Use of flow injection atmospheric pressure photoionization quadrupole time-of-flight mass spectrometry for fast olive oil fingerprinting

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The recently introduced technique of an atmospheric pressure photoionization (APPI) source coupled to quadrupole time-of-flight mass spectrometry (QqTOFMS) has been applied to fast olive oil fingerprinting on the basis of the accurate mass measurements obtained with this instrumentation. The key compounds can be characterized as $[MRH]^{R}$ (produced by proton transfer) or as $[M]^{R\cdot}$ (by charge transfer) ions in the mass spectra. $[MþH]^{þ}$ ions, however, show higher abundance, especially for triacylglycerols. Other ions present in APPI-MS are the acylium ion $[R_iCO]^{þ}$ and $[R_iCO–H_2O]^{þ}$. This latter ion is absent in the electrospray ionization (ESI)-MS spectra, and this represents valuable complementary information. Several critical parameters in the APPI source were optimized such as LC eluent composition, ion spray voltage and, especially, declustering potential. APPI-QqTOFMS allows easy discrimination among different edible oils: olive, extra virgin olive, olive-pomace, hazelnut, sunflower, corn and several mixed oils, with high throughput (approximately 1 min per sample). Cluster analysis was applied to obtain the best experimental conditions for oil discrimination on the basis of declustering potential. Principal components analyses of these APPI-MS spectra show that the approach can be used for studies of olive oil adulteration with other oils, even in the case of hazelnut oil that exhibits a high chemical similarity with olive oil.

Food quality control and authenticity are of great economical and social importance for food producers and consumers. Authenticity studies help to guarantee the quality of food products, preventing both the overpayment caused by adulteration and consumers being misled as a result of ambiguous or improper product labeling.[1] Olive oil is one of the most important constituents of the Mediterranean diet due to its potential health benefits.[2] Olive oil is particularly expensive and this may present the opportunity for producers to adulterate it with cheaper vegetable oils of lower quality. This is critical because the healthy properties of olive oil are strongly related to its composition, and the levels of triacylglycerols (TAGs) have been established as an indicator of the quality and purity of fats and oils.[3] For this reason these compounds are considered to be good fingerprints for the detection of adulteration.[4] Rapid and reliable analytical procedures for the measurements of TAGs are therefore necessary to assure the quality of this food.

A number of analytical techniques have been proposed for the fast authentication of the quality of olive oil, such as pyrolysis-mass spectrometry,[5] nuclear magnetic resonance (NMR),[6] Raman spectroscopy,[7] gas chromatography/mass spectrometry (GC/MS)[8] and direct head-space mass spectrometry (HS-MS).[9] These techniques are now being almost totally replaced by high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled to MS in combination with either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI).[10]

Atmospheric pressure photoionization (APPI) has been recently introduced by Syage and Evans[11] for non-polar compounds prior to their analysis by MS. This source represents an alternative to APCI and ESI.[12,13] The commonly used mass analyzers for this purpose are quadrupole, ion trap and time-of-flight as well as tandem mass analyzers with higher resolution, especially, triple-quadrupole and quadrupole time-of-flight (QqTOF).[14] The ESI source is suitable for polar compounds either in positive and negative ionization mode, producing ions for protonated or deprotonated molecules with little fragmentation. Ionization of lower polarity compounds is better performed by APCI, but compounds with little or no polarity are insufficiently ionized by either of these sources. To overcome this problem alternatives have been proposed, such as dissociative and

non-dissociative electron-capture ionization with APCI,[15,16] the combination of an electrochemical stage with ESI,[17,18] coordination ionspray,[19,20] and, more recently, APPI.

In 2001, Syage and Evans[11] introduced an API approach based on single-photon ionization (APPI), which vaporizes the sample using a heated nebulizer[21] prior to ionization being induced. The ionization step is initiated by radiation in the visible/ultraviolet range, generally produced by a discharge lamp. Photon absorption by a molecule M starts the photoionization process. This photoabsorption produces ejection of an electron to form the corresponding molecular radical cation, $[M^{+\cdot}]$.[22] Other molecules present in the ionization region, i.e. LC solvents, have ionization energies higher than the photon energy and the photoionization process should be specific towards the analyte. However, the presence of protic solvents and other molecules in large excess can produce further modifications in the $M^{+\cdot}$. Likewise, in the presence of water vapor or protic solvents, $M^{+\cdot}$ can extract a $H^{\cdot}$ atom to form a protonated molecule $[MH]^{+}$, when M has a high proton affinity.[22] This fact is the basis of dopant-assisted APPI introduced by Robb *et al.*[21] that uses a large quantity of a directly photoionizable compound (dopant) in the liquid stream to enhance the analyte ionization. Therefore, the use of dopant-assisted APPI improves ionization sensitivity, although it could induce adduct formation thereby increasing the complexity of the mass spectra.[22,23] Acetone, toluene and anisole have been proposed for this purpose.[23]

Until now, only a few applications based on the use ofAPPI have been reported. The initial work on dopant-assisted APPI from Robb *et al.*[21] considered two series of test compounds with different functional groups. Polycyclic aromatic hydrocarbons in sediments,[24] flavonoids[25] and steroids,[26] and several drugs, and their metabolites,[27,28] have been analyzed using this source. In addition, APPI has been used for residue analysis (patuline) in apple juice.[22]

This paper is focused on the optimization of the declustering potential to study its capability in combination with a QqTOF mass spectrometer for the authentication of complex food matrices such as olive oil. The approach avoids the use of chromatographic techniques and allows olive oil fingerprinting for fast adulteration assessment.

EXPERIMENTAL

Reagents and sample preparation

The solvents, HPLC-grade dichloromethane, methanol, acetonotrile and toluene, were purchased from Teknokroma (Barcelona, Spain). Water was purified with a Milli-Q Gradient system (Millipore, Watford, UK).

Oil samples (olive oil, extra virgin olive oil, olive-pomace, hazelnut oil, sunflower oil and corn oil) were supplied by Olibeas Cooperative (Huelva, Spain) and stored at 4°C.

All oils were 1000-fold diluted with a mixture of 60% dichloromethane/40% methanol (v/v). Any further sample preparation was not needed prior to analysis.

Instrumentation

All experiments were performed with an API QSTAR® XL Hybrid system (Applied Biosystems, Foster City, CA, USA) using an APPI source. A model KDS 100 syringe pump from KD Scientific (New Hope, PA, USA) was used to deliver the dopant to the APPI source. The samples were introduced using an autosampler module and the eluent was fluxed with a binary pump (Agilent 1100 series).

The operating conditions for the flow injection analysis were as follows: samples were injected into a 5-mL loop at a flow rate of 0.5 mL min^{-1} provided by the LC pump using 50% aqueous methanol as mobile phase. The dopant (toluene) was delivered at 0.05 mL min^{-1} by the syringe pump. Three different eluents, acetonitrile, methanol and water, were studied mixed in different proportions. The 50% (v/v) aqueous acetonitrile provided good sensitivity for the peaks in the mass spectra but, when this solvent was substituted by methanol, the signal was improved signifi- cantly. These results are in agreement with others previously reported[22] and methanol/water mixtures were selected for further experiences.

The ion spray voltage in the APPI source was fixed at 1500 V with a declustering potential (DP) of 80 V and the instrument operated in the positive ion mode. Full-scan spectra were acquired in multiple channel acquisition (MCA) for 1 min scanning the range m/z 100–1100. The ion energy (IE) was fixed at 2.0 V with a channel electron multiplier (CEM) of 2300 V. The collision energy in the product ion mode varied between the experiments and therefore was optimized as appropriate.

The ion-block temperature was maintained at 350°C to ensure that the liquid dopant was vaporized immediately and then swept by the auxiliary gas through the heated quartz tube up to the photoionization unit, together with the LC eluant.[21]

High-purity nitrogen was used as nebulizer, curtain gas and heater gas, at flow rates about 1.50, 1.13 and 6 L min^{-1}, respectively.

Chemometrics

Computations were performed by using the statistical packageSTATISTICA version 6.0 (2001) (StatSoft, Tulsa, USA). Patternrecognition methods (cluster analysis and principal compo-nents analysis) were applied to APPI-MS spectra.

RESULTS AND DISCUSSION

APPI mass spectra

Full-scan APPI mass spectra were obtained from the diluted samples using toluene as dopant. The triacylglycerols (TAGs) were identified as protonated molecules, $[MH]^{+}$ (Fig. 1). Table 1 shows the peaks for triolein (OOO), at m/z 885.8, dioleoyl-palmityl-glycerol (POO), at m/z 859.8, and dioleoyl-linoleoyl-glycerol (LOO), at m/z 883.8. Characteristic fragment ions are formed by the loss of an acid molecule from the $[MH]^{+}$ ion of TAGs, fragment 1 in Fig. 2. Other abundant fragment ions in the mass spectra of TAGs (fragment 2) are formed by subsequent losses of an acid molecule and a fragment that forms the acylium ion, $[R_iCO]^{+}$.[29] In addition, other important ions[30] are $[R_iCO]^{+}$ and $[R_iCO–H_2O]^{+}$ at m/z 265 and 247 for oleic acid, respectively (Table 2). Diacylglycerol (DAG) fragments (fragment 1) are the predominant ions and can be used for

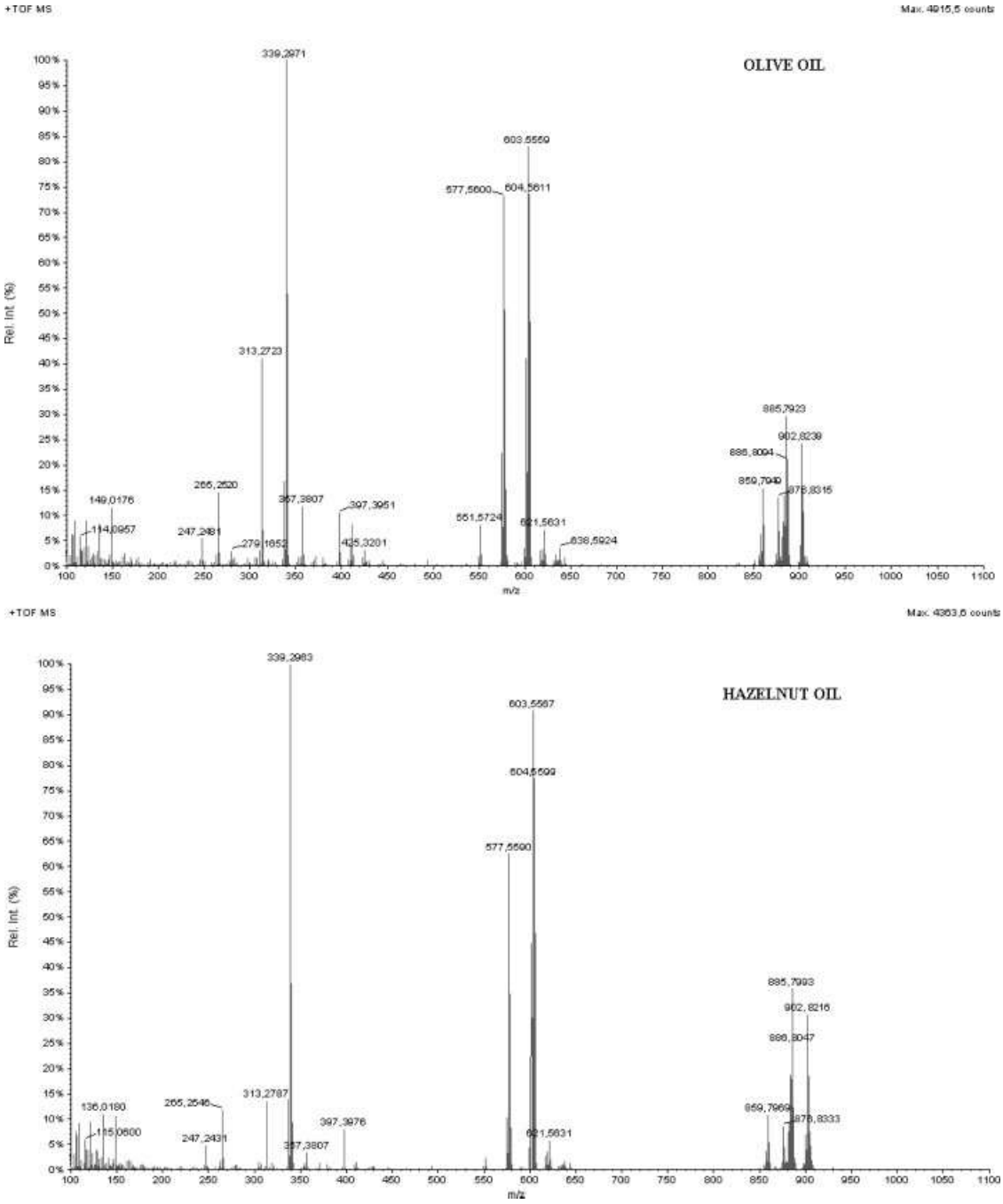

Figure 1. APPI mass spectra for the olive oil (top) and hazelnut oil (bottom).

the identification of TAG molecular species. These peaks can be observed in Fig. 1, which also shows that the elative abundance of analytes varies significantly between hazelnut and olive oils.

Optimization of DP for discrimination of edible oils

To increase the capability of the APPI source to discriminate edible oils such as olive, virgin, olive-pomace, hazelnut, corn and sunflower oils, the declustering potential (DP) was modified, since it affects the ionization efficiency and mass spectrum. The DP is very critical in the transmission

Table 1. Main ions observed in the APPI-QqTOF mass spec- tra of six vegetable oils (olive, extra virgin olive, olive-pomace, hazelnut, sunflower and corn oils)

	[MþH]þ	[MþH-RiCO2H]þ		[MþH-RiCO2-RiCO]þ	
TAGs	*m/z*	Fragment 1	*m/z*	Fragment 2	*m/z*
OOO	885.79	OO	603.55	O	339.29
LLL	879.72	OL	601.56	L	337.27
LLO	881.77	LL	599.54	P	313.27
POO	859.79	OP	577.56	Ln	335.26
LOO	883.78	SO	605.56	S	341.31

SOO	887.81	PL	575.54
PLO	857.79		

efficiency and fragmentation process of sample ions by in-source collision-induced dissociation (CID).[31] In general, the

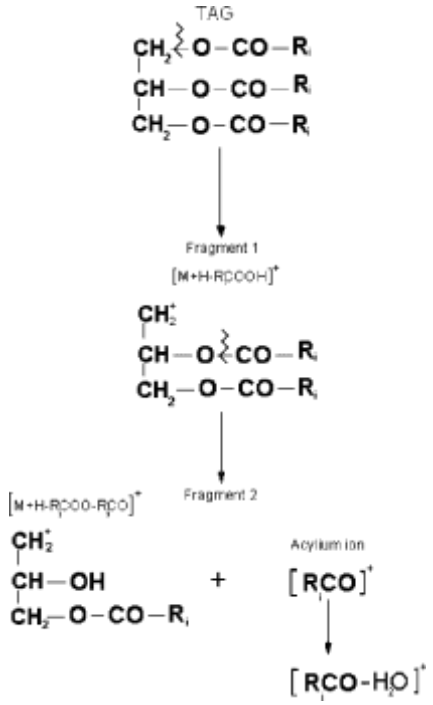

Figure 2. Fragmentation of triacylglycerols (TAGs) by positive ion mode APPI-MS.

optimum DP is compound-dependent,[29] and its effect on the mass spectra peaks and analyte discrimination is crucial. When this parameter changes in the range 80–300 V (85, 100, 200 and 300 V), different values in the intensity for the six edible oils under study were obtained depending on voltage. Analysis of these data was by simple cluster analysis, resulting in the dendrogram shown in Fig. 3, which can help to choose the best DP for oils discrimination. The optimum was achieved at 80 V which permitted the detection of olive oil adulteration even in the case of hazelnut. The worse results were found at 300 V, because the six oils were grouped in the same cluster. On the other hand, at 200 V, there is one cluster that comprises all the olive oil samples (extra virgin, olive-pomace and olive) together with hazel- nut, while sunflower and corn oils are perfectly discrimi- nated between them and with others oils.

Table 2. $[RCO]^-$ and $[RCO\text{-}H_2O]^-$ ions observed in the APPI-QqTOF mass spectra of various vegetable oils

		$[RCO]^-$	$[RCO–H_2O]^-$
Trivial name	Abbreviation	*m/z*	*m/z*
Oleic	O	265.25	247.24
Linoleic	L	263.24	245.23
Palmitic	P	239.24	221.23
Linolenic	Ln	261.22	243.21
Stearic	S	267.27	249.26

Capability of APPI-QqTOF for discrimination of edible oils

To explore the ability of the non-chromatographic APPI-QqTOF system to generate information-rich mass spectra the procedure described in the previous section was applied to hazelnut, olive, sunflower and corn oils, as well as several mixtures in different proportions. A study based on principal components analysis (PCA) was carried out, despite *a priori* knowledge about the class membership of the oil samples. PCA finds the maximum variations in the data set and forms new variables known as principal components (PCs),[32] such that each successive PC accounts for as much of the remaining variability as possible and each new variable must be totally independent of all other variables. In this case, PCA was applied to our data calculated according to the relative abundance (percentage) of each peak in the total ion chromatogram (TIC), as a semiquantitative method.

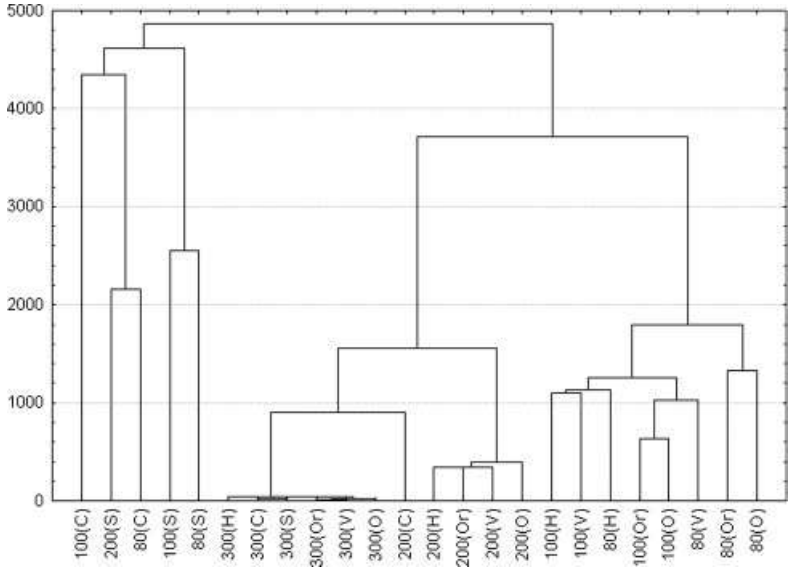

Figure 3. Dendrogram showing the relationships between the oils analyzed, and the effect of different declustering potentials (80, 100, 200, 300 V). The code refers to the type of oil analyzed [olive oil (O), extra virgin olive oil (V), olive-pomace oil (Or), hazelnut oil (H), sunflower oil (S) and corn oil (C)].

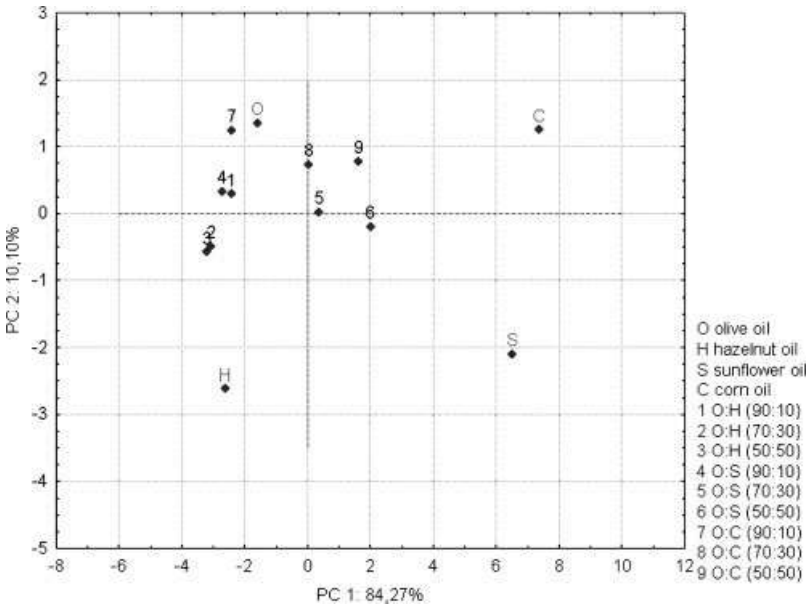

Figure 4. Principal components analysis of the analyzed oils (code in parentheses); olive oil (O); hazelnut oil (H); sunflower oil (S); corn oil (C); 1 (O:H) olive oil/hazelnut oil (90:10); 2 (O:H) olive oil/hazelnut oil (70:30); 3 (O:H) olive oil/hazelnut oil (50:50); 4 (O:S) olive oil/sunflower oil (90:10); 5 (O:S) olive oil/sunflower oil (70:30); 6(O:S) olive oil/sunflower oil (50:50); 7 (O:C) olive oil/corn oil (90:10); 8 (O:C) olive oil/corn oil (70:30); 9 (O:C) olive oil/corn oil (50:50).

The resulting ordination plot from PCA on these oils and their mixtures with APPI is shown in Fig. 4. It can be seen even from this simple PCA that a clear separation in PCA space occurs between the oils which are undoubtedly separated from olive oil. The first two PCs accounted for >94% of the overall variance.

CONCLUSIONS

APPI represents a powerful ionization source that coupled to a highly discriminant mass analyzer, namely QqTOF-MS, provides information-rich spectra. The approach is suitable for characterization of multi-component matrices, such as edible oils, and it allows unequivocal olive oil authentication, in particular hazelnut oil adulteration. In addition, the absence of chromatographic separation increases sample throughput.

The APPI source presents several important differences with respect to the most used ESI source in relation to the fragmentation pattern. For example, the production of $[M+H]^+$ and $[M]^{+\cdot}$ ions of key compounds by APPI against the ammonium adducts $[M-NH_4]^+$ in ESI. In addition, the $[R_iCO-H_2O]^+$ ion, that corresponds to loss of water by the acylium ion, can be observed in APPI-MS but is absent in ESI-MS.

Method performance, especially the capability to discriminate between oils, can be improved by careful optimization of the declustering potential that allows an easy classification of oils. Likewise, olive oils (extra virgin, olive-pomace and olive) can be easily distinguished from adulterants such as hazelnut, corn and sunflower oils, as well as their mixtures.

The main advantages of the methodology are the absence of both chromatographic separation and sample treatment. This is possible due to the high resolution of the QqTOF equipment that allows the unequivocal identification of the compounds on the basis of exact mass measurement. In addition, the method is rapid, reliable and precise. The equipment is robust, the maintenance is reduced, and information-rich mass spectra are obtained.

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