra. The relative intensity of oxidated triacylglycerols signals increased with days of exposition. Monitoring sensitivity of PESI-MS was high. This method provides a fast, simple and sensitive technique for the analysis (detection and characterization) of lipids in seed tissue and degree of oxidation of the oil samples. Copyright © 2015 John Wiley & Sons, Ltd.

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Keywords: PESI; peanuts; cashew nuts; macadamia nuts; walnuts; pistachios; almonds; ESI-MS; rancidification

Introduction

Mass spectrometry (MS) has become an indispensable analytical tool in the field of biological science. Although matrix-assisted laser desorption/ionization (MALDI) MS and electrospray ionization (ESI) MS are currently recognized as the most frequently used, the development of improved techniques is still necessary for MS measurements of biological samples, especially at the level of intact tissues and living cells. ESI is a soft ionization technique that achieves the formation of gaseous ions from solutions.[1] Hiraoka *et al.* developed the probe electrospray ionization (PESI) method, which adopts a solid needle as the electrospray emitter.[2] As this method does not use a capillary tube, clogging of the probe is avoided. In PESI, a probe needle directly contacts with the sample, and a small amount of it is picked up on to the tip of the needle when the needle is removed from the sample. Then a high voltage is applied to this needle to generate electrospray. The quality of generated electrospray and mass spectrum was influenced by the material and shape of the needle tip and applied voltage.[3] This technique is versatile in the analysis of biological samples as it requires a small amount of the material picked up without any special sample preparation,[2,4] and lower invasiveness in living biological systems. It was demonstrated, that by using a solid needle with a small tip diameter, a fine electrospray jet could be produced from the tiny liquid droplet formed on the needle tip, with the spraying condition quite similar to that of nanoESI.[3] PESI also shares the advantages of nanoESI in low sample consumption, and higher tolerability to salt buffer compared to conventional ESI. Similarly to ESI, PESI is more sensitive to the more surface-active molecules (more hydrophobic) because they are enriched on the surface of the charged droplet leading to the preferential formation of gaseous ions via the off-spring droplet fission.[4] Thus, sequential ion formation starting from surface-active (hydrophobic) molecules followed by relatively hydrophilic molecules was expected. Recently, it was found that a single-shot PESI-MS using a titanium (Ti) wire could ionize all the analytes contained in a droplet at the needle tip. The analytes undergo sequential and exhaustive ionization according to their surface activities; this phenomenon helps to alleviate the ion-suppression effect that is inherent to capillary-based ESI.[5,6]

Several designs of electrospray ion sources that use noncapillary probes had been put forward to overcome the clogging problem.[7] Shiea *et al.* succeeded in electrospraying a sample solution deposited on a copper wire ring after applying a high voltage to the copper wire, and mass spectra of proteins similar to those of conventional ESI could also be obtained.[8] The technique was further explored using optical fibers wired with copper or platinum wires,[9,10] a glass rod[11] and nanostructured tungsten oxide.[12] Electrospray from the solution deposited on microcapillar chips has also been reported..[13] Furthermore, the so-called electrospray ionization on solid substrates (solid-substrate ESI) include solid-

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substrate ESI with cellulose-based materials (cotton wick and fiber, paper, wooden-tip ESI, bamboo, etc.), online TLC-MS analysis for compound identification[14] and direct ionization (DI), for direct analysis of plant and animal tissues.[15]

Phospholipids (PL) are lipids that contain phosphoric residues, polar head groups and non-polar lipid chains[16] (i.e. phosphatidylcholine (PC), phosphatidylethanolamine (PE); see formulas in Scheme 1S in supplementary material). Due to their wide occurrence in foods and their pro- and antioxidant effects, phospholipids and triacylglycerols (TAG) are used as multifunctional additives in food,[17] pharmaceuticals[18] and other industrial applications.[19] Thin-layer chromatography (TLC)[7] and high-performance liquid chromatography (HPLC)[20] have been used for the analysis of phospholipids of plant seed oil extracts. The introduction of ESI-MS to analyze intact phospholipids has led to a new level of sensitivity for their detection.[21] Compared to those techniques, PESI-MS requires lower sample volume, shorter time analysis and minimal sample preparation.[3] Recently, easy ambient sonic-spray ionization (EASI) has been employed for direct analysis of vegetable oils,[22,23] phospholipids present in lecithins[24] and brazil nut oil.[25] No sample preparation or pre-separation was needed for EASI application.[22–25]

Lipid oxidation in foods constitutes a complex chain of reactions that yields to primary products (peroxides, hydroperoxides), which, once exposed to extended oxidation conditions, gives rise to secondary oxidation products, including aldehydes, ketones, epoxides and hydroxy compounds. Most of these secondary oxidation products produce undesirable sensation (i.e. smell, taste) and biological effects (i.e. toxicity) on animals and humans.[26] The oxidation process becomes more pronounced with the higher number of double bonds in lipids. Despite being intermediate compounds of lipid oxidation process, hydroperoxides are relatively stable (depending on the lipid structure) and can be used to assess lipid oxidation status in food samples, as long as the sample is not in an advanced oxidative status.[26] Recently, EASI has been applied for TAG oxidation monitored in fats and oils.[27,28]

Here we describe the PESI mass spectra obtained, *in situ*, of soybeans and several nuts (peanuts, walnuts, cashew nuts, macadamia nuts, pistachios and almonds) with commercial interest (i.e. food, production of oil for human consumption, cosmetic oil, etc.) using different solid needles as sampling probe. We use the intact seeds without pre-treatment and methanolic extracts as samples for comparative analyses. Additionally, methanolic extracts were exposed to air and sunlight for several days (oxidative conditions; rancidification) and analyzed by using PESI-MS and ESI-MS. Principal component analysis (PCA) was applied to PESI-MS results. The data set consists of *m/z* values and the corresponding ion intensities for same lipids detected in the seeds. The present work shows that the PESI-MS method is a useful tool for analysis of lipid content in nuts and soybean seeds and in its oil. Besides, characterization of the oil and evaluation of degree of rancidification is possible.

Experimental

Materials

All reagents used in this study were analytical grade or higher and were used without further purification. Water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Ammonium acetate, glacial acetic acid, chloroform and methanol were provided by Kanto Chemicals (Tokyo, Japan). Pentafluorophenyl triethoxysilane was purchased from Sigma-Aldrich Chemical Co., USA. Soybean and nuts were obtained from local market (Kofu, Japan). Walnuts were obtained from local market in Buenos Aires (Argentine), too.

PESI mass spectrometry

The PESI experimental procedures were similar to those described previously.[4,5] Briefly, the needle was moved up and down along a vertical axis using a custom made linear actuator system. When the needle was at the bottom position, the tip of the needle was adjusted to touch the surface of the sample. When the needle or wire was moved up to the highest position, a high voltage of about 2–3 kV was applied to it. The distance of the needle stroke was 10 mm. As electrospray emitters, disposable acupuncture needles (Seirin, Shizuoka, Japan: 0.3 × 50 mm and 0.14 × 30 mm) with submicrometer tip diameter and a tangentially cut titanium (Ti) wire (Nilaco Corporation, Tokyo, Japan) with 0.5-mm diameter were used throughout the PESI-MS experiments. The Ti wire surface was modified as described before.[5] Briefly, after the Ti wire surface was oxidized under a burner flame, the wire was incubated in 100% pentafluorophenyl triethoxysilane for 1 h at room temperature. After incubation, the wire was rinsed with methanol and pure water.

The seeds were cut in two pieces and kept on the sample holder using double fixing tape or a metallic hook (Fig. 1S in supplementary material). The sample was stitched directly by the probe needle. For each sampling location, the sample stage was adjusted in such a way that the tissue section was stitched to a minimum threshold depth where ion signals with acceptable signal-to noise (S/N) ratio were detected. The stitching depth was not precisely determined, but was estimated to be less than 0.5 mm. Since PESI is based on electrospray, it can only be applicable to liquid samples. In some cases, however, the surface of the biological samples was not juicy enough; only a small amount of biofluid could be attached to the needle tip. In general, a drop (0.5 μl) of methanol or methanol/water (1:1) was deposited over the tissue to solve that problem. The fluid picked up by the needle was electrosprayed and analyzed.

Two methods were used to acquire the mass spectra: the droplet was left to be electrosprayed until the electrospray current decreased to zero (single-shot) or the droplet was periodically renewed (continuous-shot). In the latter, the PESI mass spectra were obtained with an acquisition time of 1 s with 3 Hz of the probe motion. For PESI, the sample was sprayed by electrostatic means and was not assisted by any sheath flow or nebulizing gas.

The ions generated from the electrospray were sampled through the ion-sampling orifice with a diameter of 0.4 mm into the vacuum chamber and mass analyzed by an orthogonal time-of-flight mass spectrometer (AccuTOF; JEOL, Akishima, Japan). Spectra were obtained and analyzed with the program Mass Center (JEOL). Each experiment was repeated at least three times in order to ensure the reproducibility.

ESI mass spectrometry

Experiments were conducted with two different mass spectrometers. (i) A linear ion trap mass spectrometer (LTQ-Velos, Thermo Scientific, USA) equipped with collision-induced dissociation (CID) and pulse-Q collision induced dissociation (PQD) modes was operated in the positive ion mode. The source voltage was 3 kV, the source current was 0.2 μA, the sheath gas flow rate was 3 (a. u.) and the capillary temperature was 150 °C. A syringe from Agilent

with 2.3 mm in inner diameter was used to introduce the sample and a flow rate of 10 μl/min. Spectra were obtained and analyzed with the program Thermo Xcalibur Qual Browser. (ii) ESI-MS analyses were performed in positive ion mode using mass spectrometer Bruker micrOTOF-Q II equipped with CID. The source voltage was 4.5 kV, the end plate offset was −500 V, the collision cell RF was 300 Vpp, the nebulizer operated at 0.4 Bar, the dry heater temperature was 200 °C and the dry gas flow was 4.0 l/min. Sample flow rate of 5 μl/min and the solvent stream was methanol.

Lipid extraction from seeds

Seeds were smashed in a mortar. Lipids were extracted and washed with small portions of methanol (i.e. three seeds and total volume of methanol 2 ml). The liquid extracts were collected and then centrifuged.

Principal component analysis

Statistical analysis was performed by principal component analysis (PCA) (SPSS Statistic 17.0, IBM software, USA). The data set consists of *m/z* values of TAGs and PCs and the corresponding signal intensities of the spectra to be compared. Six different experiments, using six seeds each time, were conducted for soybeans and each variety of nuts. Mass spectra were measured sampling with the needle all over the surface and replacing the seed for a fresh one.

Results and discussion

PESI-MS experiments

In order to find the optimal conditions for seed analysis by PESI-MS the effect of changing some experimental conditions on the spectra was studied. Different materials as needle probes and different needle diameters were tested. Additionally, two methods were used to acquire the mass spectra: the single-shot mode and the continuous-shot mode. In the former, the droplet was left to be electrosprayed until the electrospray current decreased to zero, and in the latter the droplet was periodically renewed. In connection with the diameter of the probe it is interesting to point that a needle with tip diameter ≥0.3 mm was required to observe signals. No signals were observed when a smaller one (i.e. needle diameter 0.14 mm, tip diameter ~ 700 nm) was used (result not shown). The smaller needle could not penetrate into the hard seed tissue due to its flexibility. When probes of different materials (titanium wire or acupuncture needle) were used by continuous mode, spectra with similar number of lipid signals were obtained (Fig. 2S, supplementary material) although the relative intensities of signals changed when different probes were used. In PESI experiments, not only lipid but also carbohydrate signals were detected. In general, four signals corresponding to carbohydrates of low molecular masses were detected: $[Hex2 + Na]^+$ with $m/z = 365.12$ (Fig. 2Sa), $m/z = 365.23$ (Fig. 2Sb), (m/z calc. = 365.11), $[Hex2 + K]^+$ with $m/z = 381.07$ (Fig. 2Sa), $m/z = 381.18$ (Fig. 2Sb), (m/z calc. = 381.08), $[Hex3 + Na]^+$ with $m/z = 527.31$ (Fig. 2Sb), (m/z calc. = 527.16) and $[Hex3 + K]^+$ with $m/z = 543.14$ (Fig. 2Sa), (m/z calc. = 543.13). Most signals observed in the region between m/z 700 and 850 were assigned to PC and between m/z 850 and 950 to TAG. Identification of ions was based on the measured molecular mass, comparison with references and MS/MS experiments (Table 1).

Mass spectra of seeds were measured by PESI-MS and those of methanolic seeds extract by PESI-MS and ESI-MS for comparative study. The results obtained by *in situ* analysis of almonds using PESI are shown in Fig. 1a and those obtained by the ESI analysis of the methanolic extract in Fig. 1b. To record the PESI mass spectra, a piece of almond was fixed on the sample holder using tape, an acupuncture needle was used as a probe, a drop of methanol (0.5 μl) was deposited over the tissue to keep it wet and the spectra were recorded in continuous-shot mode. In this experimental conditions the species $[PC(34:2) + Na]^+$ ($m/z = 780.80$), $[PC(36:3) + Na]^+$ ($m/z = 806.79$), $[PC(36:2) + Na]^+$ ($m/z = 808.81$), $[TAG(54:4) + Na]^+$ ($m/z = 905.97$), and $[TAG(54:3) + Na]^+$ ($m/z = 907.97$) were detected (Fig. 1a, and Table 1). As is shown in Figs. 1a and 1b, PESI and ESI mass spectra were quite similar. It is worth mentioning that

Table 1. Main PC, PE and TAG ions detected by PESI-MS and ESI-MS in nuts. Positive ion mode. Assignment based on *m/z* values and MS/MS

Assignment	Adduct			MS/MS
	H^+	Na^+	K^+	
PC[34:2]	758	780	–[a]	M-59, M-183, M-205, 86, 147,441, 465, 524
PC[34:1]	760	782	–[b]	M-59, M-183, M-205, 86,147,467, 441
PC[36:3]	784	806	–[c]	M-59, M-183, M-205, 86, 147, M-283
PC[36:2]	786	808	–	M-59, M-183, M-205, 86, 147, M-283
PE[40:1]	–	824	–	M-59, M-183, M-221, 86, M-283
TAG[50:2]	–	853	–	-
TAG[52:4]	–	877	893	-
TAG[52:2]	–	–	897	-
TAG[54:7]	–	–	915	-
TAG[54:6]	–	901	917	-
TAG[54:5]	–	903	919	-
TAG[54:4]	–	905	921	M-256, M-282, M-304
TAG[54:3]	–	907	923	–
TAG[54:2]	–	–	925	–

[a] $m/z = 796$ detected in walnuts;
[b] $m/z = 798$ detected in walnuts;
[c] $m/z = 822$ detected in walnuts

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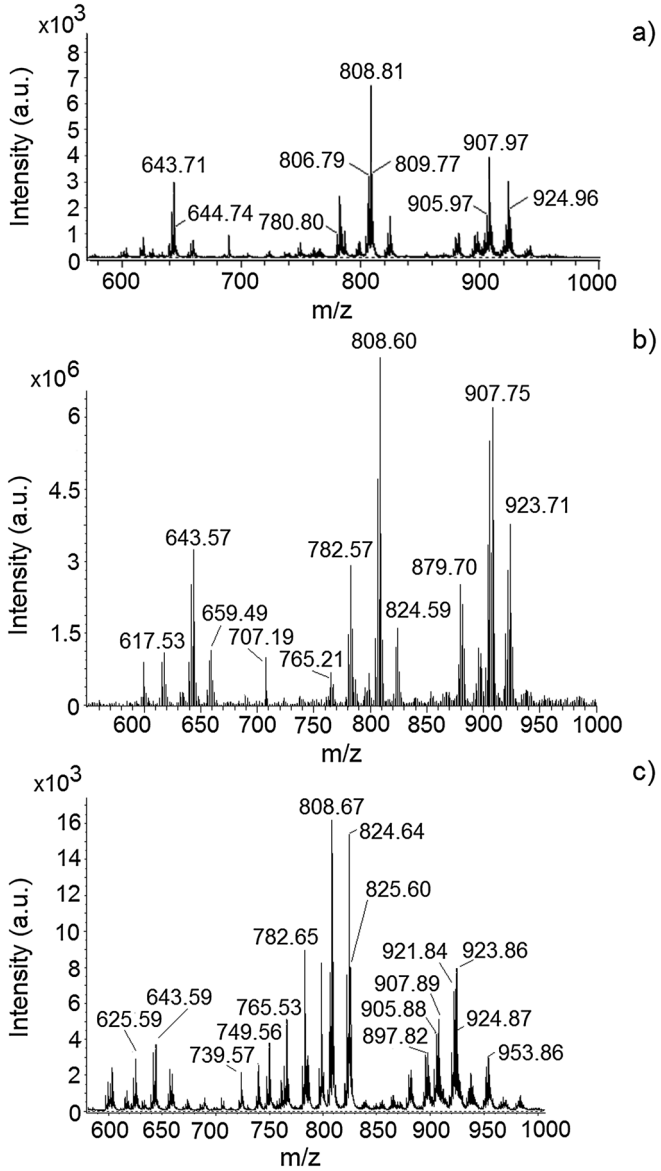

Figure 1. Positive ion mass spectra of almonds: a) PESI mass spectrum *in situ* (intact tissue). Probe: acupuncture needle, continuous-shot mode and b) ESI mass spectrum of methanolic extract. Positive ion PESI mass spectra of peanuts: c) *in situ* (intact tissue). Probe: titanium needle, continuous-shot mode.

intensities and resolution of these spectra are different since two different mass spectrometers were used for ESI and PESI measurements. Similar results were obtained for all the other seeds studied. PESI-MS experiments were also performed *in situ* by continuous-shot mode using titanium needle as probe. As an example for peanuts (Fig. 1c) most signals identified are sodium or potassium adduct of PC and TAG such as $[PC(34:2)+Na]^+$ ($m/z=780.73$), $[PC(34:1)+Na]^+$ ($m/z=782.65$) $[PC(36:3)+Na]^+$ ($m/z=806.72$), $[PC(36:2)+Na]^+$ ($m/z=808.67$), $[PE(40:1)+Na]^+$ ($m/z=824.64$), $[TAG(54:4)+Na]^+$ ($m/z=905.88$), $[TAG(54:3)+Na]^+$ ($m/z=907.89$) and $[TAG(54:3)+K]^+$ ($m/z=923.86$) (Table 1). It is worth mentioning that the lipid composition of soybeans and nuts obtained by PESI-MS is similar to those previously described by gas chromatography-MS.[19,29]

We conducted PESI experiments *in situ* in the hard nuts tissue without any sample preparation. As it is known in plant tissue the content of K^+ is higher than Na^+. Thus, the ions $[M+K]^+$ are expected from tissues not washed before the experiments. For the nuts studied the reported average content of Na^+ and K^+ in 100 g of each shows a variation of the Na^+/K^+ ratio of 1 mg of Na^+ to 70–1100 mg of K^+ (average values).[29] As is detailed in Table 1 lipids were detected as $[M+K]^+$ and also as $[M+Na]^+$. This fact suggests that different stability of the adducts may rule the efficiency of gas ion formation from the droplet, assuming that the relative distributions of Na^+, K^+, PL and TAG are similar in the tissues studied. In general PL and TAG were detected as $[M+Na]^+$ and $[M+K]^+$, mainly the former as $[M+Na\}^+$ and the latter as $[M+K]^+$ (Figs. 1, 3, 4, 3S and 4S; Table 1). Taking into account that $Na^+ < K^+$, that means that these sodiated PL and TAG adducts are quite stable. In the case of walnuts both PL and TAG were mainly detected as $[M+K]^+$ (Figs.4 and 4S; Table 1). Taking into account the fact that the content of unsaturated lipids is higher in walnuts (see *Monitoring rancidification* section) the results can be explained suggesting that in PC when the molecular weight to number of double bonds (unsaturation) ratio is high (PC[34:2] and PC[36:3], Table 1) the corresponding potassiated species with *m/z* 796 and 822 are preferentially formed (Figs. 4 and 4S; Table 1). Similar conclusion is obtained from walnut TAG analysis because the higher unsaturated species, TAG[54:7] and TAG[54:6], are detected in PESI as $[M+K]^+$ at *m/z* 915 and 917, respectively (Table 1). On the contrary TAG[54:3] yielded as stable adducts $[M+Na]^+$ ($m/z=907$) and $[M+K]^+$ ($m/z=923$) (Table 1), i.e. almonds and peanuts (Fig. 1). The degree of unsaturation in PC and TAG seems to play a key role in the efficiency of $[M+Na]^+$ and $[M+K]^+$ formation. In connection with the improvement of knowledge of the major effects governing EASI desorption/ionization efficiency, a systematic investigation of desorption/ionization efficiencies as a function of unsaturation and length for TAG as well as for diacylglycerols, monoacylglycerols and PL has recently reported.[30] Affinities for Na^+ as a function of unsaturation level were assayed via comprehensive metadynamics calculations to understand the influence of this phenomenon on the ionization efficiency. The results suggest that dipole–dipole interactions within a carbon chain tuned by unsaturation sites govern ionization efficiency of TAG and PL.

Principal component analysis

The triglyceride PESI spectra of various seeds were compared by principal component analysis (PCA) (Fig. 2). The data set consists of *m/z* values and the corresponding signal intensities for several PCs and TAGs. The positive ion PESI mass spectra of soybeans, macadamia nuts, walnuts and peanuts were recorded using acupuncture needles and continuous-shot mode in order to detect possible differences in TAGs and PCs composition. As is displayed in Fig. 2, PCA shows that the variations in the intensity distribution contain enough information to obtain a clear differentiation between the four families of seeds studied (Figs. 3S and 4S, supplementary material).

Monitoring rancidification

As an application example of PESI-MS the oxidation process of lipids in extracted oils was monitored. Methanolic extract of walnuts was chosen for the investigation of oxidation process, as it contains highly unsaturated lipids. The sample was exposed to air and sunlight for several days. With the aging of the oil, the formation of oxidated products with increasing number of hydroperoxide groups was observed in the PESI spectra (Fig. 3). As is shown in Table 2, signals of oxidized lipids were detected after light

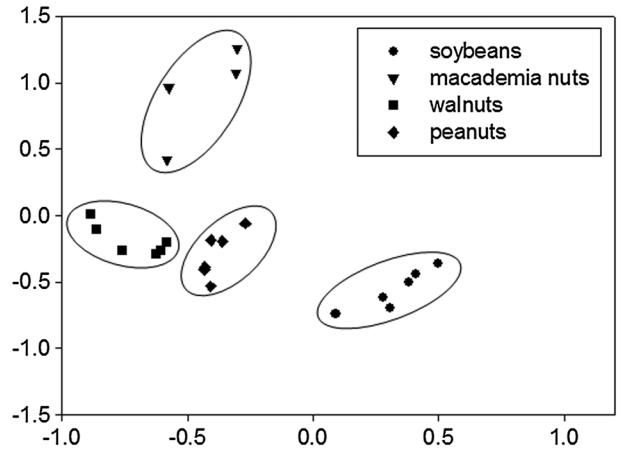

Figure 2. PCA of four different seeds. Data from positive ion PESI mass spectra *in situ* of soybeans, macadamia nuts, walnuts and peanuts. Probe: acupuncture needle, continuous-shot mode. Methanol:H_2O (1:1 *v/v*) to keep the seed surface wet.

exposition at $m/z = 933.58$ $[M + Na]^+$; $m/z = 949.56$ $[M + K]^+$; $m/z = 965.57$ $[M + Na]^+$; $m/z = 981.73$ $[M + K]^+$; $m/z = 997.56$ $[M + Na]^+$; $m/z = 1013.55$ $[M + K]^+$ and $m/z = 1029.56$ $[M + Na]^+$.[27,28] Similar experiments were performed using soybeans and peanuts methanolic extracts. The PESI spectra showed that the species detected after oxidation were similar than those present in fresh oil for both varieties of seeds studied (Figs. 5S and 6S, supplementary material). Unlike the results obtained for walnuts, no significant differences were detected for mass spectra of other nuts in the range of *m/z* 900 and 1050, before and after air exposition. In our experimental conditions oxidation was more important in walnuts compared with soybeans and peanuts. This result can be explained considering unsaturated lipid composition of soybeans, peanuts and walnuts (walnuts 47.0-g polyunsaturated fatty acids (PUFA**s**)/100 g; peanuts 15.5-g PUFAs/100 g and soybeans 3.2 g PUFAs/100 g). ESI-MS spectra recorded before and after rancidification treatment also showed the easier oxidation of walnuts (Fig. 7S, supplementary material).

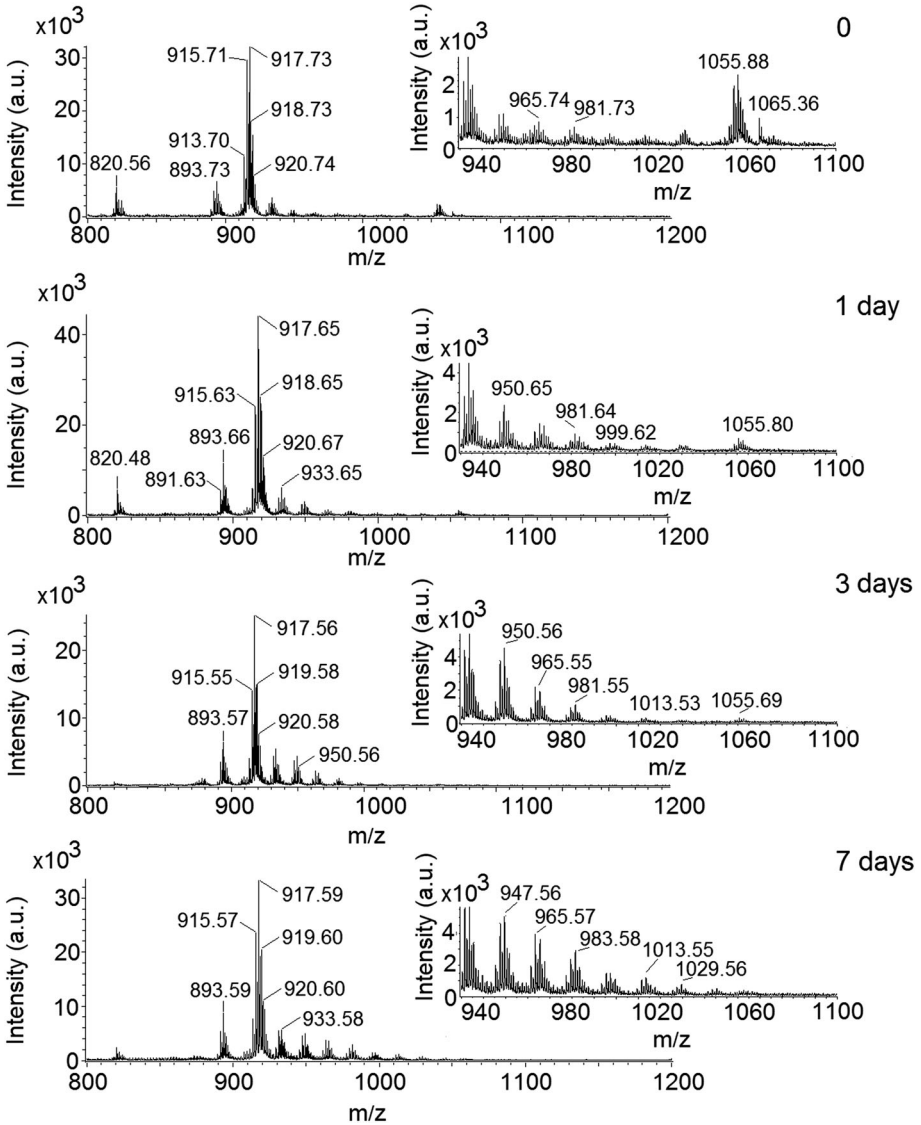

Figure 3. Comparison of PESI mass spectra of methanolic extract of walnuts after exposure to air and sunlight for: a) 0 days, b) 1 day, c) 3 days, and d) 7 days. Inset: zoom between $m/z = 930$ and $m/z = 1100$.

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Table 2. Main TAG ions detected by PESI-MS and ESI-MS in nuts after oxidation (rancidification). Positive ion mode

Assignment	Lipid		Monohydroperoxide		Bishydroperoxide		Trishydroperoxide		Tetrahydroperoxide	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
TAG[52:2]	–	897	913	–	–	–	–	–	–	–
TAG[54:7]	–	915	931	947	963	979	995	1011	1027	1043
TAG[54:6]	901	917	933	949	965	981	997	1013	1029	1045
TAG[54:5]	903	919	935	951	967	983	999	1015	1031	1047
TAG[54:4]	–	921	937	953	969	985	1001	–	–	–
TAG[54:3]	–	923	939	955	–	–	–	–	–	–

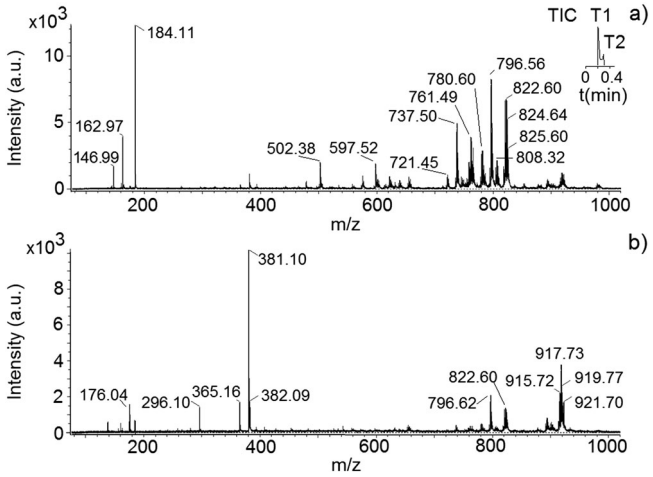

Figure 4. Positive ion PESI mass spectra *in situ* of walnuts. Probe: titanium needle, single-shot mode. a) Mass spectrum measured at T1, inset: total ion chromatogram. b) Mass spectrum measured at T2. Methanol:H_2O (1:1 *v/v*) to keep the seed surface wet.

Single-shot PESI mass spectra

As an example Fig. 4 displays the results obtained for walnuts. Inset in Fig. 4a shows the total ion chromatogram (TIC) for the single-shot PESI mass spectra measured after 10 μl of the mixture H_2O:MeOH (1:1 *v/v*) was dropped on the surface of the walnuts. TIC shows two steep stepfunction-like increases. Figures 4a and 4b show the mass spectrum measured at T1 and T2. At T1, the onset of the electrospray, signals of PC were dominant (Fig. 4a), while at T2, low weight carbohydrates and TAG were detected (Fig. 4b). Time-dependent mass spectrum between PC and TAG has been observed when surface modified titanium wire was used as a probe.[5] Here, for the first time, this effect was observed for mixtures of carbohydrates and lipids.

Conclusions

It was demonstrated that mass spectra of PL, TAG and carbohydrates of low molecular masses can be easily obtained from the hard tissue of intact soybeans and nuts using PESI-MS *in situ*. For comparative study ESI-MS and PESI-MS analyses were performed with methanolic extracts obtained from the seeds. The great advantage of PESI-MS is that minimal sample preparation is needed and minimum volume of solvent is required. Combining PESI-MS analysis of lipids and PCA a clear differentiation between soybeans and the nuts studied could be obtained.

Modification of the titanium wire surface with pentafluorophenyltriethoxysilane to make it more hydrophobic allowed to get time-dependent mass spectra for the natural mixture of carbohydrates and lipids present in seeds, when single-shot PESI mode was adopted. PESI-MS was useful for early detection and characterization of lipid oxidation products formed in methanolic extracts containing oil from seeds. Thus PESI can be employed in quality and safety control applications in food and cosmetic industry.

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