RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2009; **23**: 3057–3062
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.4220

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Direct olive oil analysis by low-temperature plasma (LTP) ambient ionization mass spectrometry

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Received 4 May 2009; Revised 21 July 2009; Accepted 22 July 2009

A fast, reagentless, and direct method is presented for the mass spectrometric analysis of olive oil without any sample pretreatment whatsoever. An ambient ionization technique, the low-temperature plasma (LTP) probe, based on dielectric barrier discharge, is used to detect both minor and trace components (free fatty acids, phenolics and volatiles) in raw untreated olive oil. The method allows the measurement of free fatty acids (the main quality control parameter used to grade olive oil according to quality classes), selected bioactive phenolic compounds, and volatiles. The advantages and limitations of the direct analysis of extremely complex mixtures by the ambient ionization/tandem mass spectrometry combination are discussed and illustrated. The data presage the possible large-scale application of direct mass spectrometric analysis methods in the characterization of olive oil and other foodstuffs. Copyright © 2009 John Wiley & Sons, Ltd.

The detection of specific chemical compounds in complex matrices, long important in the chemical research laboratory, has gradually taken on increased importance as a large-scale regulatory and commercial activity. Purchases of new items of major scientific instrumentation to facilitate organic and biological compound analysis run at approximately $US 20B/year and the associated analytical chemistry activity dwarfs this sum. These considerations demand efficiency in the total analysis protocol, an end-point value insufficiently considered by those engaged in the development of new analytical instrumentation, where attention tends to focus on analytical performance parameters such as specificity and sensitivity, and the range of applicability. Reducing sample preparation prior to analysis is a key to increased efficiency and this will also help to increase the use of automated, *in situ* and ideally low-solvent consumption ('green') procedures in the analysis of complex samples. New mass spectrometric ionization methods, in which samples are ionized in the ambient environment,[1–4] sometimes using miniature mass spectrometers,[5] offer particular promise for direct, real-time analysis. These advantages are principally the result of the fact that sample preparation is not required to implement ambient ionization mass spectrometry.

Among the available ambient ionization methods,[3] several employ atmospheric pressure plasmas, including direct analysis in real time (DART),[6] desorption atmospheric pressure chemical ionization (DAPCI),[7] flowing afterglow-atmospheric pressure glow discharge (FA-APGD),[8] plasma-assisted desorption ionization (PADI)[9] and dielectric barrier discharge ionization (DBDI).[10] Recently, our research group described a new ambient plasma-based ionization technique, which employs a low-temperature plasma (LTP) probe,[11–13] to directly analyze trace compounds on solid surfaces and in complex matrices, without the need for solvents or reagents, other than a low flow of discharge gas.

The direct analysis of foodstuffs without any sample treatment but with high molecular selectivity and sensitivity has not generally been possible by mass spectrometry (MS). Among the more challenging foodstuffs to characterize are the vegetable oils, and, in particular, olive oil. Vegetable oils are complex chemical mixtures, in which di- and triglycerides and free fatty acids are the main components, but a series of minor polar compounds is also present and their distributions are characteristic of different types of oils. The compositions of these oils depend on the vegetable, nut, or seed from which they are extracted. Olive oil, an expensive oil considered to have health benefits,[14] is sometimes adulterated with lower priced oils. This has created a need for the authentication of oil samples by methods that are fast, straightforward, and accurate to determine their provenance, quality, and possible adulteration. Ideal analytical methods for this purpose require minimal or no sample preparation, are rapid and accurate, and are capable of a high degree of automation and of *in situ* operation. Currently available methods for the characterization of vegetable oils based on MS and other spectrometric techniques (e.g. fluorescence,[15,16] vibrational spectroscopy,[17–19] and nuclear magnetic resonance spectrometries[20]) do not meet these requirements. Extraction protocols involving liquid-liquid and/or solid-phase extraction are required prior to analysis by MS,[21–25] except that

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headspace analysis can be used to characterize oils on the basis of their volatile components.[26] Electrospray ionization (ESI) has been used to characterize particular components[27] and photoelectron resonance capture ionization (PERCI) has been used for the direct MS analysis of fatty acids in olive oil without prior sample treatment but under the normal vacuum conditions of MS.[21]

In this work, an ambient ionization method, LTP-MS, is used for the direct analysis of crude olive oil without sample pretreatment. Crude olive oil samples are examined in the open environment without any previous treatment or even dilution, by deposition on a heated glass surface and exposure to the plasma. No organic solvents are used. This 'green' approach permits the direct sampling and ionization of the main chemical species used for quality control and authentication purposes although not all compounds known to be present in olive oil can be detected under the given conditions. The LTP-MS method utilizes a low-flow helium carrier gas as the discharge gas to generate a low-temperature plasma that samples, desorbs and gently ionizes compounds of interest from the oil being studied. In related unpublished work[28] direct analysis in real time (DART) has been used to characterize some of the constituents of olive oil without any prior workup.

EXPERIMENTAL

Low-temperature plasma mass spectrometry

Experiments were performed using a Thermo LTQ linear ion trap mass spectrometer (ThermoFinnigan, San José, CA, USA) tuned for the maximum signal of the precursor ion of interest. Data were acquired using the manufacturer's Xcalibur® software. LTP-MS analysis was performed in the positive and negative ion modes for all the samples studied. The instrument was set to record spectra in the automatic gain control mode for a maximum ion trap injection time of 200 ms using 2 microscans per spectrum. The main experimental parameters used were as follows for the positive ion mode: *m/z* range, 150–600; ion spray voltage, 4.5 kV; capillary temperature, 200°C; tube lens, –65 V; capillary voltage, −15 V. For the negative ion mode: ion spray voltage, −4.5 kV; capillary temperature, 200°C; tube lens voltage, +65 V; capillary voltage, +15 V. Tandem mass spectrometry (MS/MS) experiments were performed using collision-induced dissociation (CID) in order to confirm the presence of particular analytes in the studied samples. These experiments were performed using an isolation window of 1.5 *m/z* units and 25–35% collision energy (manufacturer's units).

The LTP probe (Fig. 1) is described in detail elsewhere.[11,12] It consists of a glass tube (o.d. 6.35 mm; i.d. 3.75 mm) with an internal grounded electrode (stainless steel, diameter: 1.57 mm) centered axially, and an outer electrode (copper tape) surrounding the outside of the glass tube. The wall of the glass tube serves as the dielectric barrier. An alternating high voltage, in the range 5–10 kV, at a frequency of ca. 2.5 kHz, is applied to the outer electrode with the center electrode grounded to generate the dielectric barrier discharge. The discharge alternating current (AC) voltage was provided by a custom-built, variable frequency, variable voltage power supply with total power consumption below 3 W. Helium (flow rate 0.4 L/min) was normally used as a discharge gas and as the agent to transport analyte ions to the mass spectrometer. The olive oil samples were placed on the sample holder, typically 5 mm away from the LTQ inlet. The LTP probe was placed with its end 4 mm away from the surface with an angle to the sample surface of ca. 30°.

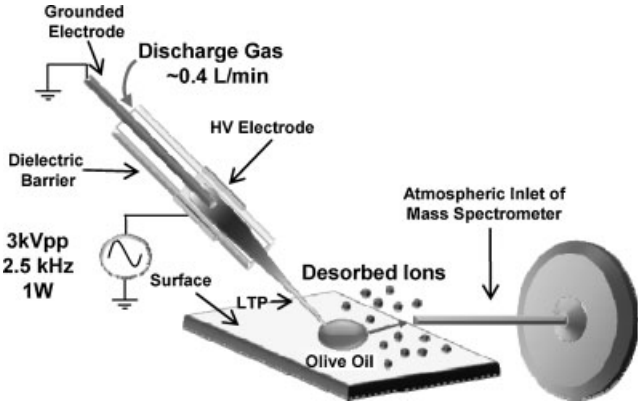

Figure 1. Schematic of the configuration of the LTP probe used for ambient ionization mass spectrometry of crude olive oil.

Direct LTP-MS analysis of crude olive oil

Olive oil samples were purchased from different local markets. No sample treatment was used. Aliquots (3 μL of each solution) were deposited using a micropipette on a microscope glass slide (beveled micro slides, size 75 × 25 mm, thickness 1 mm, Gold Seal®; Becton, Dickinson and Co., Franklin Lakes, NJ, USA).

RESULTS AND DISCUSSION

Olive oil is rich in unsaturated fatty acids (especially oleic and linoleic acid) and in phenolic compounds, which act as natural anti-oxidants. The free fatty acids, volatiles and phenolic constituents in crude olive oil constitute fundamental parameters for quality control and authentication purposes. The compounds detected by LTP-MS were the free fatty acids, some of the compounds responsible for the aroma of extra-virgin olive oil, and some of the main phenolic compounds characteristic of olive oil (Table 1). A typical

Table 1. Some compounds characterized by LTP-MS in untreated virgin olive oil

Compound	MW
Oleic acid	282
Linoleic acid	280
Palmitic acid	256
Tyrosol	138
Hydroxytyrosol	154
p-Hydroxybenzoic acid	138
Coumaric acid	164
Ferulic acid	194
Syringic acid	198
Hexanal	100
2-Hexen-1-ol	100
2-Hexenal	98

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DOI: 10.1002/rcm

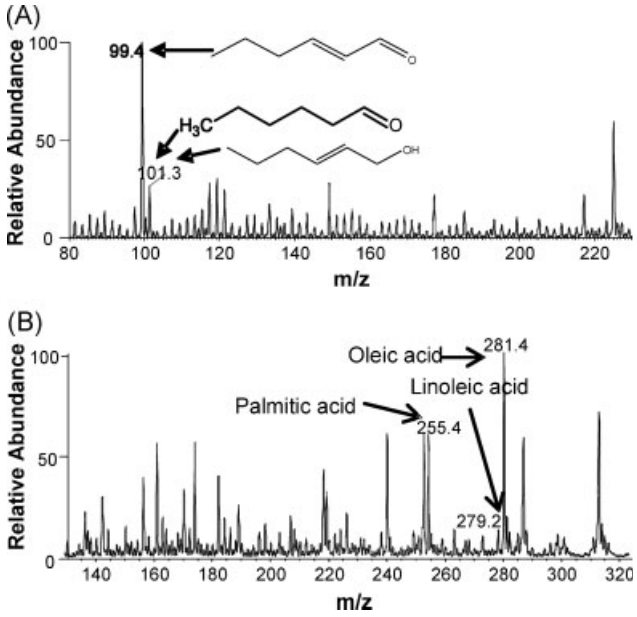

Figure 2. LTP-MS full-scan spectra of crude virgin olive oil in the positive (A) and negative (B) ionization mode.

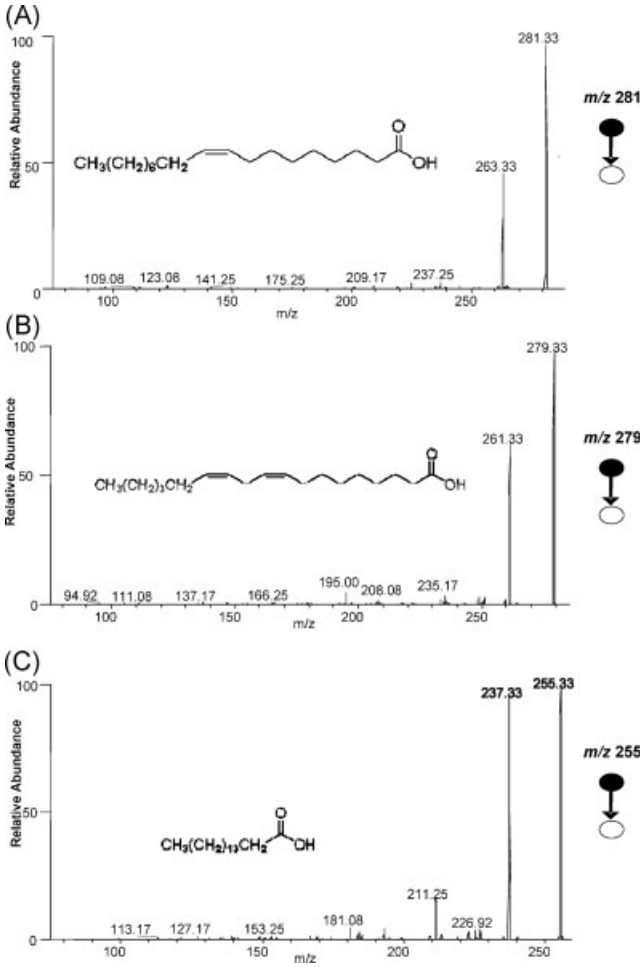

Figure 3. LTP-MS/MS spectra (negative ion mode, $[M–H]^-$) of free fatty acids found in crude virgin olive oil: (A) oleic acid, (B) linoleic acid, and (C) palmitic acid.

full-scan positive ion mass spectrum of an Italian extra-virgin olive oil sample with low acidity (Fig. 2(A)) gives information on the presence of volatiles (hexanal, 2-hexen-1-ol and 2-hexenal), while in the negative ion mode ions for the deprotonated molecules of the main free fatty acids (oleic acid, linoleic acid and palmitic acid) are evident (Fig. 2(B)). The phenolic compounds are present at lower concentrations, so that MS/MS experiments are required to detect them in the complex matrix (see below).

Free fatty acids

When evaluating the quality of virgin olive oil, the free fatty acid (FFA) content (the combination of all acids expressed as the percentage of oleic acid) is a major quality criterion. As previously mentioned, the direct reagent-free determination of FFA content in crude olive oil has only once been described by any mass spectrometric technique.[21] This is because fractionation, purification or dilution steps are usually required.[22–25] We used an oil sample with low acidity (ca. 0.2%, expressed as oleic acid percentage in the sample). The main fatty acids characteristic of crude olive oil (oleic, linoleic and palmitic acids) were detected in both the positive and the negative ion modes (in both full-scan and MS/MS experiments (Fig. 3)), and with or without heating the glass surface on which the sample was deposited. The experiment is characterized by being performed under ambient conditions.

To evaluate the ability to measure FFA content, we used spiked samples prepared by adding oleic acid to different aliquots of the studied virgin olive oil sample – with 0.2% intrinsic oleic acid – to obtain samples ranging in FFA content from 0.5 to 2%. These samples gave signals of the target ions characteristic of the fatty acid and displayed a linear concentration response (Fig. 4). Note that the intercept in this plot corresponds to the actual concentration of oleic acid in the original olive oil sample. This attractive feature of the proposed approach might be useful for the quality control of crude oils and their classification according to their degree of acidity, and also for the control of processes which might lead to the elimination of free fatty acids in the edible vegetable oil industry. Another potential application of this readily obtained LTP data is the use of the concentration profile of these three main fatty acids, in order to detect adulterations with cheaper seed oils which have characteristically different distributions of fatty acids.

Phenolics

Phenolic compounds have attracted much attention because of their health attributes. Amongst the various components of the unsaponifiable fraction of olive oil, phenolics are important because they are natural antioxidants. Their chemical nature and amounts are factors to be considered in the evaluation of the quality of an extra-virgin olive oil due to their role as natural antioxidants, and their nutritional value, flavor, and organoleptic features. The direct LTP-MS analysis of the main phenolic compounds including phenolic acids and phenolic alcohols is possible from the MS/MS data shown in Fig. 5. Phenolics occur in olive oil at trace levels and typical concentrations in crude olive oil vary, depending on the origin and variety of the sample, but the usual values are

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DOI: 10.1002/rcm

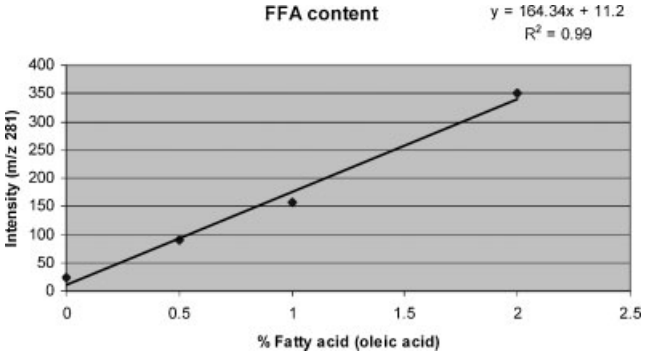

Figure 4. Linear concentration response calibration curve of free fatty acids in spiked virgin olive oil.

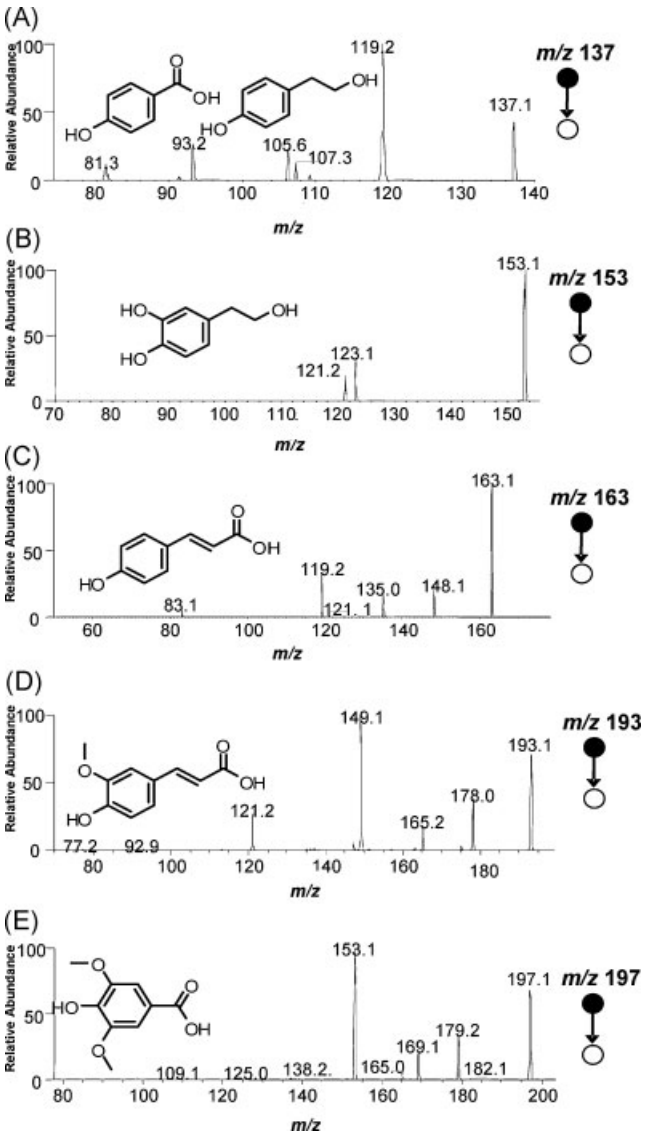

Figure 5. LTP-MS/MS spectra (negative ion mode, $[M–H]^-$) of phenolics found in crude virgin olive oil: (A) tyrosol and *p*-hydroxybenzoic acid, (B) hydroxytyrosol, (C) coumaric acid, (D) ferulic acid, and (E) syringic acid.

in the high $ng\,g^{-1}$ range. Because of their relatively low concentrations compared with free fatty acids, we used LTP-MS with a heated substrate (at 150°C) and MS/MS experiments for the identification of the phenolics. Figure 5 shows the MS/MS spectra (negative ion mode) collected from an Italian virgin olive oil sample, in which the signal-to-noise ratios obtained for most of the known phenolic constituents were distinctly higher than the limit of detection. The data show the ability of the proposed approach to detect many of these important compounds. The main phenolic acids identified were benzoic acid derivatives (*p*-hydroxybenzoic acid, syringic acid, coumaric acid and a derivative (ferulic acid)). In addition, two phenolic alcohols (tyrosol and hydroxytyrosol) were identified. It must be noted, however, that without more comparison data with authentics it is not possible to rule out contributions from isomeric compounds to these spectra.

The MS/MS product ion spectrum of the ion at *m/z* 137 combines features of the MS/MS spectra of both deprotonated tyrosol and deprotonated *p*-hydroxybenzoic acid (Fig. 5(A)) which are present in this olive oil sample. A characteristic product ion with *m/z* 93 is generated by neutral loss of CO_2 from the deprotonated molecule of *p*-hydroxybenzoic acid (confirmed by MS/MS on the ion of *m/z* 137 in a solution of the neat standard); the ions at *m/z* 107 and *m/z* 105 are generated, respectively, by neutral losses of methanol and formaldehyde from deprotonated tyrosol. These ions, including *m/z* 93 and 81, are all present in relative intensities that are consistent with those from neat tyrosol standards. The fragmentation pattern of deprotonated hydroxytyrosol is similar; its MS/MS spectrum reveals the product ions at *m/z* 123 and 121 which correspond to the same neutral losses of methanol and formaldehyde, respectively (Fig. 5(B)). The MS/MS spectra of other phenolics are characterized by loss of simple neutrals CO_2 (loss of44 Da) and CO (loss of 28 Da). In particular, the MS/MS spectra of deprotonated coumaric acid (Fig. 5(C)) and deprotonated ferulic acid (Fig. 5(D)) are analogous. The loss of CH_3 (15 Da) is characteristic of only syringic and ferulic acids. Therefore, the product ion with *m/z* 148 in the case of coumaric acid (Fig. 5(C)) probably corresponds to an interfering species. The main product ion in the MS/MS spectrum of deprotonated syringic acid (Fig. 5(E)) is detected at *m/z* 179, corresponding to water loss, a process not evident in the MS/MS data from neat standards. On the other hand, product ions characteristic of syringic acid (*m/z* 153 (CO_2 loss); *m/z* 182 (CH_3 loss) and *m/z* 138) are present but at low abundances, and partially interfered with by a species yielding an unspecified water loss. Thus, most, but not quite all, of the MS/MS features observed in the crude oil sample were consistent with experiments performed with neat standards of the known phenolic constituents. These results show the capability of LTP-MS as a rapid method of screening and estimating the approximate concentration of particular phenolic compounds in a given sample (total phenolic content), by using product ion relative intensities and comparing these with those of neat standards. This attractive feature of the proposed approach can be used for the prediction of the origin and variety of the olives used to produce the crude olive oil for authentication purposes.

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DOI: 10.1002/rcm

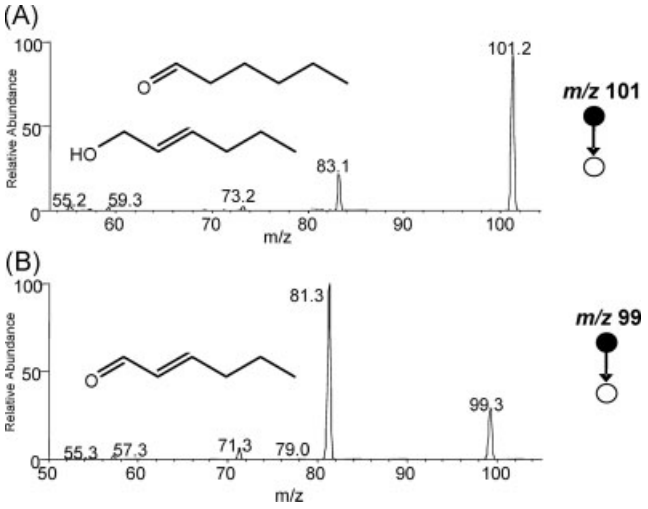

Figure 6. LTP-MS/MS spectrum (positive ion mode, $[M+H]^+$) of volatile compounds: (A) hexanal and 2-hexen-1-ol and (B) 2-hexenal.

Volatiles

In addition to the free fatty acids and the phenolics, we detected in the same crude olive oil sample (and confirmed with standards) signals that correspond to the three major volatile compounds (hexanal, 2-hexen-1-ol and 2-hexenal) in the full-scan positive ion mode (and also in the MS/MS mode); these were seen both with and without heating of the glass substrate. These three compounds are typical virgin olive oil volatiles, and the use of the profile and concentration of these species has been suggested for fingerprinting and authentication purposes.[29,30]

The approach used here shows limitations as a dedicated (univariate calibration based) method to determine individually the main volatile species characteristic of olive oils. In this sense, the similarities between the MS/MS spectral features of many of the volatile species make it impossible to rule out contributions from isomeric compounds to these spectra using LTP-MS/MS. For appropriate identification a dedicated GC/MS method, and one that would have to use multivariate calibration, would be required.

The MS/MS spectrum of protonated hexanal is characterized by few product ions; *m/z* 83 corresponds to loss of water, while the ions at *m/z* 59 and 55 correspond to losses of CH_2CO and ethanol, respectively. The MS/MS spectrum of the ion *m/z* 101 includes product ions from both hexanal and 2-hexen-1-ol; the MS/MS spectrum of protonated 2-hexenal is similar to that of hexanal (Fig. 6). These data show the ability of LTP-MS to detect these volatile species. However, it should be noted that over 20 volatile species can be present in olive oil samples, having similar features and MS/MS spectra.

CONCLUDING REMARKS

LTP-MS is a fast, powerful tool for the direct analysis of components of complex matrices such as vegetable oils, and appears useful for quality control and authentication of virgin olive oil. The method has limitations as well as advantages but the data clearly show proof-of-principle of LTP probe analysis of complex non-aqueous matrices. We used olive oil as a model and showed the detection of different species at different concentration scales. Accurate quantitation of particular phenolic compounds will require more detailed sample workup, but the very rapid screen carried out here allows rough estimates to be made of the concentration range of particular analytes in terms of antioxidant activity (total phenolic content), fatty acids and volatiles.

The main advantage of the proposed ambient mass spectrometry technique is the elimination of sample preparation steps, plus the simplicity and environmentally friendly features of the method,[31] which include the absence of reagents and/or organic solvents except for a low flow of a discharge gas such as helium. The approach used here can probably be extended to other fields like petroleomics and mineral oil and lubricant characterization.

Acknowledgements

This work was funded by Thermo Fisher Inc. F.M. and G.S. acknowledge funding from the National project: MIUR-FIRB, RBIP06SXMR_004. J.F.G.-R. acknowledges a postdoctoral Fulbright Program scholarship funded by '*Secretaría de Estado de Universidades e Investigación*' of the Spanish Ministry of Education and Science.

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DOI: 10.1002/rcm