Direct analysis of olive oil and other vegetable oils by mass spectrometry: a review

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Abstract

Virgin olive oil (VOO) is a highly valued vegetable oil often subjected to fraud practices such as adulteration with lower prized oils such as seed oils and refined olive oil. Thus, there is a need to provide rapid tools that allow high-throughput authentication and quality control of VOO as well as other valued edible oils. The characterization of the chemical composition of edible oils is challenging due to the complexity of the matrix. Different methods have been used for both edible oil quality control and authentication purposes including Raman and infrared spectroscopy, molecular fluorescence, nuclear magnetic resonance as well as chromatographic methods. One of the key features is whether the analyses are performed directly in the oil matrix without further treatment or, sample preparation and processing is mandatory including dilution steps or dedicated sample preparation stages. Given the complexity of the matrix, the first approach is difficult to tackle with. Another feature is the scope of the analysis, whether the entire fat composition (*eg.* saponifiable fraction) is analyzed or, on the contrary, specific fractions that may contain useful information for quality control and authentication purposes are targeted. Mass spectrometry offers unique features -such as specificity, sensitivity and speed of analysis- that map well against this challenge, either those based on atmospheric pressure ionization methods such as electrospray and atmospheric pressure chemical ionization, or those occurring under vacuum environment such as matrix assisted laser desorption ionization (MALDI) for nonvolatile species or headspace sampling-mass spectrometry using electron impact ionization (HS-MS) or chemical ionization (proton transfer reaction mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS)) for volatile fraction analysis. In addition, more recent atmospheric pressure methods (Ambient MS) enable direct analysis with minor or even no sample manipulation. The aim of this article is to provide a critical overview on all these methods and their potential use for edible oil characterization, highlighting the strengths and weaknesses of the different approaches

Keywords: olive oil; mass spectrometry; authentication; adulteration; ambient mass spectrometry; MALDI.

1. Introduction

Virgin olive oil (VOO) is probably the most representative and iconic component of the Mediterranean diet, being highly appreciated for its nutritional value and beneficial role on the prevention of cardiovascular diseases [1-4]. VOO is a natural juice extracted from *Olea europaea* L. fruits, exclusively using mechanical methods [5]. No industrial processes or chemicals are added, thus providing an extra value reflected in its higher price compared to most edible oils. For this reason, the assessment of VOO quality, safety and authenticity is of the utmost importance from both economic and health perspectives. Considerable effort has been put during the last decades in this direction [6-9]. Organizations such as European Union [10], the International Olive Council (IOC) [11] and The Codex Alimentarius (FAO-WHO) [12] have established guidelines and regulations, updated periodically, to protect the quality and authenticity of VOO and to prevent fraud [13-14].

Table 1 includes the main analytical methods used officially for VOO authentication purposes [11]. Most of the methods proposed by the IOC rely on the use of gas chromatography (GC) and high-performance liquid chromatography with nonspecific detectors such as flame ionization detector (GC) or refraction index (HPLC). Nevertheless, despite the continuous effort towards the development of new tools for VOO authentication purposes, appropriate solutions for some specific issues have not been found yet [13]. Current gaps include the detection of selected blends of VOO with other lower quality vegetable oils (VOs) -including refined olive oils (OOs)-, the specific detection of soft deodorized (refined) OOs used to adulterate VOO, or the verification of the geographical origin of VOOs. Thus, the development of analytical solutions improving the detection of common and emerging frauds in the olive oil sector is of the utmost importance.

VOO testing is not an easy task since it is a complex matrix mainly constituted by triacylglycerides (TAGs), and other components such as fatty acids (FAs), hydrocarbons, sterols, phenolic compounds or fatty alcohols. Depending on the scope of the analysis, different approaches have been attempted such as fingerprinting, profiling, or targeted analysis, each of those seeking a different range of compounds, from the whole sample to specific compound-class fractions [15]. Fingerprint analysis involves the use of total data obtained without single compound identification. On the other hand, profile analysis comprises the measurement of a previously defined class of analytes. Finally, the targeted analysis implicates determination of a single or small set of target analytes.

Analytical methods used for the characterization of edible oils for quality control and authentication purposes have been previously studied [13-18]. Faria *et al.* [14] classified them according to the technique used, shorting them out as chemical (chromatographic, spectroscopic/spectrometric) or biological methodologies (DNA-based techniques). Gas chromatography (GC) and liquid chromatography (LC) have been extensively used for the determination of both major and minor compounds in edible oils as well as for authentication, traceability and quality control purposes [17]. GC is very convenient for several assays such as the determination of fatty acids using a derivatization step or for volatile analysis. On the other hand, LC is useful for most non-volatile components including TAGs, free fatty acids (FFAs) or phenolic species. As an alternative, spectroscopic techniques (NMR, atomic absorption, ICP-MS, IR (MIR, NIR) and Raman spectroscopy or molecular fluorescence) are used in the field of edible oil with different purposes such as the determination of moisture content, total fat content, free fatty acid content, oxidation indexes and authentication/fraud detection. Finally, DNA-based methods (biological methods) can provide information about the cultivar (botanical) identity.

Regardless the technique used, one of the key features of edible oil chemical methods is whether the analyses are performed directly in the oil matrix without further treatment or on the other hand, sample preparation and processing -including dilution steps or dedicated sample preparation stages- are mandatory. Given the complexity of the matrix, the first approach is difficult to tackle with. Another feature is the scope of the analysis, whether the entire fat fraction composition (*eg.* saponifiable fraction) is analyzed or, on the contrary, specific fractions that may contain useful molecular information for quality control and authentication purposes are targeted.

Mass spectrometry (MS) offers unique features that map well against the challenge of characterizing edible oils, with the ability of simultaneous, fast analysis of low concentration levels of organic molecules with very high specificity. MS has been widely applied in direct edible oils analysis by means of atmospheric pressure ionization methods such as electrospray (ESI) or atmospheric pressure chemical ionization (APCI), which requires simple sample workup based only on either a dilution or a simple liquid-liquid extraction. Additionally, other approaches operated under vacuum conditions, such as headspace sampling mass spectrometry (HS-MS) for direct volatile analysis (with electron impact or chemical ionization), or MALDI, mainly for TAGs profiling have been also extensively used for vegetable oil characterization, as they require no sample treatment at all (headspace sampling) or scarce sample treatment (matrix addition and dilution in the case of MALDI). Furthermore, the relatively recent ambient mass spectrometry methods enable direct analysis with minor or even no sample manipulation [19-23]. The aim of this article is to provide a critical overview on all these methods and their potential use for edible oil characterization, highlighting the strengths and weaknesses of the different approaches. As the ionization method used in mass spectrometry determines the compound classes sought in each type of analysis, the article is organized following this criterion. Amongst the methods discussed we should include direct infusion atmospheric pressure ionization using either ESI, APCI and atmospheric pressure photoionization (APPI), ambient desorption/ionization mass spectrometry methods using ESI or APCI-like ionization mechanisms, and also vacuum methods such as MALDI, together with specific methods for volatiles such as HS-MS and proton transfer reaction mass spectrometry (PTR-MS).

[Table 1]

2. Direct infusion mass spectrometry analysis of edible oils using atmospheric pressure ionization sources

The development of novel analytical methodologies that lead to improved assays, involving minimal sample preparation and reduced reagent consumption, high throughput and enhanced automation is demanded [24-26]. Direct infusion mass spectrometry is a technique which offers fast and reproducible analysis avoiding chromatographic separation and providing expedite data acquisition using mass spectrometry with atmospheric pressure ionization sources such as ESI, APCI and atmospheric pressure photoionization (APPI). This approach has been proposed for direct analysis of edible oils (Table 1) [27-52]. Studies can be roughly classified according to the compound class targeted, either if methods are focused on main oil components (*eg.* FAs) or TAGs) or, on the contrary on minor species present at low concentration levels (*eg.* phenolic compounds, tocopherols, …). Different groups/compound families have been studied (FAs, phenolic compounds, amino acids, sterols, tocopherols, TAGs, etc.) in vegetable oils (and also, in animal origin oils). The studies were focused on the potential of the technique to evaluate the quality, the oxidation status, adulterations, identification of olive oil commercial classes, classification of botanical varieties or geographic origin for authentication purposes. Sample treatment is needed in most cases. It usually involves the implementation of high dilutions or a liquid-liquid extraction. A summary of the main aspects of these studies is shown in **Table 2** [27-52].

[Table 2]

Olive oils. Different studies have been performed in order to evaluate OO quality and also to discriminate botanical varieties or assess the geographical origin. They are mostly based on the profile and relative abundances of FFAs in OOs (including EVOOs) by means of direct infusion electrospray tandem mass spectrometry using ion traps as mass analyzer (ESI-MS/MS (IT) [27-30]. Thus, peak abundances corresponding to FFAs were employed as variables to perform linear discriminant analysis (LDA) capable to predict OO commercial quality grade according to European Union standards [28]; and FA profile followed by statistical treatment allowed discrimination between different botanical varieties of OOs [27-29]. For all these studies, a simple oil dilution in a basic alcoholic mixture was performed.

Lerma-García *et al.* also studied the oxidative status of EVOOs during their storage using direct infusion APCI-MS/MS [30]. EVOO samples, with distinctly different content of phenolic compounds were stored in an oven (60°C) during seven weeks. Peaks corresponding to FFAs, tocopherols, phenolic compounds, and their oxidized forms were used as variables. Two LDA models were constructed, the first one using both EVOO samples with different content of polyphenols, and taking only into account FAs and their oxidized products. The second model was built with EVOO samples with phenolic compounds, considering all measured peaks as variables. Both models led to a correct classification, with better Wilks' lambda value for the latter.

The bulk mass spectra from oil polar fraction obtained by ESI-MS/MS were used by Alves *et al.* [31] to develop a method for adulteration detection of EVOO, and to discriminate between different olive oil (OO) grades (EVOO and ordinary quality OO). MS data were subjected to two exploratory statistical approaches, Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) [31], or Partial Least

Squares (PLS) discriminant analysis [36], showing, in both studies, sample aggrupation correlated to OO quality. Adulteration studies were performed using binary mixtures of EVOO and ordinary quality OO or other vegetable oils (VOs), allowing adulteration detection at concentrations as low as 1% (w/w).

The determination of phenols in olive oil samples usually involves an extraction to separate them from the fatty matrix. Electrospray ionization (ESI) was used in the negative ion mode by Lara-Ortega *et al.* [32] to study the phenolic compounds profile of three different categories of olive oil (EVOO, VOO and *lampante* OO) from hydroalcoholic extracts (diluted 1:10). Although a fairly similar pattern was observed for the three classes, significant differences in peak distribution, and intensities was noticed.

The botanical origin of different VOs (including OO) has been studied by direct infusion mass spectrometry (DI-MS) using other minor components such as amino acids (AAs) from of hydrolyzed protein extracts [33], sterols [34], and the more polar components fraction [35]. Profile analysis may involve sample treatment in order to obtain an enriched extract in the selected analytes; for example, acidic hydrolysis of protein content, previous MS infusion was carried out to obtain AAs profile in OOs. These profiles were used to construct an LDA model capable to discriminate VO botanical origin [33]. For sterol profile, fraction isolation by Thin Layer Chromatography (TLC), and subsequent dilution were needed to DI-MS. These fractions were infused using both ESI and APPI sources, and the corresponding data were used to build an LDA model, which led to perfect classification based on botanical origin [34]. LLE and acidification of hydroalcoholic layer previous to electrospray high resolution mass spectrometry (ESI-HRMS) analysis using a Q-TOF analyzer was carried out by Ramos-Catharino *et al.* [35] to discriminate well-defined groups from different botanical origin in VOs (including OO) by PCA, even profiles obtained could differentiate VOO from refined oils, being useful for aging [35,36], and also adulteration detection by implementing PLS discriminant analysis [37,38].

Ultra-high-resolution mass spectrometry using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICRMS) enabling resolving power above 300.000 (FWHM) was proposed by Marshall and co-workers [42] to unravel the complexity of different vegetable oils. This enhanced selectivity not only enabled a thorough profiling of TAGs and DAGs but also minor components such as tocopherols were distinctly detected. The authors envisaged the ability of this approach for the detection of adulterations. Follow-up studies using the same approach were proposed by Li et al. [43] using free fatty acids to reveal key differences in the molecular compositions of the various vegetable oils tested.

Additional studies of OO adulteration by means high resolution mass spectrometry, although using benchtop Q-TOF instruments with lower resolving power, were performed by Goodacre *et al.* [39] and Gómez-Ariza *et al.* [40], using simple oil dilution (1000-fold) before direct infusion HRMS. Oil fingerprints along to PCA led to promising results, showing possible discrimination between OO and other oils frequently used as adulterants, including refined hazelnut oil (HO) [40]. Likewise, Gómez-Ariza *et al.* [41] used the triacylglycerol profiles obtained by direct infusion HRMS using both ESI and APPI sources with the same authentication purpose. Despite, the ESI spectra of both OO and HO shared many features -such as the main peak attributed to triolein ammonium adduct ion (*m/z* 903)-, the peak corresponding to trilinoleoylglycerol (*m/z* 897) was only present in HO, being possible its use as OO adulteration marker among others. In addition, the use of both sources (ESI and APPI) led to complementary results, since MAGs and DAGs detection is more sensitive using APPI source, whilst ESI source was more effective for TAGs detection. Finally, statistical treatment by PCA and LDA of triacylglycerol peaks abundances showed the ability to detect the presence of other adulterant oils in OO.

The use of ion mobility spectrometry adds an additional dimension to *m/z* separation, thus, representing an interesting alternative given the complexity of the studied samples. Arce and co-workers [44] have recently proposed the combined use of electrospray ionization, differential mobility analysis (DMA) -a class of ion mobility spectrometry (IMS)- and mass spectrometry for chemical fingerprinting of olive oils for authentication purposes (ESI-DMA-MS). Two different approaches were tested: (*i*) sample dilution and; (*ii*) liquid-liquid extraction with MeOH/water to include mainly the fraction of polar compounds. To examine the feasibility of the approach, thirty samples were tested using PCA and orthogonal PLS-DA. The second approach were found more effective (89%) - than direct dilution (67%)- to classify between EVOO, VOO and *lampante* olive oil samples, being the combined information leading to correct classification of all the samples. The results show that ESI-DMA-MS can become an effective tool for olive oil sector, although a more comprehensive study is needed.

Other edible oils. FFA and TAG profiles have been also the main compound classes used in direct infusion MS studies for other vegetable oils. Lerma-García *et al.* used ESI-MS FFA profiles of diluted samples [45] to develop a method for pumpkin seed oils classification according to their species (*Cucurbita maxima*, *Cucurbita pepo*, and *Cucurbita moschata*), and among *Curcubita moschata* oils according to their genetic varieties (RG, Inivit C-88, and Inivit C-2000). Fatty acid profile and subsequent statistical analysis (LDA) was capable to differentiate between both *Cucurbita* species and *Curcubita. moschata* genetic varieties.

TAG profile was also proposed for the characterization of grape seed oil samples from hybrid grape varieties by ESI-MS/MS [46], with simple oil dilution. Structural TAG identification was carried out by MS/MS experiments indicating the presence of mainly four fatty acids linked to the glycerol skeleton, displaying significant differences on their relative abundances. Physical and biochemical properties of TAGs are dependent of FA nature, and their substitution position on the glycerol skeleton. These positions are designed as *sn*-1 and *sn*-3 (outer positions) and *sn*-2 (inner position). The characterization of lithiated TAGs adducts, used to enhance ionization efficiency of TAGs, by ESI-MS^3 enabled regioisomer distinction in linseed oil [47] and fish oil [48]. This adduct formation strategy can occasionally be unfavorable, especially when different ions can be obtained from the same molecule. This holds true particularly for complex sample matrices and low-resolution MS systems. With the aim to simplify the collected MS data, Tonin *et al.* [49] proposed the addition of 18-crown-6 ether and trifluoroacetic acid in a methanolic solution of soybean oil, favoring $[TAG+H]^+$ ions against adduct formation with Na^+ and K^+ ions.

Despite most of the work has been focused to main fat components, the use of direct infusion mass spectrometry after dedicated sample workup has been also proposed to detect key trace compounds in olive oil and other vegetable oils. Sindona and co-workers proposed a method for the quantitation of oleuropein, a key phenolic component in EVOO, using LLE fractionation and ESI-MS/MS [50]. The same group also proposed a method for the quantitation of rotenone, an insecticide, using APCI-MS/MS after a C18 column cleanup step [51]. Finally, Marina and co-workers have proposed a method for seed oil adulteration in EVOOs based on the detection of five nonprotein amino acids and three betaines using flow injection ESI-MS/MS, enabling the detection of as low as 2% (w/w) seed oil in EVOO, being ornithine the key marker to identify that adulteration [52].

3. Edible oil analysis by ambient desorption ionization mass spectrometry

A relatively recent subdiscipline of mass spectrometry is Ambient Mass Spectrometry (Ambient MS), a term coined first by R. Graham Cooks and co-workers [53,54], which refers to the ability of interrogating samples in their native state (solid, liquid or gas) using atmospheric-pressure sampling mass spectrometry with minimal or even no sample preparation [53,54]. In this experiment, sample/analyte ionization takes place at ambient conditions together with the desorption of the species from the condensed-phase sample; ions are thus generated outside the MS instrument and subsequently mass analyzed. These reasons make ambient mass spectrometry techniques an interesting alternative to classic atmospheric pressure ionization methods (*eg.* ESI, APCI).

In the last decade, a large number of ambient desorption/ionization methods have been developed in the field of food quality and safety testing in general and for edible oil analysis in particular. Most of these methods can be roughly shorted out in two main classes: (i) ESI-based and, (ii) APCI-based ambient mass spectrometry techniques [55-57] attending to the primary ionization process in which they are based. Briefly, the former are techniques dependent of a solvent spray, meanwhile in the latter, an electrical discharge is the responsible of generating gas phase ions. The more relevant aspects from selected studies on edible oils using ambient desorption/ionization methods reported are summarized in **Table 3** [58-81]. They are discussed according to the previous classification as follows.

[Figure 1] and [Table 3]

3.1. Electrospray-based ambient MS methods

Desorption Electrospray Ionization (DESI) Mass Spectrometry **(Figure 1)** is probably the most widely used ambient MS technique. It is based on a pneumatically assisted electrospray beam focused onto a surface, where the charged solvent droplets impinge the sample, collecting and ionizing sample compounds present which are transferred to gas-phase and mass analyzed [55-57]. Gerbig *et al.* [58] used this source to study the composition and oxidation behavior of TAGs in edible oils and margarine samples without sample preparation. Ammonium acetate was added into the spray solvent in order to enhance ammonium adduct signals. TAG profiles led to a clear separation between different edible oils by PCA. Detection and identification of oxidation products from oxidized oil samples was also reported [58].

Solvent-assisted desorption/ionization interface was described by Mirabelli *et al.* [59] for fatty acid studies in complex mixtures such as olive and fish oils. This technique is based on the use of a solvent sprayer as in DESI, but avoiding high voltage request. The authors tested several substrates, including glass, metal, filter paper, or silica and C-18 TLC plates, among others, being the latter those which showed optimum performance, along with an important reduction of signal suppression.

Despite the high viscosity of vegetable oils, direct analysis was proposed by microjet sampling in combination with extractive electrospray ionization mass spectrometry (EESI-MS) **(Figure 2)** [60-61]. In this technique, a nitrogen stream forms bubbles inside bulk viscous liquids, generating an aerosol by microjetting mechanism, which is merged with an extractive electrospray plume, being the result somewhat representative of the bulk liquid composition. The extraction event provides extra selectivity, although it also involves biased composition information. Notably, spray solvent composition exerted strong influence on the extracted molecules, being capable to extract compounds with varying polarities, and therefore, generating rich molecular information for VOO classification and adulteration detection [61].

[Figure 2]

Desorption sonic spray ionization [62], which was later renamed as Easy Ambient Sonic Spray Ionization (EASI) [63], is an ambient MS method that only requires the action of coaxial gas flow to promote charged droplets by charge statistical imbalance distribution. Edible oil analysis was studied by Haddad *et al.* [62] using EASI-MS for on-spot detection of TAGs and DAGs in TLC plates. Simas *et al.* [63] also used EASI-MS to obtain TAGs and FFA profile in VOs by directly focusing the EASI source to an oil drop. The combination of TAGs qualitative analysis in VOs performed with EASI-MS, GC, and theoretical TAG prediction by a mathematical algorithm led to a satisfactory correlation /classification [64]. TAG profile obtained by EASI-MS was also found useful for monitoring of quality in VOs, adulteration, and oxidation without sample preparation [69]. Likewise, Cabral *et al.* [70] used the impression of a cut seed onto a paper or a seed directly located under the ionization source to obtain typical diterpenoid profiles for quality control of *P. pubescens* seed oil. On another hand, taxonomic markers in oils, mainly FAs and phenols, obtained by LLE with a basic hydromethanolic mixture were determined by EASI-MS in order to discriminate between vegetable and animal origin oils [67], and assess and confirm the geographical origin of OOs [68].

[Figure 3]

Paper spray (PS) is a versatile and low-cost ambient ionization platform, introduced by R. Graham Cooks group in 2010 [82], that provides many practical advantages. It uses a disposable paper triangle as substrate held in front of the mass spectrometer, where the sample aliquot is loaded. Then, ions are directly generated for MS analysis by applying a high voltage to the wetted paper. Samples can be loaded onto the paper by direct addition (analyte ions can be generated with small volumes (< 10 μL) or the paper can be used as swab for surface sampling.

Lara-Ortega *et al.* [32] used this technique to obtain TAGs and DAGs profiles in three commercial categories of OO without any sample treatment except dilution (1000-fold). Main peaks were assigned to TAGs and DAGs as ammonium adducts, achieving signal enhancement by silver adduct ion formation [32]. On the other hand, Sindona and co-workers from University of Calabria [71] proposed a high-throughput method based on paperspray (PS-MS/MS) using isotope dilution analysis with deuterated standards for the determination of hydroxytyrosol and tyrosol content in EVOOs. The method intended to serve the European regulation EU 432/2012 on health claims, which allows to report on the front label of olive oil, the positive health effects associated. It relies on a two-step analysis, first to estimate the free form of tyrosol and hydroxytyrosol and then, their ester conjugates after hydrolysis treatment. Appropriate LODs were obtained showing the feasibility of the approach. The same group also used PS-MS/MS and deuterated standards for the high-throughput determination of tocopherols in EVOO [72]. Olive oil dilution (*ca*. 1:25) with acetone was performed prior to sample spotting onto the paper substrate. MS/MS experiments were performed in multiple reaction monitoring scan mode; in particular, the transitions m/z 429 → m/z 163 for α-tocopherol and m/z 435 → m/z 169 for the labeled internal standard were monitored, in order to achieve the greatest specificity and the highest sensitivity. Appropriate LOQ values were obtained, demonstrating that this approach can be applied for the rapid screening of tocopherols in different vegetable oils. The results were compared with analyses performed by traditional chromatographic methods. Another interesting "reactive" PS-MS/MS high-throughput assay including *in situ* derivation with methoxyamine was proposed by the same group for the determination of dialdehydes oleocanthal and hydroxyoleocanthal in EVOO [73].

3.2 Atmospheric pressure chemical ionization-based ambient MS methods

The more popular APCI-based ambient MS method is Direct Analysis on Real Time (DART™) **(Figure 4**), commercially available since 2005 [83]. The basic DART source consists of a tube divided into several chambers through which a gas (typically He or N_2) flows. This gas is introduced into a discharge chamber containing a cathode and an anode, where a DC potential of several kilovolts is applied, initiating an electrical discharge containing excited-state species (metastables), ions and electrons, which are carried to a second chamber where a second perforated electrode is used to remove ions from the gas stream. The gas flow then passes through a third region that can be optionally heated. Gas exiting through the third perforated electrode or grid is directed toward the mass spectrometer atmospheric pressure inlet. An insulating cap protects the sample and operator from any exposure to the grid. There are different assemblies which allow the interrogation of solid, liquid and gas samples.

[Figure 4]

DART have been applied to edible oil analysis either for fingerprinting purposes or for target analysis [74-78]. Olive oil authentication by statistical discrimination between several vegetable oils and edible oil mixtures of EVOO and hazelnut oil was achieved with DART using either TAG or polar compounds profile [74]. A DART source combined with high resolution mass spectrometry was used to monitor chemical changes in vegetable oils during their thermal oxidation [75], enabling chemical fingerprinting covering of a wide range of compounds such as TAGs, phytosterols, free fatty acids and their corresponding oxidation products. Another study carried out by Alberici *et al.* [76]

focused on the detection of phytosterols combined with the use of PLS allowed sample aggrupation according to fat type. Refined olive oil samples were not correctly clustered with other edible oil classes, but a PLS model constructed only with OO samples from different categories showed clear discrimination of refined samples. Alternatively, DART-MS has been also applied to target analysis of key species in edible oils such as the mono and diesters from 3-monochloropropane-1,2-diol (3-MCPD), species found in refined edible oils and several fat-containing foodstuffs [77]. A preliminary sample treatment was applied using a silica gel column or aminopropyl solid-phase extraction cartridges for 3-MCPD di- and mono-esters, respectively. The proposed method enabled semi-quantitative examination of 3-MCPD di-esters fraction, needing samples with a relatively high contaminant content for correct determination. However, poor ionization for 3-MCPD mono-esters hindered their monitoring at cutoff concentration levels. DART has been also proposed for the profiling of phenolic compounds in EVOO [78].

Besides DART, the more commonly used APCI-based ambient MS methods are those based on Dielectric Barrier Discharge (DBD) principle. Dielectric Barrier Discharge Ionization (DBDI) has gained attraction in recent years as a versatile ionization method available in different formats (ambient ionization probes, GC-MS or LC-MS interfaces), intended for many applications including ambient mass spectrometry imaging, explosive detection or food safety [20-22]. The (dielectric barrier) discharge is formed between two electrodes, with at least one dielectric layer which separates the electrode from the plasma [57]. DBD is induced by applying an AC voltage in the designed electrode configuration, with helium or argon used preferentially as discharge gases. Different publications have shown the potential of DBDI for the determination of compounds characterized by a wide range of polarities (more or less polar pesticides, PAHs, pharmaceuticals etc.). Among different possible configurations, DBDI [79] and low temperature plasma (LTP) probe [80] have been used for edible oil analysis. The main differences between both ionization sources are the dimensions (LTP is larger), and electrode configuration (HV and ground electrodes).

The combination of neutral desorption with DBDI-MS was developed by Zhou *et al.* [79] for the study of hogwash and edible oils samples. Samples were placed into a glass vial, and directly impinged by a nitrogen gas stream, leading analyte(s) desorption, which were then transported to the DBDI source using a sample transfer line. FFAs were decisive markers to discriminate between hogwash and qualified edible oils samples using PCA. On the other hand, García-Reyes *et al*. [80] proposed a simple method for direct olive oil analysis using the LTP probe. Free fatty acids, phenolics, and volatile compounds, some of the targeted compounds tested for quality control and authentication purposes, were easily determined. A sample drop (3 μL of oil) is pipetted onto a glass slide microscope, which was directly interrogated with the LTP probe in front of the atmospheric pressure inlet of a mass spectrometer. Full-scan spectrum in positive ion mode gave evidence of volatile compounds, whilst in negative ion mode spectrum led to ions coming from main FFAs. Phenolic compounds detection, which are present at lower concentrations, was accomplished by MS/MS experiments with a heated substrate. A follow-up study by Lara-Ortega *et al.* [32] used LTP to study phenolic profiles from olive oil hydroalcoholic extracts, and triacylglycerol profile from raw olive oil samples. In this work, the performance of LTP was also compared to paper spray for the direct olive oil analysis for quality control and authentication purposes. Both approaches allowed the analysis of olive oil without (raw oil) or after a simple dilution. Interestingly, significant differences were found in the information that can be extracted from both LTP and PS-MS. Above a value of *m/z* 500, scarcely any olive oil compound was efficiently desorbed using LTP. This fact involves the loss of most of the information related to intact TAGs along with possible MAGs and DAGs. Therefore, paper spray in this aspect outperformed LTP, as a higher range of species are ionized and can be used for sample classification. On the other hand, nonpolar species such as key hydrocarbon squalene were only detected with the plasma-based method, highlighting the complementariness of both approaches.

Desorption atmospheric pressure photoionisation (DAPPI) [81,84] is also an APCI-like ambient MS source, which offers good sensitivity for polar, non-polar, and neutral compounds [84]. It is based on a nebulizer microchip acting as vaporized solvent supplier, along with a photoionization lamp. DAPPI and DESI were studied by Suni *et al.* [81] for lipid analysis in fish oil capsules, and butter. Efficient desorption and ionization of both polar and non-polar lipids was achieved by DAPPI, although with higher fragmentation due to the thermal desorption process. The group of Facundo Fernandez and co-workers proposed the combination of laser-induced acoustic desorption with microplasma-based atmospheric pressure photoionization (LIAD-APPI) [85] using a nebulized sweep jet to aid dopant introduction and ion transmission. It has been applied to the analysis of model, nonpolar lipid compounds. Specifically, several sterols, sterol esters, and triacylglycerols were detected using dopants such as anisole and toluene, as a proof-of-principle demonstration of the applicability of LIAD-APPI on actual samples.

Finally, a somewhat difficult to classify method is Matrix Assisted Ionization (MAI) proposed first by Trimpin and McEwen [86], has been reported for the characterization of polar and non-polar lipids in complex mixtures of edible oils [87]. This technique originally named Matrix Assisted Ionization in Vacuum (MAIV) [88,89], or more recently, MAI [90,91] is based on direct introduction of the sample, previously co-crystallized with matrix, only using the MS vacuum (without laser). This approach leads to gas-phase ion generation by analyte/matrix crystals sublimation. In this case, ions are produced in the intermediate pressure region of the ion inlet during sampling and ion transport into the mass spectrometer. Liyanage *et al.* [87], registered the TAGs profile corresponding to different edible oils by MAI-HRMS, being possible oil identification taking into account these profiles. For this purpose, 3-nitrobenzonitrile was used as matrix, prepared with or without addition of ammonium acetate. Diluted edible oils and matrix were blended, and spotted onto a MALDI target to allow air dry. TAG profiles obtained without ammonium acetate by both MAI and MALDI were very similar, with sodium adducts being the predominant ions observed.

4. MALDI and other laser-based methods

4.1. MALDI

Traditional lipid analysis generally relies on saponification, formation of fatty acid methyl esthers (FAMEs) detected by GC. This type of analysis does not allow detection of the actual triacylglycerols (TAGs), but only the total percentage of individual fatty acids. Alternatively, HPLC with refraction index detector is proposed for TAG separation, although the resolution of selected TAGs is complex to achieve. TAG analysis using matrix assisted laser desorption ionization (MALDI-TOFMS) [92, 93] offers interesting advantages such as fast and easy sample preparation as no analyte purification or derivatization are required and appropriate resolution in the TAG mass range is achieved. Due to the TAG preserved structure during MALDI-TOFMS analysis, it also provides structural information for authentication purposes such as the assessment of the geographical origin of edible oils.

MALDI is a soft ionization method based on a pulsed laser striking the sample surface composed by the analyte along with an excess of matrix which triggers molecule desorption and subsequent ionization. Sample (analyte) and matrix (typically a high UV-light absorbing organic molecule) aliquots are placed onto a conducting metal target plate. After laser irradiation at an appropriate wavelength, matrix molecules absorb the energy, convert it to heat energy, transferring analyte molecules to gas phase, being also ionized during the process. This ionization technique, mostly occurring in vacuum conditions, has an inherent pulsed nature, so it is usually combined with time-of-flight mass analyzers (MALDI-TOFMS). Its main application has been the analysis of large biomolecules and (bio)polymers. It has also been used for analyzing TAGs and other lipids as well as proteins in oils. A summary of different studies of MALDI applied to edible oil characterization are shown in **Table 4** [94-125].

[Table 4]

4.1.1. Olive oil

The two main compound classes tested in the reported studies with MALDI are TAGs and phospholipids. TAG profile obtained by MALDI-TOFMS was used: to distinguish between different OO varieties from Israel [95] and Tunisia [96]; to discriminate OOs from different growing areas in the Croatian coast [97], or the assess the olive oil composition changes during olives ripening [98]. In addition, TAG structure determination has been also accomplished using MALDI-TOF/TOF-MS [99] and MALDI-TOFMS as support tool for high resolution Nuclear Magnetic Resonance (HR-NMR) [100]. MALDI-TOFMS TAG profile along with PCA have been also used for EVOO adulteration studies [101]. TAG profile obtained by MALDI-TOFMS permitted the recognition of different EVOOs and also enabled the detection of blends as low as 1% of foreign oil addition. Combined with PCA, MALDI-TOFMS TAG profile was found more effective to classify EVOOs from different Croatian areas than fatty acid profile or NIR spectroscopy [126].

One of the strengths of MALDI-based approaches is the relative simplicity of sample preparation, which is mainly based on sample dilution together with the addition of the matrix solution. This makes TAG analysis in edible oil samples (including OOs) by MALDI a straightforward tool, which is usually combined with different chemometric approaches for data analysis including LDA [112], Euclidian distances [113], PCA and

HCA [114,115]. The method published by Ng *et al.* [114] was also used to build a spectral database suitable to classify 900 edible oil samples [116], including cooked oils and gutter oils. Finally, thermal stress characterization on edible oils was also studied by MALDI-MS [117,118].

On the other, besides TAGs, in the case of phospholipid profiling, selective extraction procedures are required. Different strategies have been reported such as the use of TiO_2 nanoparticles as sorbent for matrix solid-phase dispersion (MSPD) procedures [102], or polar fraction enrichment by μ-SPE [103]. Phospholipid compounds probed by MALDI have been used as adulteration markers of OOs with HO [104] or seed oils [127]. A modification of Bligh-Dyer extraction procedure [128], introducing an ionic liquid as extraction solvent, which can be used as MALDI-TOFMS matrix was also proposed to study EVOO adulteration with HOs [104], and corn oil [105]. Adulteration of OOs with HO may involve health issues by the incorporation of hazelnut-derived allergens. Different MALDI-TOFMS methods have been developed enabling analysis of protein and allergens in EVOOs adulterated with HO [106,107].

Alternatively, the saponification and MALDI-TOFMS qualitative analysis of fatty acid composition of vegetable oils such as plan kernel oil, palm oil, olive oil, canola oil and castor oil has been also proposed [129], although the more common and straightforward approach is the direct analysis of TAGs as it provides intact molecule structural information that can be useful for authentication purposes.

4.1.2 Other edible oils

MALDI-MS TAGs profile has been also applied to studies with different oils such castor oil [120], canola oil [109], typical Indian edible and non-edible plant oils [121], seed oils [122], pomegranate oil [123], or grape seed oils [124] (**Table 4**). These studies led to detect main TAGs in each oil, including nitrogen or sulfur derivatives from processing reactions [119] and for instance, allowing rapid differentiation of grape seed varieties [124]. In addition, TAG profile was used to detect adulteration of poppy seed oil with sunflower oil [125]. Recently, Kuo *et al.* proposed a robust and simple MALDI-TOFMS assay for rapid fingerprinting of triacylglycerols (TAGs) in different edible oils [130]. Spectral similarity analysis was performed to quantitatively reveal correlations among edible oils, enabling the reliable classification of commercial edible oils from both animal and vegetable origin. As an example, the quantitative evaluation of a binary mixture of olive oil and canola oil was successfully conducted.

4.2. Other surface-based direct mass spectrometry methods under vacuum conditions

Minor components such as squalene and its analogues are also of interest for edible oil characterization. Despite MALDI-TOFMS has been proven as an interesting alternative approach for TAGs characterization, the matrix produces several background signals in the low *m/z* range, which has hindered its application to the analysis of relatively low molecular weight compounds. A possibility to circumvent this drawback was proposed by Zambonin *et al*. by direct laser/desorption ionization (LDI) of edible oils using a standard stainless-steel target plate [131]. Silver trifluoroacetate was used to yield silver adducts ions of squalene and related squalene oxide forms in the positive ion mode. The same approach was also tested for the characterization of olive and sunflower oil before and after thermally assisted oxidation, targeting in these cases higher molecular weight species such as DAGs and TAGs [132]. A similar approach, but using a TLC target plate compatible with MALDI, was proposed by Catharino *et al* [133]. The approach, so called sorptive tape-like extraction laser desorption ionization mass spectrometry (STELDI-MS) enabled the detection of fatty acids and phenolic compounds used to differentiate oil blends from EVOO, *lampante* OO, soybean oil and hazelnut oil. Finally, other laser-based approaches proposed for TAG profiling includes nanostructure initiator mass spectrometry, a matrix-free technique used for TAG profile of olive oil and soybean oil [134], the use of functionalized (porous) silicon (DIOS) as substrate for laser desorption ionization mass spectrometry [135], time-of-flight secondary ion mass spectrometry (TOF-SIMS) [136] and photoelectron resonance capture ionization mass spectrometry (PERCI-MS) [137].

5. Other direct MS methods for volatile analysis of vegetable oils with ionization under vacuum conditions

5.1. Electron impact ionization-based Head-space Mass Spectrometry (HS-MS). The *Volatolome* of EVOO samples is very rich, and provide useful molecular information for sensory analysis, although the species are not stable over the entire shelf life of the sample, which reduces its analytical usefulness for authentication purposes. One of the methods which provides fast response and selective data due to mass spectrometry is the coupling of a headspace sampler to a mass spectrometry (HS-MS) operated with a vacuum ionization source (*eg.* EI). HS-MS combined with LDA to detect the adulteration of virgin olive oil with sunflower oil and olive pomace oil [138]. A headspace module was connected to a standard mass spectrometry system with electron impact ionization (*eg.* classic GC-MS Agilent 5973 MSD) by means of an appropriate transfer line and a set of solenoid valves to adjust the pressure during headspace (sample) injection. The same authors also reported the use of HS-MS and LDA to allow the differentiation of monovarietal Portuguese EVOOs for protected designation origin (PDO) purposes [139]. Follow-up studies enabled the correct classification of samples from five different Mediterranean areas (Italy (Liguria and Apulia), Spain, Greece and Tunisia) using PCA and LDA treatment [140]. The same HS-MS setup was used by Valcárcel and co-workers for the detection of EVOO adulteration with hazelnut oil using PLS and PCA as multivariate regression techniques [141], and also for the classification of EVOOs on the basis of its PDO, olive botanical variety and geographical origin, using soft independent modeling of class analogy (SIMCA) data treatment [142]. Another related application of HS-MS was the characterization and quantitation of both positives (fruity) and negatives (viz. fusty, muddy sediment, musty, rancid and vinegary and vegetable water) sensory attributes of monovarietal EVOOs and its comparison with sensory assessment methodology [143,144]. Good prediction and correlation were obtained with the instrumental approach compared to the official sensory analysis in most cases, emphasizing the potential of these approaches for quality control of edible oils, although the main weakness is connected to the instability and uneven degradation of the volatile compounds targeted over the shelf life of commercial EVOOs. Only with sample sets for calibration and prediction under comparable conditions reliable data and classification is attained. The same purpose (classification of EVOO samples according to the presence of negative sensory attributes (off-flavors)) was recently pursued by extending the information from HS-MS with other spectroscopic techniques such as mid infrared spectroscopy and UV-Vis using data fusion and PLS-DA [145].

5.2. Chemical-ionization based HS-MS analysis. Besides the classic HS-MS method described above based on electron impact ionization, there are alternative methods also intended for comprehensive olive oil volatile analyses using a more gently chemical ionization (CI) as primary ionization method. These CI-based HS methods are proton transfer reaction-mass spectrometry (PTR-MS) [146-149] and selected ion flow tube mass spectrometry (SIFT-MS) [150-152]. Both methods, based on similar principles although implemented in different commercial platforms, are flow-tube based mass spectrometric techniques that are used to detect and quantify volatile organic compounds (VOCs) in whole air in real time. Subtle difference between both methods are the different reagent ions generated in the ionization chamber and the possibility of mass isolating this CI reagent ions prior to contact the sample stream. In the case of the original PTR-MS, protonated water is the main reagent ion whereas in SIFT-MS, due to the use of a supply of Ar or He in the reagent ion source, and presumably the formation of excited Ar/He metastables, H_3O^+, NO^+ and O_2^+ are generated. Besides, continuous reagent ion selection and real-time reagent ion switching are also feasible. A scheme of the instrumentation assembly of SIFT-MS is included in **Figure 5**.

[Figure 5]

The general procedure is somewhat similar in both methods. An aliquot of *ca.* 5-10 g of EVOO is placed in a 500-mL headspace vial/bottle. The sample is incubated at *ca.* 30 ºC for 30-40 minutes to allow volatile equilibrium. Then, the headspace is continuously introduced into a drift tube of the instrument at a flow of *ca.* 50 mL/min, brought into contact and mix with a stream of the generated reagent ions (*eg.* H_3O^+), which are able to ionize all the volatile species with proton affinity higher than water. The generated ions are mass separated in a quadrupole mass spectrometer and finally detected. Both CI-based techniques do have the advantage of producing simpler mass spectra that EI-based HS-MS method due to the minimum fragmentation associated with CI methods. This should be helpful in providing more useful datasets with less interfered *m/z* values and less redundant signals, making multicomponent analyte mass spectra simpler and easier to interpret, which eventually will enable better classification of samples [149].

PTR-MS has been successfully used to distinguish between EVOO and defective samples (*lampante* OO) [146], to classify samples according to the country, region or district origin -with different success rates- [147,148], and according to olive variety (monovarietal EVOOs) [149]. SIFT-MS has been also used for volatile analysis in VOO

[150], to monitor olive oil oxidation [151] and, recently, for sample classification according to country and regional origin (origin-labeled olive oil) [152].

6. Critical assessment and concluding remarks

Despite the continuous effort towards the development of new tools for VOO authentication purposes, appropriate solutions for some specific issues have not been found yet. Thus, the development and/or improvement of analytical solutions for OO quality and authenticity assessment are timely. The detection and quantitation of selected blends of OOs with other vegetable oils (seed oils) or refined olive oils and the verification of geographical origin of virgin olive oils are amongst the more relevant challenges to address. Most of the current official methods rely on the use of GC and HPLC with nonspecific detectors. These Official methods should be open to techniques other than chromatography since they can offer rapid, robust, and precise analyses of many of the series of chemical compounds discussed in this review.

Mass spectrometry methods, either those based on atmospheric pressure ionization (such as ESI, APCI and Ambient MS methods), or those occurring under vacuum environment, offers several features that map well against this purpose. The ionization step is critical as it determines the type of species subjected to analysis. A wealth of ionization choices is presented, enabling whole oil analysis targeting nonvolatile species such as TAGs (*eg.* MALDI) to small volatile aromas present in the headspace of the sample (HS-MS, PTR-MS).

[Table 5]

Table 5 summarizes the different MS-based direct methods discussed in this review. A comprehensive comparison and critical assessment of the main advantages and drawbacks is difficult, though, given the complexity, variability and heterogeneity of the samples as well as the varied nature of the studies with different purposes (botanical, geographical origin, adulteration, classification of commercial categories, etc.). Some of the methods enable direct analysis with no prior preparation whereas, in other cases, sample dilution or rapid sample treatment protocols using solvent extraction or solid sorbents are required (viz. for the extraction of relevant polar compounds such as polyphenols). The choice of sample workup is of the utmost importance, as incomplete or inadequate sample status may raise instrumental issues such as the presence of carryover effects, due to undesired sample deposition on critical parts of the mass spectrometer. The rich molecular information gathered from the variety of methods discussed allow different applications ranging from quality control, detection of adulteration, assessment of geographical and/or botanical origin, and even the classification according to commercial olive oil categories. In summary, although MS is not yet included in official olive oil quality control and authentication methods, it will undoubtedly come into play -either alone of combined with separation techniques- and become a gold standard as it did in many other disciplines such as pesticide testing or antidoping control. There are too many advantages not to benefit from for such as challenging task VOO authentication represents.

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Figure captions

Figure 1. Schematic of typical DESI experiment. The sample solution is deposited from solution and dried onto a PTFE surface and an appropriate solvent is sprayed at a flow rate of 3 to 15 μL/min under the influence of a high voltage (4.0 kV). Adapted from Ref. [53] with permission. The nominal linear velocity of the nebulizing gas was set to 350 m/s.

Figure 2. Schematic illustration of the concept and setup of EESI-MS. Inset: compositions and environment of molecules present in bulk liquid, liquid-gas interface, and headspace. The temperature of the heated column is 80 ° C. Adapted from Ref. [60] with permission.

Figure 3. Schematic of the TLC-EASI-MS system in operation. The desorption/ionization of the analytes from the surface of the TLC plates is performed by the charged droplets produced by sonic spraying an acidic water/methanol solution in a N_2 (or air) assisted process. The charged droplets are created during sonic spray by a static imbalance of charges. The EASI source uses only a Swagelok T-element, ferrules, and tubing for the gas flow and a fused-silica capillary at the sonic spray exit. Adapted from Ref. [62] with permission.

Figure 4. Schematic representation of a DART source. Adapted from Ref. [83] with permission.

Figure 5. Diagram of the SIFT-MS instrument used in this study. Direction of the flow is right to left. Adapted from ref. [150] with permission.

Table 1. Selected International Olive Council (IOC) official methods for quantifying major and minor components in virgin olive oil for purity assessment and authentication purposes[1]. The COI/T20 documents can be downloaded from the website: http://www.internationaloliveoil.org/estaticos/view/224-testing-methods [11,13].

Compound class targeted	**Rationale of the determination**	**Brief method description (Reference method document)**
Fatty acids including *Trans* fatty acids	Assessment of fat composition. Determination of possible oil adulterants. The *trans*- form may appear -above a certain threshold- as a result of certain fat manipulations (refining)	GC with FID detector Derivatization with MeOH/KOH (methyl esters) (COI/T20/Doc. No. 33)
Triacylglycerols	Assessment of fat composition. Identification of synthetic fats from free fatty acid esterification with glycerol (due to altered combination of fatty acid molecules and their positions in the glycerol moiety with regards to biosynthesized). Detection of hazelnut oil adulteration	HPLC (C18 column) with refraction index detector Cleanup on a 1g (SPE) silica cartridge (COI/T20/ Doc. No. 20 Rev 3) (IOC/T20/ Doc. No. 25)
Triacylglycerols Diacylglycerols		GC-FID with silylation. (COI//T.20/Doc No. 32)
2-glyceryl monopalmitate (%)	Assessment of fat composition. The presence of 1% saturated fatty acids in the position-2 of the glycerol is connected to adulteration of olive oil with strange oils (like re-esterified edible oils by synthetic means).	GC-FID LC or SPE separation after hydrolytic reaction with pancreatic lipase. Silylation (COI//T.20/Doc No. 23)
Waxes	Assessment of fat composition. Waxes are biosynthesized in olives from saturated very-long-chain FAs. Their presence in OOs is relevant because their concentration differs among the olive oil categories, and information about its presence can be used for both quality assessment and authentication purposes. Waxes concentration is up to 10-fold higher in solvent extracted oils with respect to pressure extracted oils. Combined with erythrodiol and uvaol content are used for the detection of VOO adulteration with olive pomace oils	GC-FID Fractionation by LC on a silica gel column (COI//T.20/Doc No. 18 Rev 2)
Waxes, Fatty Acid Methyl Esters, Fatty Acid Ethyl Esters	Assessment of fat composition. Simultaneous method for waxes, fatty acid (as methyl esters), and fatty acid ethyl esters for olive oil authentication purposes such as (i) to distinguishing between olive oil and olive-pomace oil, (ii) for the detection of the presence of lower-quality oils (ordinary, *lampante*) in EVOOs, and for the detection of the fraudulent addition of some deodorized oils to EVOOs	GC-FID Fractionation by LC on a silica gel column (COI//T.20/Doc No. 28 Rev 1)
Stigmastadienes	Purity assessment. Detection of adulteration with refined oils. Sterene stimasta-3,5-diene, produced due to dehydration of sterols, is used as a marker for the detection of refined edible oils in VOOs	GC-FID Isolation of unsaponifiable fraction and separation of

		steroidal hydrocarbon fraction on silica column (COI//T.20/Doc No 11 Rev 2)
Aliphatic hydrocarbons and sterenes	Purity assessment. Detection of adulterations. Detection of desesterolized seed oils in refined oils	GC-FID Separation of unsaponifiable fraction on LC Si-column impregnated with silver nitrate (COI//T.20/ Doc No. 16 Rev 1)
Sterols and triterpene dialcohols *(erythrodiol + uvaol)*	Purity assessment. Main compound class from unsaponifiable fraction for authentication purposes. The profile and concentrations of selected sterols are useful for vegetable oil authentication. Detection of presence of olive-pomace oil and seed oils in VOOs (*eg*. grapeseed oil).	GC-FID Separation of unsaponifiable fraction (on TLC or LC column) and silylation (COI//T.20/ Doc No. 30 Rev 1)

[1] Besides purity assessment methods, EVOO quality control methods are also applied to assess the different (commercial) olive oil categories (extra virgin olive oil, virgin olive oil, ordinary virgin olive oil, *lampante* olive oil (not commercial), olive oil (a mixture of refined olive oil +VOO), pomace oil (a mixture of refined pomace olive oil +VOO)). They are based on acidity titration (free fatty acid content), oxidation status (peroxide value and UV absorption measurements at 232 and 270 nm), and sensory assessment methodology (*Panel Test*). For details, see refs. [11] and [13].

Table 2. Edible oil analysis using direct infusion mass spectrometry using ionization methods at atmospheric pressure [27-52].

Compound class	Sample type	Detection method	Sample treatment	Purpose of the study	Reference
FFAs	EVOOs	ESI-MS/MS (IT)	Oil dilution (PrOH/MeOH)	Botanical varieties and maturity index classification	[27]
FFAs	OOs	ESI-MS/MS (IT)	Oil dilution (PrOH/MeOH)	OO quality and binary mixtures of different quality grade evaluation	[28]
FFAs, and phenolic compounds	EVOOs	ESI-MS/MS (IT)	Oil dilution (PrOH/MeOH)	Classification by botanical origin	[29]
FFAs, tocopherols, phenols, and their oxidized forms	EVOOs	APCI-MS/MS (IT)	Oil dilution (PrOH/MeOH)	EVOOs classification by oxidative status	[30]
Bulk mass spectra from polar fraction	EVOOs and ordinary quality OOs	ESI-MS/MS (IT)	LLE (MeOH/H_2O)	Adulteration studies	[31]
Phenolic compounds	EVOOs, VOOs, and lampante oils	ESI-MS/MS (IT)	LLE (MeOH/H_2O) and dilution	Phenolic compounds profile of three different OO classes	[32]
AAs	VOs	ESI-MS/MS (IT)	Precipitation, hydrolysis, and dilution	Classification by botanical origin	[33]
Sterols	VOs	ESI-MS/MS (IT)and APPI-MS/MS	Sterol band isolation by TLC, and dilution	Classification by botanical origin	[34]
Polar compounds	VOs	ESI-QTOF-MS	LLE (MeOH/H_2O)	Classification, control quality, aging evaluation, and adulteration detection	[35]
Bulk mass spectra from polar fraction	OOs	ESI-MS/MS (IT)	LLE (MeOH/H_2O)	Discrimination to assess botanical adulteration	[36]
TAGs	EVOO	ESI-MS/MS (IT)	LLE (MeOH/H_2O, 1:1)	Detection and quantitation of adulteration of EVOO with inexpensive edible oils (5-20% w/w) (soybean, corn, sunflower and canola)	[37]
TAGs	EVOO	ESI-MS/MS (IT)	LLE (MeOH/H_2O, 1:1, 1% formic acid)	Detection and quantitation of adulteration of EVOO with inexpensive edible oils (1-20% w/w) (soybean, corn, sunflower and canola)	[38]
Bulk mass spectra	OOs	ESI-TOF-MS or ESI-QTOF-MS	Oil dilution (CH_2Cl_2/NH_4OAc)	Discrimination by botanical origin	[39]

Acylglycerols	OOs	ESI-QTOF-MS and APPI-QTOF-MS	Oil dilution (CH_2Cl_2/NH_4OAc)	Discrimination by botanical origin for adulteration detection	[40]
TAGs	VOs	APPI-QTOF-MS	Oil dilution (1:1000) (CH_2Cl_2/MeOH, 60/40 v/v) Mobile phase (flow injection): 50/50 acetonitrile/ H_2O (v/v)	Fast oil fingerprinting of VOs (EVOO, OO, olive pomace, hazelnut, sunflower, corn and mixed oils)	[41]
TAGs and minor components such as tocopherols	Olive oil	ESI-FTICR-MS	Oil dilution (1:1000) (CH_2Cl_2/MeOH)	Detailed compositional fingerprints of vegetable oils and detection of olive oil adulteration with soybean oil	[42]
FFAs and TAGs	VOs	ESI-FTICR-MS	Dilution with CH_2Cl_2 followed by dilution with MeOH	TAGs fingerprinting of VOs (soybean, rapeseed, corn, sunflower, peanut, linseed and olive oil)	[43]
TAGs and polar phenolic fraction	OOs with different categories	ESI-DMA-MS	Oil dilution (1:1000) (CH_2Cl_2/MeOH) (TAGs)/ LLE (MeOH/H_2O, 1:1) (polar compounds)	Classification of olive oil from different categories (EVOO, VOO and lampante olive oil)	[44]
FFAs	Pumpkin seed oils	ESI-MS/MS (IT)	Oil dilution (PrOH/MeOH)	Classification according to different species, botanical variety and genetic modifications	[45]
TAGs	Grape seed oils from hybrid grapevine	ESI-MS/MS (IT)	Oil dilution (THF/MeOH/H_2O)	TAG profiling	[46]
TAGs	Linseed oil	ESI-MS/MS (IT)	Oil dilution (CH_2Cl_2/LiI)	FA content and TAG regioisomerism determination	[47]
TAGs	Fish oil	ESI-MS/MS (IT)	Oil dilution (MeOH)	FA content and TAG regioisomerism determination	[48]
TAGs	Soybean oil	ESI-MS/MS (TQ)	Oil dilution (MeOH)	Mass spectra simplification for DI analysis of complex matrices	[49]
Oleuropein	VOOs	ESI-MS/MS (IT)	LLE extraction (MeOH). Cleanup using LLE with acetonitrile/hexane 4:6 (v/v)	Quantitation of high nutritional value antioxidant from different cultivars	[50]
Rotenone	OO	APCI-MS/MS	Dilution with acetonitrile and cleanup using a C18 column in pass-through mode	Rapid identification of selected pesticide residues in OO and olives	[51]

Nonprotein amino acids and betaines	VOs	ESI-MS/MS (TQ)	Dilution with MeOH/$CHCl_3$ (2:1, v/v) Multiple LLW with MeOH/$CHCl_3$/H_2O Butyl ester derivatization	Nonprotein amino acids used a markers for the detection of adulteration of OOs with seed oils	[52]

List of abbreviations: AA(s): Amino Acid; **APCI**: Atmospheric Pressure Chemical Ionization; **APPI**: Atmospheric Pressure Photoionization; **DI**: Direct Infusion; **ESI**: Electrospray Ionization; **EVOO(s)**: Extra Virgin Olive Oil; **FA**: Fatty Acid(s); **FFA(s)**: Free Fatty Acid; **FFA(s)**: **FT-ICR**: Fourier transform ion cyclotron resonance; **IT**: Ion Trap; **LLE**: Liquid-Liquid Extraction; **MeOH**: Methanol**; MS**: Mass Spectrometry**; NH4OAc:** Ammonium acetate**; OO(s)**: Olive Oil; **PrOH**: Propanol**; Q**: Quadrupole; **TAG(s)**: Triacylglyceride(s); **THF**: Tetrahydrofuran**; TLC**: Thin Layer Chromatography; **TOF**: Time of Flight; **TQ**: Triple-Quadrupole; **VO(s):** Vegetable oil(s) including OO; **VOO(s)**: Virgin Olive Oil(s).

Table 3. A summary of ambient MS methods for edible oil analysis [32, 58-81].

Compounds	**Matrix**	**Ambient MS technique / Spray solvent or gas discharge / MS system**	**Sample treatment**	**Study**	**Reference**
Electrospray-based ambient MS methods					
TAGs	Edible oils and margarine	DESI / MeOH/H_2O mixture / LTQ-Orbitrap-MS	Not required	TAG analysis and oxidation behavior	[58]
FAs	OO and fish oil	Solvent assisted desorption/ionization / different solvent mixtures and additives were tested / Q-IT-MS	FAs extraction by saponification, and their separation by TLC	FA measurements	[59]
EVOO fingerprint	EVOO	EESI / acidic hydromethanolic mixture / Q-TOF-MS	Solvent extraction	Quality and discrimination of OOs	[60]
Edible oil fingerprint	Edible oils	EESI / different solvent mixtures / Q-TOF-MS	Solvent extraction or dilution	Edible oil fingerprint and discrimination between EVOO and its adulterations	[61]
TAGs and DAGs	Edible oils	EASI / acidic hydromethanolic mixture / TQ-Trap-MS	Separation by TLC	Analyte characterization in complex samples	[62]
TAGs and FFA profile	VOs	EASI / MeOH / Q-MS	Not required	Characterization of VOs	[63]
TAGs	VOs, hydrogenated oils, and cocoa butter	EASI / MeOH / Q-MS	Not required	TAGs quantification	[64]
TAGs	Brazil Nut Oil	EASI / MeOH / Q-MS	Not required	Quality, adulteration, and oxidation study	[65]
TAGs	Andiroba oil, castor oil, and coconut seed oil	EASI / MeOH / Q-MS	Oil dilution	Oil characterization	[66]
FAs and phenol profile	OO, HO, soybean oil, grape seed oil, canola oil, butter, and lard	EASI / basic hydromethanolic mixture / IT-MS	Oil dilution	FA and phenol profiling	[67]
FFAs and phenolic compounds	EVOO	EASI / basic methanolic solution / Q-TOF-MS	LLE	EVOO quality control and geographical origin	[68]
TAGs and their hydroperoxides	VOO, soybean oil and lard	EASI / MeOH / Q-MS	Not required	TAGs oxidation monitoring	[69]
Diterpenoid profile	*P. pubescens* seed oil	EASI / MeOH / IT-MS	Not required	Quality control, authenticity, and origin certification	[70]

Phenols, and acylglicerol compounds	EEVO, VOO, and lampante OO	PS / acidic MeOH / IT-MS	Not required for acylglycerol study. LLE for polar fraction	Phenolic profile and acylglycerol profile	[32]
Hydroxytyrosol and tyrosol (free and total)	EVOO	PS	Dilution with hexane and SPE using silica (SepPak)	Free and total content of tyrosol and hydroxytyrosol	[71]
Tocopherols	EVOO	PS-MS/MS (TQ)	Oil dilution with acetone	Vitamin E content	[72]
Hydroxyoleocanthal and oleocanthal	EVOO	PS-MS/MS (TQ)	Not required (sample directly spotted onto the paper (*in situ* derivatization with reagents)	Quantitation of phenolics	[73]
Atmospheric Pressure Chemical Ionization-based Ambient MS methods					
TAG profile and polar compounds	VOs	DART / He / TOF-MS	Oil dilution for TAG profile or LLE for polar compounds	Discrimination between VOs and OO adulteration detection	[74]
Oil fingerprint	VOs	DART / He / Orbitrap-MS	Oil dilution	Establishment of compositional differences between oils during their thermal oxidation	[75]
Phytosterols	VOs, margarines, butters, and animal oil	DART / He / QTOF-MS	Sample dilution	Discrimination between different samples	[76]
3-MCPD esters	Crude and refined palm oil, refined sunflower oil, refined rapeseed oil, and EVOO	DART / He / Orbitrap-MS	3-MCPD esters isolation by chromatographic fractionation or SPE	3-MCPD esters analysis	[77]
Phenolic compounds	32 edible oils	DART / He / LTQ-Orbitrap-MS	LLE	Phenolic compound characterization in EVOO. Statistical classification according olive variety	[78]
FFAs	Hogwash and edible oils	DBDI / Ar / IT-MS	Not required	Statistical discrimination between hogwash and edible oils	[79]
FFAs, phenolic and volatile compounds	OO	LTP / He / IT-MS	Not required	Main component analysis for quality control in OO without sample treatment	[80]

Phenol, and acylglycerol compounds	EVOO, VOO and lampante OO	LTP / He / IT-MS	Not required for acylglycerol study. LLE for polar fraction enrichment	Mass spectra profiles of phenolic compounds and acylglycerols	[32]
FAs, vitamins, TAGs, steroids, phospholipids, and sphingolipids	Fish oil and butter	DAPPI / different solvents/ IT-MS	Not required	Lipidic analysis	[81]

List of abbreviations: 3-MCPD: 3-chloropropane-1,2-diol**; CNTF**: Carbon Nanotube Film**; DAG(s)**: Diacylglycerides; **DAPPI**: Desorption Atmospheric Pressure Photoionization; **DART**: Direct Analysis in Real Time; **DBD:** Dielectric Barrier Discharge; **DBDI**: Dielectric Barrier Discharge Ionization; **DESI:** Desorption Electrospray Ionization; **EASI**: Easy Ambient Sonic Spray Ionization; **EESI**: Extractive Electrospray Ionization; **EVOO**: Extra Virgin Olive Oil; **FA(s)**: Fatty Acid(s); **FFA(s)**: Free Fatty Acid(s); **HO**: Hazelnut Oil; **IT**: Ion Trap; **LLE**: Liquid-Liquid Extraction; **LOD(s):** Limit(s) of Detection; **LTP:** Low Temperature Plasma; **MeOH**: methanol; **OO(s)**: Olive Oil(s); **PS**: Paper Spray; **Q:** Quadrupole; **TAGs:** Triacylglycerides; **TLC**: Thin Layer Chromatography; **Q-TOF**: quadrupole time-of-flight; **TQ**: Triple-Quadrupole; **VO(s)**: Vegetable oil(s); **VOO**: Virgin Olive Oil.

Table 4. An overview of MALDI-MS methods applied to the characterization of edible oils [94-125].

Compounds	**Sample matrix**	**MALDI-MS / laser**	**Previous treatment / Matrix for MALDI**	**Purpose of the study**	**Reference**
TAGs	OO standard	MALDI-TOFMS / N_2 (337 nm)	Diluted oil / Four matrices: DHB, CHCA, dithranol, and $K_4Fe(CN)_6$/glycerol	Analysis of TAGs and whole oils	[94]
TAGs	EVOO	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / DHB	TAG profile of OOs from Israel Negev desert	[95]
TAGs	OOs	MALDI-TOFMS / N_2 (337 nm)	Fat dilution / DHB, using NaI as cationization agent	Comparative study of TAGs by HPLC and MALDI-TOF-MS from Tunisian crops	[96]
TAGs	OOs, sunflower oil, and sesame oil	MALDI-SpiralTOFMS / Nd-YLF (349 nm)	Diluted oils / DHB, using sodium trifluoroacetate as cationization agent	Screening of different VO types including OOs from different geographical areas	[97]
TAGs	EVOOs	MALDI-TOF/TOFMS / Nd:YAG (355 nm)	Diluted oils / DHB with sodium acetate by sandwich spotting method	TAG and DAG profile modifications during olive ripening	[98]
TAGs	OO	MALDI-SpiralTOFMS equipped with TOF/TOF option / Nd-YLF (349 nm)	Diluted oils / DHB and sodium trifluoroacetate as cationization agent	TAGs structural analysis	[99]
TAGs	Vegetable and seed oils	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / DHB	TAGs structural analysis combining HR-NMR and MALDI-TOFMS	[100]
TAGs	EVOO	MALDI-TOFMS / Nd:YAG (355 nm)	Diluted oils / DHB and sodium trifluoroactetate as cationization agent	EVOO adulteration detection by TAG profiles	[101]
Phospholipid profile	OO	MALDI-TOF/TOF-MS / Nd:YAG laser (355 nm)	Extraction by MSPD, using TiO_2 nanoparticles as sorbent / DHB by sandwich method	Development of MSPD procedure, using TiO_2 nanoparticles	[102]
Oil polar fraction	EVOO	MALDI-TOFMS / N_2 (337 nm)	µ-SPE / DHB prepared in acidic media	Adulteration studies of EVOO with HO	[103]
Phospholipids	EVOO	MALDI-TOFMS / N_2 (337 nm)	Modified Bligh-Dyer method / Ionic liquid composed by TBA and CHCA	Adulteration studies of EVOO with HO	[104]
Phospholipids	EVOO	MALDI-TOFMS / laser not reported	Modified Bligh-Dyer extraction method / Ionic liquid composed by TBA-CHCA	Adulteration studies of EVOO with corn oil	[105]

Oil proteins	EVOO, OO, and HO	MALDI-TOF/TOF-MS / laser not reported	LLE, gel electrophoresis, and “in gel” trypsin digestion / CHCA	Adulteration studies of EVOO with HO	[106]
Oil proteins	EVOOs, and HOs	MALDI-TOFMS / N_2 (337 nm)	Extraction by precipitation, and “in solution” trypsin digestion / CHCA	Test different protocols for protein oil extraction, and study of EVOO adulterations with HOs	[107]
FAs	Vegetable and seed oils	MALDI-TOFMS / N_2 (337 nm)	Saponification, and solution / *meso*-tetrakis(pentafluorophenyl) porphyrin	Determination of FAs composition in VOs	[108]
TAGs	Olive, canola, and castor oil	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / CHCA	TAG determination	[109]
TAGs	Olive, sunflower, safflower, walnut and linseed oils	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / DHB	Characterization of different oils	[110]
TAGs	EVOO	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / DHB with sodium acetate. Different spotting procedures tested	TAGs determination by MALDI-TOFMS minimizing compound fragmentation by using a nitrocellulose film	[111]
TAGs	VOs*	MALDI-TOFMS / N_2 (337 nm)	Diluted oils / DHB	Statistical discrimination between VOs	[112]
TAGs	VOs*, hydrogenated VOs, shortening, butter, and lard	MALDI-TOFMS and MALDI-FTICRMS / (laser not reported)	Sample dilution / DHB	Characterization of edible oils	[113]
TAGs	VOs*, lard, butter, and gutter oil	MALDI-TOFMS / UV (337 nm)	Not required / DHB	Rapid screening of mixed edible oils and gutter oils	[114]
TAGs	VOs*, and home-made lard	MALDI-FT-ICRMS / smartbeam laser (355 nm)	Sample dilution / DHB	Rapid characterization of TAGs in edible oils	[115]
Characteristic peaks and spectral features	VOs*, fish oil, butter, margarine, lard, and repeatedly cooked edible oil	MALDI-TOF/TOF-MS / smartbeam laser (355 nm)	Not required / DHB	Spectral database establishment for edible oils classification	[116]
TAGs and derivate compounds	OO, and linseed oil	MALDI-TOFMS / laser not reported	Diluted oil / DHB with trifluoroacetic acid	Study of thermal stressing in VOs	[117]
Profile of polar and non-polar fractions	Refined sunflower oil and EVOO	MALDI-TOFMS / N_2 (337 nm)	LC separation on silica column. Oils, and their fractions dilution / DHB with NaCl as cationization agent	Study of heated VOs	[118]

TAGs, DAGs, decomposition and combination compounds	Soybean oil fried with chicken breast meat	MALDI-TOFMS / N_2 (337 nm)	Diluted oil / DHB with trifluoroacetic acid	Analysis of compounds in deep-fat frying oil	[119]
TAGs	Castor oil	MALDI-TOFMS / N_2 (337 nm)	TAG fractions separation by LC / $Na_4[Fe(CN)_6]$	Castor oil characterization	[120]
TAGs	VOs	MALDI-TOFMS / N_2 (337 nm)	Dilution oil / $Na_4[Fe(CN)_6]$	Determination of volatile compounds and TAG composition from Indian plant oils	[121]
TAGs	Corn, colza, peanut, and soybean oils	MALDI-FT-ICRMS / Nd:YAG (355 nm)	Diluted oil / DHB	Quantitative determination of TAGs, using MALDI target plate precoated with 2B pencil graphite	[122]
TAGs	Pomegranate oil	MALDI-TOFMS / N_2 (337 nm)	Diluted oil / DHB	TAGs profile of pomegranate oil	[123]
TAG profile	Grape seed oils	MALDI-TOFMS / N_2 (337 nm)	Diluted oil / $Na_4[Fe(CN)_6]$	Analysis of TAG composition	[124]
TAGs	Poppy seed oils	MALDI-TOFMS / N_2 (337 nm)	Diluted oil / $Na_4[Fe(CN)_6]$	Adulteration detection of poppy seed oil with sunflower oil	[125]

List of abbreviations: CHCA: α-cyano-4-hydroxycinnamic acid; **DAGs**: Diacylglycerides; **DHB**: 2,5-dihydroxy benzoic acid; **EVOO:** Extra Virgin Olive Oil; **FAs**: Fatty Acids; **HO**: Hazelnut Oil; **HPLC**: High Performance Liquid Chromatography; **FT-ICR-MS:** Fourier Transform Ion Cyclotron Resonance Mass Spectrometry; **LLE**: Liquid-Liquid Extraction; **MALDI-TOFMS:** Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry; **MSPD**: Matrix Solid Phase Dispersion; **HR-NMR**: High Resolution Nuclear Magnetic Resonance; **OO**: Olive Oil; **SPE**: Solid Phase Extraction; **TAGs:** Triacylglicerides; **TBA**: Tributylamine; **VOs(*):** Vegetable oils, including olive oil; **VOO**: Virgin Olive Oil.

* Including OO

Table 5. Critical assessment of different approaches for direct mass spectrometry analysis of olive oil and other edible oils.

Compound class targeted	MS methods available	Strengths	Weaknesses
TAGs	MALDI ESI / APCI / APPI Ambient (ESI-based methods) Ambient (heat-assisted plasma-based methods)	High-throughput and straightforward data acquisition using MALDI, ESI/APCI and ambient MS methods TAGs are stable compounds over the shelf-life of the oil sample Specific information of individual TAGs isomers possible if appropriate MS/MS experiments are planned and/or high-resolution MS(/MS) is used High sensitivity in most ionization methods used, allowing high dilution factors without compromising sample information acquired Ambient MS methods allow high-throughput data acquisition with minor sample preparation allowing successful characterization and classification of different olive oil samples Possibility to use disposable substrates (*eg*. paperspray and other ambient MS methods) that avoids the introduction of oil matrix in the MS instrument and possible carryover effects. Only an aliquot of the sample is actually inserted in the MS instrument.	Difficult to distinguish between isomers (lost information unless dedicated MS/MS data are acquired) Quantitation issues with MALDI and ambient MS methods (matrix effects) TAGs are not efficiently ionized in ESI, so adduct ion formation (ammonium and lithium adduct ions) is often used to improve detectability Carryover effects on direct bulk analysis of untreated (diluted) samples
Phenolics	ESI Ambient (ESI-based methods, *eg*. DESI and PS) Ambient (plasma-based methods)	Despite the intrinsic instability of phenolic compounds as antioxidants, different reports have shown the usefulness of phenolic profiles for authentication purposes, particularly for geographical and botanical origin assessment ESI-based methods in the negative ionization mode generated relatively simple mass spectra	Phenolic compounds degrade easily so they are not stable over the shelf life of the oil Need some sample preparation (eg. liquid partitioning with water/methanol)

		Relatively high concentrations in VOO despite it is a minor compound class Rapid screening methods have been proposed to prove the “freshness” of VOOs with minor sample workup [71]	Secoiridoid isomers with m/z 377 and 361 difficult to distinguish without chromatographic separation Concentrations are not very high depending on the sample type making dilutions less convenient Unavailability of standard makes MS-based quantitation challenging
Fatty acids	ESI/APCI/APPI	The concentration of free fatty acids is relatively high and large dilutions are possible without diminishing the gathered molecular information for characterization purposes ESI-based methods in the negative ionization mode generated relatively simple mass spectra	Carryover effects on direct bulk analysis of untreated (diluted) samples, unless disposable MS devices/substrates are used
Volatile (aromas)	HS-MS (electron impact ionization) PTR-MS and SIFT-MS (Chemical ionization)	Volatile fraction of VOOs provides extremely useful information that can be correlated with sensory analysis (Panel Test) to speed up the processing of classification of VOO samples according to sensory features (positive attributes and defects) Several examples on the literature with success on the authentication of VOO samples from different geographical and/or botanical origin based on the use of direct MS methods testing volatiles. Headspace sampling reduces dramatically the impact of the oil matrix on the actual MS measurements. No carryover effects are expected so that large runs of samples can be acquired without major instrument maintenance operations. The use of soft ionization (PTR-MS or SIFT-MS) produces simpler mass spectra (than EI) providing more useful datasets with less interfered m/z values and less redundant signal, making data processing more effective and enabling better classification of samples	Additional (headspace) equilibrium/incubation step (*ca.* 30-40 min) required per analysis Volatile compounds are not stable over the shelf life of the oil

Sterols	APCI/APPI Ambient MS (plasma-based)	Sterol fraction of VOOs and other edible oils is abundant enough and stable over the shelf life of the product so that can be used for authentication purposes. Different examples in the literature have shown the usefulness of sterols for authentication purposes and for the detection of adulteration APCI/APPI as well as ambient plasma-based ionization methods are capable of efficiently ionizing sterol (better than ESI) Selected ambient MS methods may allow direct detection of sterol fraction from TLC plates so that many sample treatment operations (collection of the TLC spot, dilution or derivatization) may be skipped	Dedicated workup for specific isolation of sterol fraction from edible oils (saponification, TLC or column chromatography fractionation). The structures of sterols as well as their mass spectra features are similar so dedicated MS/MS experiments are needed for individual characterization
Tocopherols	ESI/APCI/APPI Ambient MS (plasma-based and paperspray)	Rapid screening methods have been proposed to prove the "freshness" of VOOs [72] with minor sample workup. The potential of tocopherol fraction for authentication purposes of different edible oils have been also envisaged, although these compounds are not stable (highly sensitive to light). MS/MS spectra and high-resolution MS allow the characterization of most individual tocopherols without separation	Tocopherols are not stable over the shelf life of the oil