



Ambient mass spectrometry employing direct analysis in real time (DART) ion source for olive oil quality and authenticity assessment

Lukas Vaclavik, Tomas Cajka, Vojtech Hrbek, Jana Hajslova*

Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technicka 5, 166 28 Prague 6, Czech Republic

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ABSTRACT

A novel approach for the authentication of olive oil samples representing different quality grades has been developed. A new type of ion source, direct analysis in real time (DART), coupled to a high-resolution time-of-flight mass spectrometer (TOFMS) was employed for the comprehensive profiling of triacylglycerols (TAGs) and/or polar compounds extracted with a methanol–water mixture. The main parameters influencing the ionization efficiency of TAGs were the type of sample solvent, degree of sample dilution, ion beam temperature, and presence of a dopant (ammonia vapors). The ionization yield of polar compounds depended mainly on a content of water in the extract and ion beam temperature. Using DART–TOFMS, not only differentiation among extra virgin olive oil (EVOO), olive pomace oil (OPO) and olive oil (OO) could be easily achieved, but also EVOO adulteration with commonly used adulterant, hazelnut oil (HO), was feasible. Based on the linear discriminant analysis (LDA), the introduced method allowed detection of HO addition of 6 and 15% (v/v) when assessing DART–TOFMS mass profiles of polar compounds and TAGs, respectively.

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1. Introduction

The authenticity of olive oil as associated with genetic variety, geographical origin, and/or quality grade is an issue of high concern [1]. As regards the latter aspect, various types of olive oils can be distinguished. The European Union Legislation [2] defines several categories of virgin olive oil: extra virgin olive oil (EVOO), virgin olive oil (VOO), and lampante virgin olive oil (LVOO). Additionally, refined olive oil (ROO, refined VOO), and olive oil (OO, a mixture of ROO and VOO) are recognized. Olive pomace oils are classified as crude olive pomace oil (COPO, solvent-treated olive pomace), refined olive pomace oil (ROPO, refined COPO), and olive pomace oil (OPO, a mixture of ROPO and VOO).

Because of its unique taste and flavor, EVOO is the most highly prized of all olive oil grades. Not surprisingly, it is occasionally adulterated by fraudulent producers with less expensive vegetable and/or seed oils to increase their economic profits. A “sophisticated” type of adulteration is based on addition of hazelnut oil (HO) or lower quality grade olive oils because of similarity in their chemical composition [1,3,4]. Besides of a general inadmissibility of such approach, a serious consequence of using unrefined HO is a potential hazard for those consumers, who are allergic to hazelnuts proteins [5–7].

A lot of scientific effort has been spent to develop rapid, reliable, and cost effective analytical approach applicable for the authentication of plant oils. Besides of spectroscopic techniques employing nuclear magnetic resonance (NMR) [8,9], Raman [10], or infrared spectra [11,12], methods employing gas chromatography–mass spectrometry (GC–MS) [4,13], and high-performance liquid chromatography (HPLC) hyphenated to MS with atmospheric pressure chemical ionization (APCI) [14–16], have been implemented for this purpose. Several procedures such as matrix assisted laser desorption/ionization mass spectrometry (MALDI) [16], direct head-space mass spectrometry (HS–MS) [17,18], and/or direct infusion MS allow reduction of analysis time thanks to elimination of chromatographic separation step. In studies employing direct infusion MS, the utilization of triple-quadrupole (QqQ), quadrupole–time-of-flight (Q–TOF), or ion trap mass analyzers have been published [19–24]. Both electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) ion sources were used in this setup. Alternatively, coupling of flow injection analysis (FIA) to Q–TOF equipped with APPI source has been described [25]. Based on the profiling/fingerprinting of volatiles [17,18], triacylglycerols (TAGs) [19,20,25], phenolic compounds together with fatty acids [21,22], aminoacids [23], or sterols [24], these rapid approaches enable to classify various edible plant oils according to their botanical origin and quality grade as well as to detect olive oil admixtures with common adulterants. Since a large volume of data is typically obtained during these measurements, smart chemometric tools have to be used to establish mathematical model for classification of samples.

* Corresponding author. Tel.: +420 220 443 185; fax: +420 220 443 184.
E-mail address: jana.hajslova@vscht.cz (J. Hajslova).

The most frequently utilized strategies for this purpose involve linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), canonical variate analysis (CVA), or artificial neural networks (ANNs) [26].

Over the few recent years, a large number of novel ambient desorption ionization techniques, such as desorption electrospray ionization (DESI) [27], atmospheric-pressure solids analysis probe (ASAP) [28], direct analysis in real time (DART) [29] and many others [30], have become available. Their main advantages compared to conventional techniques, involve the possibility of direct sample examination in the open atmosphere, minimal, or no sample preparation requirements, and, remarkably high sample throughput. DART, which was investigated in this study, represents one of APCI-related techniques employing a glow discharge for the ionization. Metastable helium atoms, originated in the plasma, react with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species [29]. DART ion source was shown to be efficient for soft ionization of a wide range of both polar and non-polar compounds. Until now, several papers have been published describing DART applications for rapid analysis of explosives [29], pharmaceuticals [31–33], flavor and fragrances [34], fatty acid methyl esters originated from bacterial lipids [35], soft drinks [36], and pesticides [37]. In addition to these uses, also the reading of the thin-layer chromatography plates was successfully realized by DART [38]. Several other application notes are available now on the manufacturer's website [39]. One of these reports briefly described the ionization of TAGs with a DART ion source, demonstrating the differences in mass spectra of various edible oils.

In this pilot study, automated ambient mass spectrometric method employing high-resolution TOFMS equipped with DART ion source was used for classification of olive oil samples of different quality grades (EVOO, OPO, and OO) and HO samples. EVOO/HO mixtures were also examined in order to investigate the possibility of EVOO adulteration detection. To demonstrate discrimination capability, ion profiles of (i) whole oils (dominated by TAGs), and (ii) methanol–water extracts (containing minor polar compounds), were collected for processing by LDA.

2. Materials and methods

2.1. Chemicals and samples

HPLC-grade toluene, isooctane, ethyl acetate and methanol were purchased from Merck (Darmstadt, Germany). Water used for extractions was purified with a Milli-Q purification system (Millipore, Eschborn, Germany); an aqueous ammonia solution (25%, w/w) was supplied by Penta (Chrudim, Czech Republic).

Samples of extra virgin olive oil (EVOO, $n=6$), olive oil (OO, $n=6$), olive pomace oil (OPO, $n=9$) and hazelnut oil (HO, $n=6$), representing products from several countries (Italy, Greece, Spain, and France), were purchased from reliable market sources and stored in dark and dry at 4 °C. All oils were processed and analyzed minimally 6 months before their expiry date. Admixtures of EVOO with HO were prepared from selected samples in the volume ratios of 50:50, 75:25, 80:20, 85:15, 90:10, 92:8, 94:6, and 98:2. Each admixture was prepared in triplicate.

2.2. Sample preparation

For a preliminary evaluation of the influence of various solvents on the ionization process, EVOO sample was diluted with toluene, isooctane and ethyl acetate in the range from 1:1 to 1:50 (v/v). To enable automated sample introduction, oil had to be diluted with solvent at least in ratio 1:1 (v/v) to decrease its viscosity. For optimal TAGs profiling, all studied oils were 50-fold diluted with toluene.

To extract polar compounds, 1 mL of oil sample was placed into a 15-mL plastic cuvette and shaken automatically for 2 min with 3 mL of a methanol–water mixture (80:20, v/v). The phases were then allowed to separate (2 min) and the upper hydro–alcoholic layer was taken for the DART–TOFMS analysis.

2.3. Instrumentation and testing conditions

For the experiments, DART–TOFMS system consisting of a DART ion source (IonSense, Saugus, MA, USA), an AccuTOF LP high-resolution time-of-flight mass spectrometer [JEOL (Europe), SAS, Croissy sur Seine, France] and an HTC PAL autosampler AutoDART-96 (Leap Technologies, Carrboro, NC, USA), was used.

The operating conditions of a DART ion source were as follows: positive ion mode; helium flow: 4.0 L min⁻¹; discharge needle voltage: 3.0 kV; perforated and grid electrode potentials: +150 and +250 V, respectively. Conditions of TOFMS: cone voltage: +20 V, monitored mass range: m/z 50–1000; acquisition rate: 5 spectra min⁻¹; resolving power: approx. 6000 FWHM (full width at half maximum). The distance between the DART gun exit and mass spectrometer inlet was 10 mm. Sample introductions ($n=5$, each sample) were carried out automatically using Dip-itTM samplers (IonSense, Saugus, MA, USA). The sampling glass rod was immersed for 1 s into the sample hole of a deepwell micro-plate (Life Systems Design, Merenschwand, Switzerland) containing approx. 600 μ L of respective sample, and transferred to the optimized position in front of the DART gun exit. The sample was then desorbed from the glass rod surface within 30 s, while the spectral data were recorded. To perform a mass drift compensation for accurate mass measurements and elemental composition calculations, a polyethylene glycol (average relative molecular weight 600, Sigma–Aldrich, Steinheim, Germany) 200 μ g mL⁻¹ solution in methanol, was introduced manually at the end of each analysis run. To assess an inter-day repeatability of measurements, selected samples of diluted oils and methanol–water extracts were analyzed within 5 successive days.

To document the influence of gas beam temperature on the signal intensity, EVOO sample (diluted in toluene, as described above) and its methanol–water extract, were analyzed at different temperatures ranging from 100 to 450 °C. For all follow-up analyses of oils and oil extracts, the gas beam temperature was set to 350 and 220 °C, respectively. To produce ammoniated ions of TAGs, 2 mL autosampler vial containing 25% (w/w) aqueous ammonia solution (dopant), was placed 4.5 mm below the ion source exit. Aqueous methanolic extracts of polar compounds were analyzed without a use of any dopant.

2.4. Data processing and chemometric analysis

The Mass Center software version 1.3 (2006) (JEOL, Tokyo, Japan) was used for data processing. Mass spectral data were obtained by averaging of the mass spectra recorded during the exposure of the sample to the DART gas beam; background ions were subtracted and a mass drift was corrected. LDA was performed employing the software package statistiXL version 1.8 (2008) (statistiXL, Broadway—Nedlands, Australia). Constant row sum transformation of the data obtained from repeated sample introduction was carried out, i.e., an absolute intensity of each ion (variable) was normalized to the sum of absolute intensities of all selected variables. The transformed data were averaged for each sample prior to chemometric analysis.

3. Results and discussion

To our knowledge, DART–TOFMS has not been used until now for olive oil profiling aimed at its quality and authenticity assessment.

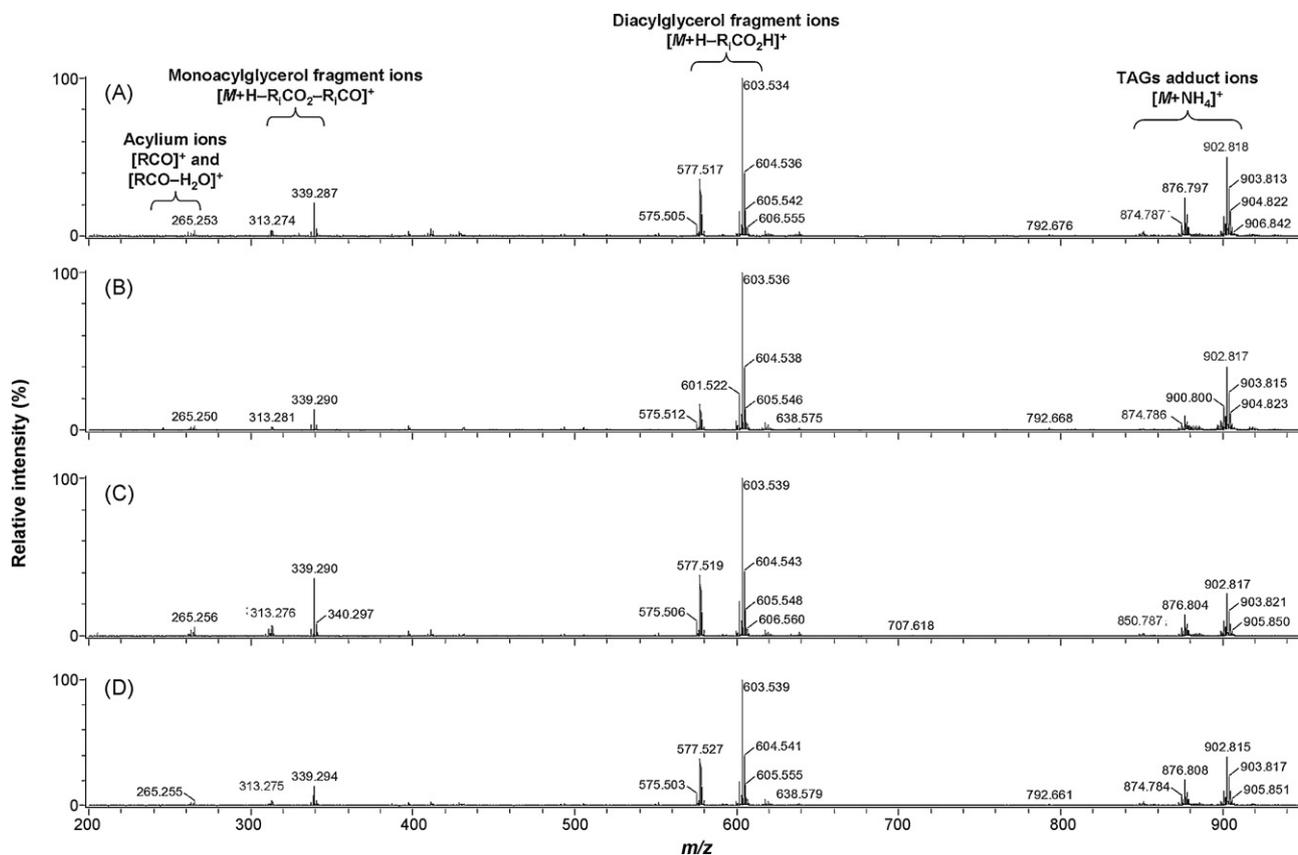


Fig. 1. DART-TOFMS mass spectra of oils diluted with toluene 1:50 (v/v) at 350 °C: (A) extra virgin olive oil, (B) hazelnut oil, (C) olive pomace oil, and (D) olive oil.

To obtain the most diagnostic markers, mass spectral fingerprints of both TAGs and polar components (fraction extracted from oil with a methanol–water mixture) were studied in detail. The discussion of experiments performed within this pilot study is presented below.

3.1. DART-TOFMS mass spectra of oils

As described in those few available studies concerned with DART performance [29,32,33], the type of ions and their intensity depend on various factors, including sample solvent, presence of dopant, and/or temperature of gas (helium) beam. Therefore, in the first phase of our experiments, we investigated the relationship between the settings of various DART operational parameters and the features of mass spectra generated under particular conditions. To overcome sampling difficulties encountered with rather too viscous test material by a glass rod (uneven, thick layer of oil on its surface was prone to forming drops), some dilution was needed prior to measurement. Protonated molecular ions $[M+H]^+$ corresponding to individual TAGs (which are the main components of edible oils) were observed in the high m/z region of mass spectra when conducting measurement of oils dissolved in toluene (1:50, v/v). However, the intensity of these ions was relatively low. Significant improvement of TAGs signals was obtained when ammonia vapors were used as a charge-transfer reagent (dopant). In Fig. 1, typical DART-TOFMS mass spectra of three brands of olive oils (EVOO, OPO, OO) and HO, a potential adulterant, are shown. $[M+NH_4]^+$ ions were approx. 10 times more intensive compared to $[M+H]^+$, moreover, the use of dopant enabled detection of even minor TAGs (Fig. 2). Similar effect, an enhancement of ESI sensitivity thanks to TAGs ammonium adducts formation, when ammonium-ion-containing modifier (ammonium formate) was added into the mobile phase, was observed in one of earlier published studies [40] assessing

detection limits of lipids in LC-MS analysis employing various ionization approaches. Based on measurements of accurate masses of molecular adducts $[M+NH_4]^+$, we tentatively identified altogether 9 TAGs in all test samples: triolein (OOO, m/z 902.82), palmitoyldiolein (POO, m/z 876.80), linoleyldiolein (LOO, m/z 900.80), palmitoylolestearin (POS, m/z 878.82), palmityllinoleyllolein (PLO, m/z 874.79), dilinoleyllolein (LLO, m/z 898.79), dipalmitoylolein (PPO, m/z 850.79), trilinolein (LLL, m/z 896.77) and palmitoyloleylpalmitolein (POPo, m/z 848.77). Without any exception, the most abundant TAG ion in mass spectra, m/z 902.82, corresponded to triolein. However, alike in other experiments employing direct infusion MS for plant oils fingerprinting; differentiation of positional isomers of TAGs with two or three different fatty acids is not possible by DART technique, due the absence of separation of isobaric molecules prior to ionization and MS detection. In any case, the relative intensities of molecular adducts, as well as other ions present in mass spectra, differed among the tested samples, thus offering a good promise for authentication.

As shown in Fig. 1, extensive fragmentation of TAGs yielding several types of ions (some of them more intensive than molecular adduct) occurred under the experimental DART conditions. The most abundant were diacylglycerol fragment ions $[M+H-R_1CO_2H]^+$ formed by the loss of one fatty acid molecule from the glycerol backbone. Diolein fragment ion $[OO]^+$ (m/z 603.53) was the base peak in all of mass spectra, less intensive monoacylglycerol ions $[M+H-R_1CO_2-R_2CO]^+$, acylium ions of corresponding fatty acid $[RCO]^+$, and $[RCO-H_2O]^+$ ions could also be detected. The TAGs fragmentation patterns obtained by DART were similar to those recorded in studies employing the key chemi-ionization techniques, APCI or APPI [19,41]. The list of ions identified in mass spectra of examined oils, together with their relative intensities and intra-day repeatabilities of measurements (expressed as relative standard deviation, RSD, $n=5$), are summarized in Table 1. It

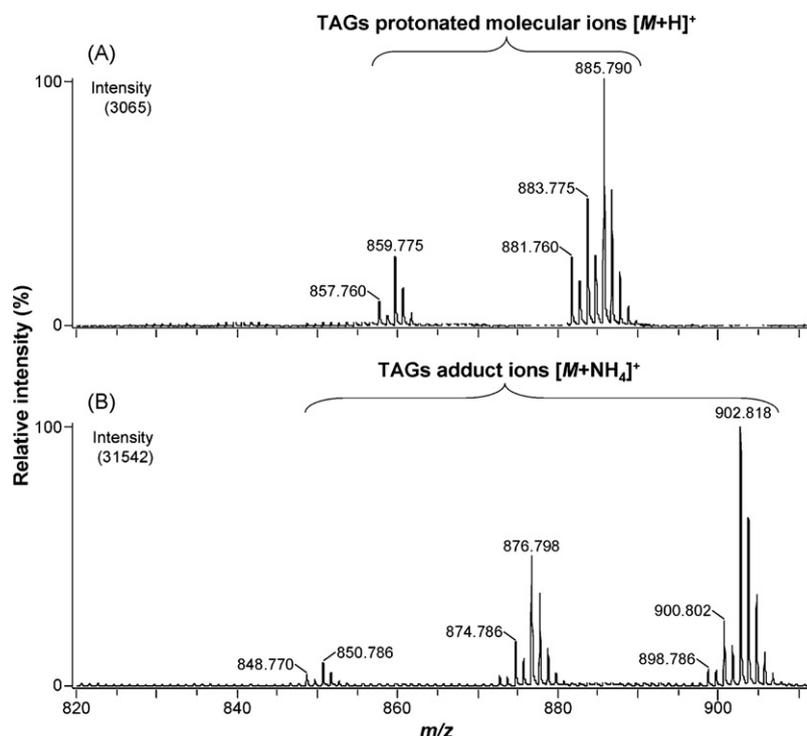


Fig. 2. Comparison of DART-TOFMS mass spectra (m/z 820–910) of extra virgin olive oil diluted with toluene (1:50, v/v) at 350 °C: (A) without dopant and (B) with dopant.

should be emphasized that no statistically significant differences of intra- and inter-day repeatabilities were observed.

Considering the need to achieve maximum diversity among sample fingerprints, we also attempted to induce even more

extensive TAGs fragmentation, and, on this occasion, enhance the intensity of minor, potentially diagnostic ions. Generally, the abundances of fragment ions can be increased by in-cell or, alternatively, in-source collision-induced dissociation (CID) [42,43]. Employing

Table 1

Typical relative intensities (RI, $n = 5$) and relative standard deviations (RSDs) of TAGs adduct and fragment ions observed in DART-TOFMS mass spectra of studied oils diluted with toluene 1:50 (v/v); gas beam temperature 350 °C.

m/z	EVOO		HO		OPO		OO		Identification
	RI (%)	RSD (%)							
<u>902.82</u>	60.6	7.2	40.6	8.1	31.7	10.0	35.2	10.1	[OOO+NH ₄] ^{+a}
<u>900.80</u>	10.2	10.9	13.0	9.8	7.5	11.9	8.6	10.6	[LOO+NH ₄] ^{+a}
<u>898.79</u>	2.6	12.8	4.8	11.7	2.4	12.6	2.8	12.8	[LLO+NH ₄] ^{+a}
<u>896.77</u>	n.d.	–	2.1	14.3	n.d.	–	n.d.	–	[LLL+NH ₄] ^{+a}
<u>878.82</u>	10.2	11.5	5.3	13.0	4.9	12.8	4.5	12.4	[POS+NH ₄] ^{+a}
<u>876.80</u>	25.4	8.6	6.2	12.6	9.3	10.3	11.0	9.8	[POO+NH ₄] ^{+a}
<u>874.79</u>	5.8	9.6	2.6	11.8	3.6	10.9	4.4	9.7	[PLO+NH ₄] ^{+a}
<u>850.79</u>	2.4	12.5	n.d.	–	1.1	14.2	1.4	13.9	[PPO+NH ₄] ^{+a}
<u>848.77</u>	1.2	15.7	n.d.	–	0.5	14.6	0.7	15.0	[POPo+NH ₄] ^{+a}
<u>603.53</u>	100.0	–	100.0	–	100.0	–	100.0	–	[OO] ^{+b}
<u>601.52</u>	15.8	3.5	23.7	3.1	20.5	2.6	23.1	2.8	[OL] ^{+b}
<u>599.50</u>	2.3	5.8	4.7	5.1	3.2	4.9	3.8	5.3	[LL] ^{+b}
<u>577.52</u>	35.6	2.9	15.0	3.2	32.9	2.1	37.1	1.9	[PO] ^{+b}
<u>575.50</u>	5.2	4.4	2.1	7.5	4.9	5.3	5.1	4.7	[PL] ^{+b}
<u>551.50</u>	2.0	6.2	n.d.	–	1.7	7.3	2.2	6.3	[PP] ^{+b}
<u>549.49</u>	0.8	13.2	n.d.	–	0.7	13.0	0.8	12.5	[PPo] ^{+b}
<u>339.29</u>	22.1	10.4	10.2	13.4	33.7	7.3	21.8	8.7	[O] ^{+c}
<u>337.27</u>	2.3	11.3	2.3	12.0	3.9	10.4	3.0	9.9	[L] ^{+c}
<u>313.27</u>	3.5	12.3	1.5	13.2	6.5	8.9	4.9	9.3	[P] ^{+c}
<u>311.26</u>	0.4	14.7	n.d.	–	2.1	11.8	2.0	10.9	[Po] ^{+c}
<u>265.25</u>	4.3	16.3	4.7	15.1	8.3	12.5	5.6	13.6	[O] ^{+d}
<u>263.24</u>	2.3	17.2	3.2	16.5	4.3	15.8	3.3	16.4	[L] ^{+d}
<u>247.24</u>	1.1	21.5	0.9	22.0	1.2	20.9	1.2	19.8	[O] ^{+e}
<u>245.23</u>	0.6	23.1	0.5	24.6	0.9	22.2	0.5	25.3	[L] ^{+e}

Fatty acid residues in TAGs and fragment ions are indicated by a capital letter according to the following legend: O = oleic; L = linoleic; P = palmitic; S = stearic; Po = palmitoleic. n.d., not detected. Underlined ions were used for construction of LDA model.

^a Triacylglycerol ammonium adduct.

^b Diacylglycerol fragment ion.

^c Monoacylglycerol fragment ion.

^d Acylium ion [RCO]⁺.

^e [RCO–H₂O]⁺.

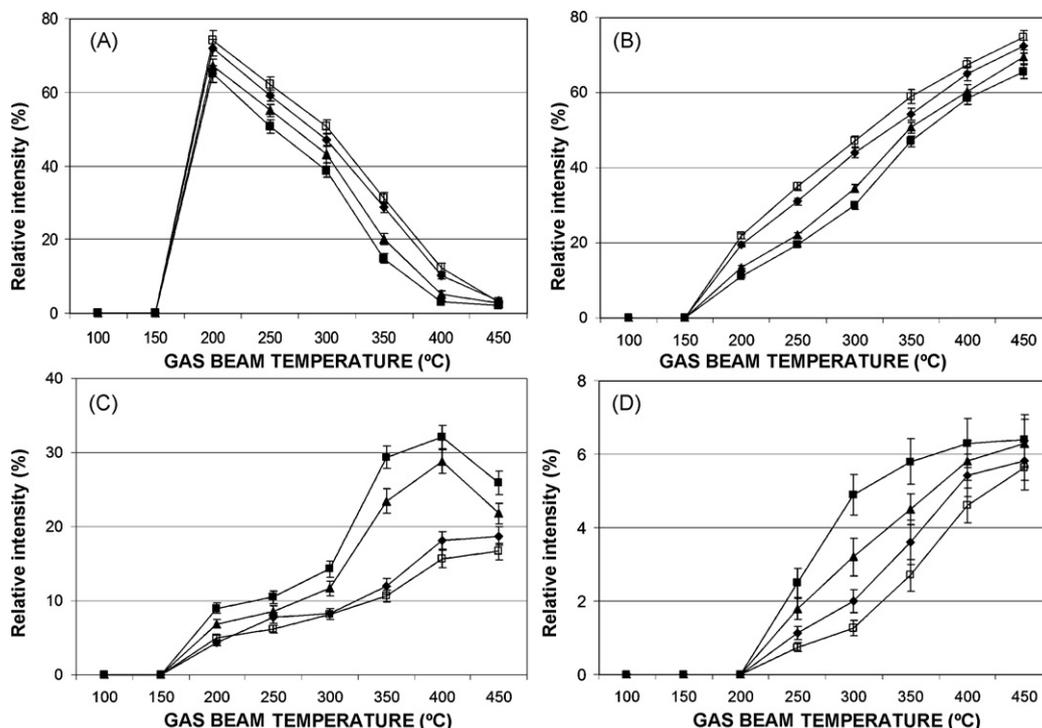


Fig. 3. The impact of DART gas beam temperature and dilution of extra virgin olive oil on the relative intensities of selected ions. (A) Triolein ammonium adduct ion $[OOO+NH_4]^+$, (B) diolein fragment ion $[OO]^+$, (C) monoolein fragment ion $[O]^+$, (D) oleic acid acylium ion. The degree of oil dilution with toluene (v/v): (■) 1:1; (▲) 1:5; (◆) 1:20; (□) 1:50. Error bars are standard deviations ($n=5$).

the latter approach, we changed the cone voltage value of mass spectrometer in the range of +20 up to +150 V. However, in this way, the only outcome obtained was an enhanced formation of diacylglycerol fragment ions on account of parent molecular adducts; while relative intensities of other ions remained almost unchanged.

The impact of other parameters that might affect discrimination capability of DART–TOFMS mass spectra was studied in detail, too. Changing the temperature of the DART gas beam from 100 to 450 °C, EVOO samples diluted in a growing degree (1:1; 1:5; 1:20; 1:50, v/v) by toluene, isooctane, and/or ethyl acetate were ionized, and intensities of generated ions then assessed.

Regardless the dilution, the total ion current (TIC) recorded during analysis of samples (dissolved in toluene) was increased with growing DART gas beam temperature as the thermo-desorption of analytes was supported. However, as illustrated in Fig. 3A–D for triolein, the relationship between relative abundances of observed TAGs adduct/fragment ions (normalized to the sum of their intensities) and operational parameters was quite complex. For ionization of triolein molecule and obtaining ammonium adduct ion $[OOO+NH_4]^+$, DART gas beam temperature of at least 200 °C was needed (Fig. 3A). At higher temperatures, the decrease of relative intensity of this ion was observed, due to a growing extent of fragmentation processes. Interestingly, the degree of oil dilution positively correlated with the relative abundance of TAG adduct ion. This effect can be explained by facilitation of analytes desorption in the presence of volatile solvent. As mentioned above, the fragment ion relative intensities increased with the growing temperature within tested range. While in a case of diolein fragment ion $[OO]^+$, the effect of dilution was in agreement with the trend observed for triolein adduct ion (Fig. 3B), oleic acid acylium ion $[RCO]^+$ (Fig. 3D), as well as monoolein fragment ion $[O]^+$ (Fig. 3C), showed opposite tendency. Based on these observations, the helium beam temperature 350 °C and dilution 1:50 (v/v) were chosen as optimal settings to acquire intensive, information-rich mass spectra with satisfactory representation of both TAGs ammonium adduct and fragment ions applicable for samples discrimination by statistical tools.

In addition to toluene, isooctane and ethyl acetate were used for dissolving of EVOO sample to the measurement of DART mass spectra. Regardless the sample dilution, no statistically significant changes in relative abundances of fragment ions and TAG adducts in mass spectra were observed comparing to those obtained by the analyses of respective samples diluted in toluene. On the other hand, the repeatability of relative intensities of TAGs adduct ions was shown to be solvent-dependent. For instance, the RSD of triolein ammonium adduct relative intensity in the sample diluted with toluene was as low as 8.5%, while significantly higher values were obtained for analyte solutions prepared in isooctane and ethyl acetate (13.6 and 18.6%, respectively). It seems, the higher boiling point of solvent used (110.6, 99.3, and 77.1 °C for toluene, isooctane, and ethyl acetate, respectively), the better repeatability of measurements.

3.2. DART–TOFMS mass spectra of the methanol–water extracts

Depending on a botanical origin of an oil crop and its follow-up processing technology, plant oils may contain various minor polar components, which can be partitioned into polar solvent. In the current study, we used a methanol–water mixture (80:20, v/v) for isolation of “phenolic” fraction. Although some authors [21] recommend employing even more polar extraction mixtures (with water content up to 50%), we did not find any additional, “new” compounds in such extracts. Moreover, the increase of water content hampered the efficiency of ionization process. Under experimental conditions of this study, numerous ions conceivable for the differentiation among the examined oils were detected in the m/z range of 100–500; some of them were highly diagnostic, as they were present only in one group of oils. Fig. 4 illustrates relatively large differences in typical mass spectral fingerprints obtained by DART–TOFMS analyses of oil extracts at 220 °C. Considering the influence of DART gas beam temperature on the number and relative intensity of detected ions, changing this parameter from 220 °C (used for optimized method) up to 450 °C did not result in detection

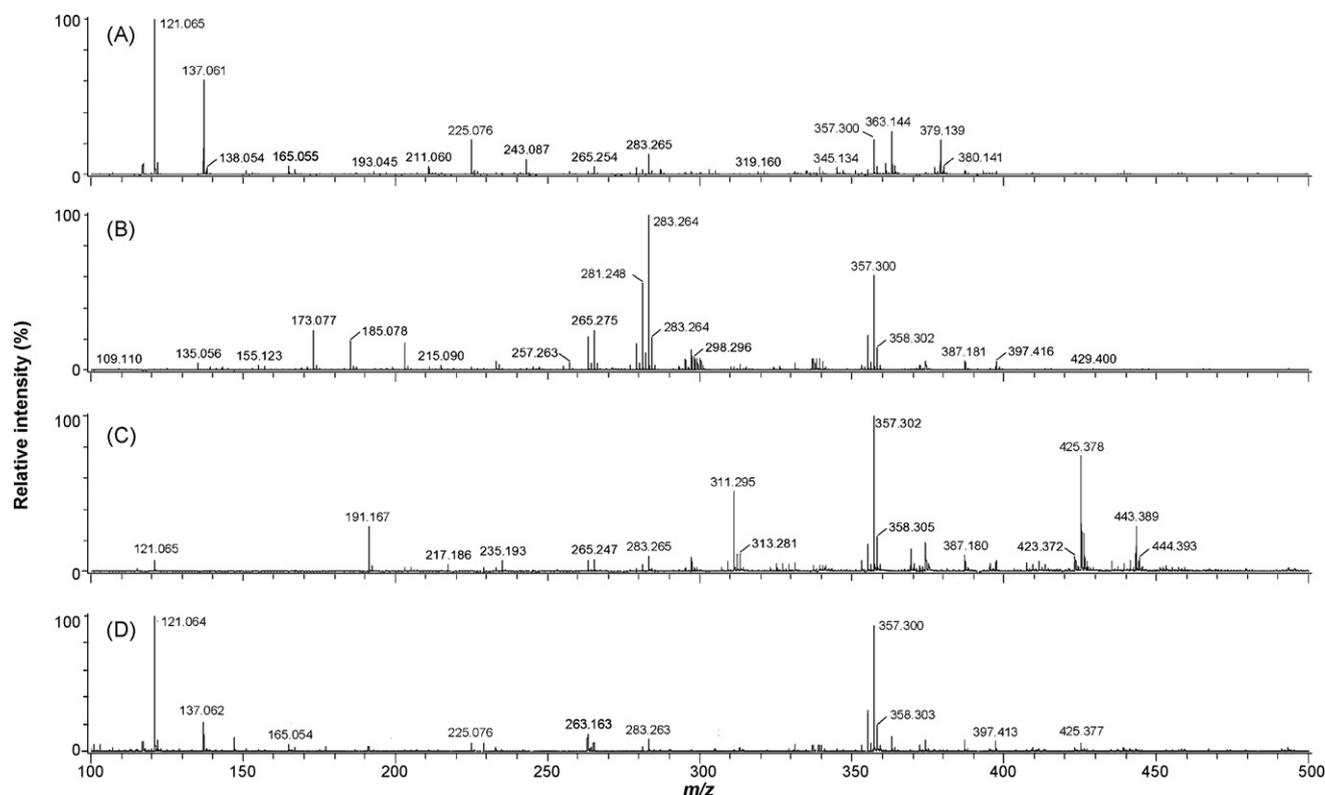


Fig. 4. DART-TOFMS mass spectra of the methanol–water extracts at 200 °C obtained from: (A) extra virgin olive oil, (B) hazelnut oil, (C) olive pomace oil, and (D) olive oil.

of additional ions, moreover, thermal degradation of all originally (at lower temperature) abundant ions occurred. Tentative identification of several compounds or the estimation of the most probable elemental compositions of unknowns (the best hit in terms of mass accuracy suggested by the software) was enabled by accurate mass measurement. Table 2 summarizes major ions detected in mass spectra of examined extracts. Alike in case of TAGs analysis, both intra- and inter-day repeatabilities of measurements were similar.

For instance, ions with m/z 121.07 and 137.06 can be attributed to $[M+H-H_2O]^+$ of tyrosol and hydroxytyrosol, respectively, compounds which are considered as typical olive oil markers [44].

Besides of these two major constituents of phenolic fraction, protonated molecular ions $[M+H]^+$, corresponding to coumaric acid (m/z 165.06), sinapic acid (m/z 225.08), and elenoic acid (m/z 243.09), were detected in positive mass spectra of EVOO extract. Worth to notice, that phenols, tyrosol and hydroxytyrosol, were abundant in EVOO and OO, whereas in OPO, their concentration was very low. In addition to (targeted) phenolic compounds, also oleic (m/z 283.26) and linoleic (m/z 281.25), free fatty acids, together with monoolein (m/z 357.30) occurred in aqueous methanolic extract; all these compounds were detected as $[M+H]^+$ ions. Protonated molecular ions of fatty acids dominated mass spectra of HO; monoolein ion, observed,

Table 2

Typical relative intensities (RI, $n=5$) and relative standard deviations (RSDs) of major ions observed in DART mass spectra of methanol–water extracts of studied oils; gas beam temperature 220 °C.

m/z	EVOO		HO		OPO		OO		Identification
	RI (%)	RSD (%)							
<u>121.07</u>	100.0	–	1.1	14.3	10.0	9.0	100.0	–	Tyrosol ^a
<u>137.06</u>	64.5	8.1	0.9	15.1	1.9	13.8	25.0	8.1	Hydroxytyrosol ^a
<u>165.06</u>	7.9	9.4	n.d.	–	n.d.	–	4.4	11.8	Coumaric acid ^b
<u>191.17</u>	n.d.	–	n.d.	–	32.4	7.2	3.8	12.0	C ₁₀ H ₂₃ O ₃ ^c
<u>225.08</u>	26.9	7.3	n.d.	–	n.d.	–	10.1	9.3	Sinapic acid ^b
<u>243.09</u>	14.7	8.5	n.d.	–	n.d.	–	n.d.	–	Elenoic acid ^b
<u>281.25</u>	2.5	12.6	61.4	6.9	4.1	12.3	4.2	12.9	Linoleic acid ^b
<u>283.26</u>	13.9	7.9	100.0	–	12.1	10.2	13.4	8.7	Oleic acid ^b
<u>311.30</u>	n.d.	–	n.d.	–	55.0	5.7	n.d.	–	C ₂₀ H ₃₉ O ₂ ^c
<u>345.13</u>	6.1	11.3	n.d.	–	n.d.	–	2.1	14.5	C ₁₉ H ₂₁ O ₆ ^c
<u>357.30</u>	20.3	8.2	65.1	7.2	100.0	–	89.9	4.4	Monoolein ^b
<u>363.14</u>	25.4	7.7	n.d.	–	n.d.	–	10.5	9.2	C ₁₉ H ₂₃ O ₇ ^c
<u>379.14</u>	19.4	7.5	n.d.	–	n.d.	–	n.d.	–	C ₁₉ H ₂₃ O ₈ ^c
<u>387.18</u>	3.2	13.0	10.6	8.2	13.1	9.6	11.5	9.0	C ₂₂ H ₂₇ O ₆ ^c
<u>425.38</u>	n.d.	–	n.d.	–	83.7	5.8	7.6	10.7	Erythrodiol/uvaol ^a
<u>443.39</u>	n.d.	–	n.d.	–	24.9	7.3	n.d.	–	Erythrodiol/uvaol ^b

n.d., not detected. Underlined ions were used for construction of LDA model.

^a $[M+H-H_2O]^+$.

^b $[M+H]^+$.

^c Ion probable elemental composition.

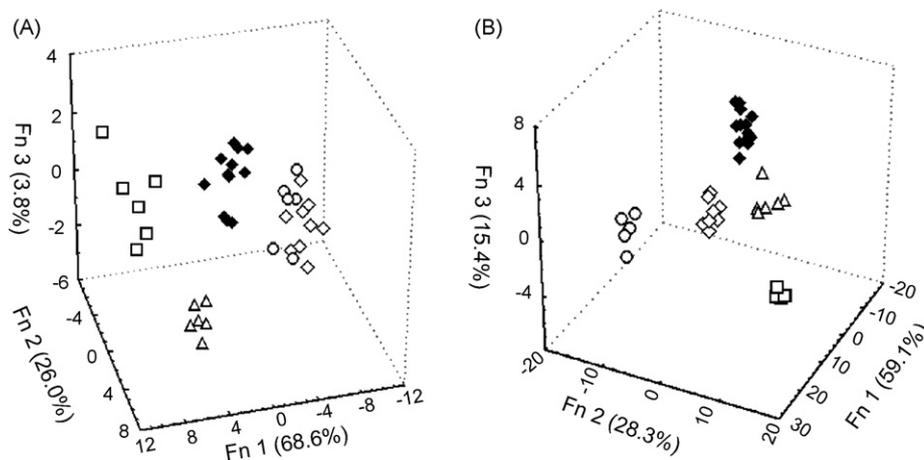


Fig. 5. Score plot of three discriminant functions of LDA calculated from DART–TOFMS spectral data. (A) TAGs and (B) polar compounds: (Δ) extra virgin olive oil; (\square) hazelnut oil; (\diamond) olive pomace oil; (\circ) olive oil; (\blacklozenge) extra virgin olive oil/hazelnut oil mixtures.

without exception, in all oil extracts, was less abundant in EVOO. Isomeric triterpene dialcohols erythrodiol and uvaol yielded two ions: $[M+H]^+$ with m/z 443.39 and $[M+H-H_2O]^+$ with m/z 425.38. These compounds, detected mainly in OPO samples, occur at high concentrations in a skin of olive fruits and are, consequently, at higher amount extracted by organic solvent during production of this olive oil brand [44]. Other intensive, but not yet identified ions, were those of m/z 191.18 and 311.30 diagnostic for OPO; m/z 345.13 and 363.14 present only in EVOO and OO; m/z 379.14 diagnostic for EVOO, and m/z 387.18 detected in all extracts (their probable elemental compositions are presented in Table 2).

3.3. Chemometric analysis

In the final phase, the potential of DART–TOFMS approach to generate information, on the basis of which classification of olive oils and detection of EVOO adulteration is possible, was tested using LDA. This supervised pattern recognition technique was applied to both spectral data of diluted oils (TAGs—data set A) and methanol–water extracts (polar compounds—data set B) in order to obtain reliable classification models for particular oils (EVOO, OPO, OO, HO) and EVOO/HO mixtures. Intensities of ions shown in Table 1 ($n=24$) and Table 2 ($n=16$) were selected as variables and pre-treated as described above. EVOO/HO mixtures and each type of oil were assigned as a class. To avoid the model overfitting, the number of initially selected variables was reduced during the stepwise algorithm of LDA. Reduction of variables from 24 to 11 and from 16 to 12 for data set A and B (selected ions are underlined in Tables 1 and 2), respectively, was shown to give the best overall results in terms of low misclassification of sample origin using the leave-one-out cross-validation of the LDA model. The possibility of overfitting was also checked by calculation of the criterion $(n-g)/3$, where n is the number of objects and g represents the number of classes [45]. For the data set A and data set B these values were 12 and 11, respectively, thus the dimensionality did not exceed the critical values in both cases. Along the first discriminant function, the ions m/z 900.80 and 902.82 for TAGs and m/z 357.30 and 443.39 for polar compounds were the most important variables for sample discrimination. A prediction ability of 100% was achieved in case of data set A, for the EVOO/HO mixtures, in the range 50:50 to 85:15 (v/v), while for data set B in the range 50:50 to 94:6 (v/v). In other words, employing DART–TOFMS mass profiles of TAGs and polar compounds, the proposed method enables reliable detection of HO in EVOO at levels 15 and 6% (v/v), respectively. Fig. 5 shows score plot of three discriminant functions documenting satisfactory resolution among the classes.

4. Conclusions

The novel DART–TOFMS method enabled to classify reliably the quality grade of olive oils from various countries of origin. LDA was employed as a chemometric tool for assessment of TAGs and polar compounds spectral profiles. The potential of this new approach to detect adulteration of EVOO with HO has been demonstrated. Addition as low as 6% (v/v) of this adulterant to EVOO could be recognized based on the mass spectra of polar fraction (aqueous methanolic extract), TAGs spectral profiles were rather less diagnostic. Thanks to none or a very simple sample preparation followed by automated sample introduction in front of a DART ion source, high throughput of measurements is achievable. The follow up research will focus on evaluation of DART–TOFMS potential to distinguish admixtures of lower grade olive oils with EVOO. The interannual crop variability as well as differences associated with processing practices employed by various producers has to be assessed, too.

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