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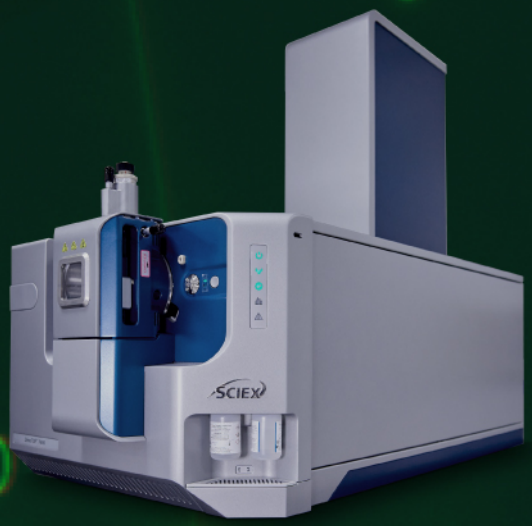

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REVIEW ARTICLE

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Bio-phenols determination in olive oils: Recent mass spectrometry approaches

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Abstract

Extra virgin olive oil (EVOO) is largely used in Mediterranean diet, and it is also worldwide apprised not only for its organoleptic properties but also for its healthy effects mainly attributed to the presence of several naturally occurring phenolic and polyphenolic compounds (bio-phenols). These compounds are characterized by the presence of multiple phenolic groups in more or less complex structures. Their content is fundamental in defining the healthy qualities of EVOO and consequently the analytical methods for their characterization and quantification are of current interest. Traditionally their determination has been conducted using a colorimetric assay based on the reaction of Folin-Ciocalteu (FC) reagent with the functional hydroxy groups of phenolic compounds. Identification and quantification of the bio-phenols in olive oils requires certainly more performing analytical methods. Chromatographic separation is now commonly achieved by HPLC, coupled with spectrometric devices as UV, FID, and MS. This last approach constitutes an actual cutting-edge application for bio-phenol determination in complex matrices as olive oils, mostly on the light of the development of mass analyzers and the achievement of high resolution and accurate mass measurement in more affordable instrument configurations. After a short survey of some rugged techniques used for bio-phenols determination, in this review have been described the most recent mass spectrometry-based methods, adopted for the analysis of the bio-phenols in EVOOs. In particular, the sample handling and the results of HPLC coupled with low- and high-resolution MS and MS/MS

Abbreviations: 3,4-DHPEA-EA, oleuropein aglycon; AMS, ambient mass spectrometry; DAD, photodiode array detectors; DAPPI, desorption atmospheric pressure photoionization; DART, direct analysis in real time; D-LFSESI, desolvating low-flow secondary electrospray ionization; DESI, desorption electrospray; DMA, differential mobility analysis; EASI, easy ambient sonic-spray ionization; EFSA, European Food Safety Authority; EI-MS, electron ionization mass spectrometer; ESI, electrospray ionization source; EVO, virgin olive oil; EVOO, extra virgin olive oil; FC, Folin-Ciocalteu; FID, flame ionization detector; FID, flame ionization detector; FT-OT, Fourier transform orbital traps; GC/MS, gas chromatography mass spectrometry; GC/MS/MS, gas chromatography tandem mass spectrometry; HILIC, hydrophilic interaction liquid chromatography; HRMS, high-resolution mass spectrometry; HRMS/MS, high-resolution tandem mass spectrometry; HTYR, hydroxytyrosol (3,4-dihydroxyphenylethanol); IMMS, ion mobility mass spectrometry; LC-MS, liquid chromatography mass spectrometry; MRM, multiple reaction monitoring; NMR, nuclear magnetic resonance; p-HPEA-EA, ligstroside aglycon; PS, paper spray; PS-MS/MS, PS tandem mass spectrometry; QQQ, triple quadrupole; SIM, selective ion monitoring; TAGs, triacylglycerols; TOF, time of flight; TYR, tyrosol (*p*-hydroxyphenylethanol); UV, ultraviolet.

analyzers, of ion mobility mass spectrometry and ambient mass spectrometry have been reported and discussed.

KEYWORDS
bio-phenols, LC-MS, mass spectrometry, olive oil, polyphenols

1 | INTRODUCTION

Extra virgin olive oils (EVOOs) are much appreciated products of the Mediterranean diet as their consumption has been associated with healthy nutritional effects (Di Nunzio et al., 2018; Psaltopoulou et al., 2011). Most of these effects have been attributed to their content in phenolic compounds that also contribute to the organoleptic aspects of EVOOs as their bitterness and their pungency (Andrewes et al., 2003; Beauchamp et al., 2005; Di Nunzio et al., 2018, Pedan et al., 2019). These substances are responsible of the extraordinary chemical stability of EVOOs (Ben Mansour et al., 2017; Tura et al., 2007) through a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelation (El Riachy et al., 2011; Presti et al., 2017).

Bio-phenols are part of the so called "nutraceutical components" (Piccolella & Pacifico, 2015). The word nutraceutical is a combination of nutrition with pharmaceutical terms, and it defines a food (or part of it) that provides health benefits (DeFelice, 1995). These bioactive compounds are naturally occurring and could be extracted from different food sources. In the nutraceutical scenario phenolic compounds and bio-phenols represent, among the most important antioxidant natural substances for olive oils (Pacifico et al., 2019; Piccolella & Pacifico, 2015). For this reason, the European Food Safety Authority (EFSA) has allowed the acknowledgment of health claims based on olive oil bio-phenols content (Agostoni et al., 2011). The list of health claims, the conditions and the restrictions of their use are reported in EU Regulation (European Commission Regulation EC No. 432/2012).

Authorized health claim, listed in Regulation 432/2012, refers to the level of phenolic compounds in olive oil and their impact on the protection of blood lipids from oxidative stress.

The health claim can be used only for "olive oil containing at least 5 mg of Hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil. To bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil" (Caprioli et al., 2019; European Commission Regulation EC No. 432/2012).

These conditions induced a growing interest of the producers, consumers, and control organisms, to accurately determine the content of bio-phenols in EVOOs.

A vast literature recently reports on the healthy properties of EVOOs. An inverse association between olive oil intake and the insurgence of some diseases as cancer or cardiovascular pathologies have been evidenced by several epidemiological surveys (Di Nunzio et al., 2018; Emma et al., 2021). Other studies correlated EVOO consumption with the reduction of the age-related occurrence of chronic inflammatory disorders and inflammatory bowel diseases (Buckland & Gonzalez, 2015; Cougnard-Grégoire et al., 2016; Guasch-Ferré et al., 2014; Psaltopoulou et al., 2011; Schwingshackl et al., 2015). Further EVOO bio-phenols have been associated to the modulation of the expression of atherosclerosis-related genes (Celano et al., 2018;). In preclinical studies, EVOOs showed also anti-inflammatory activity linked to the inhibition of COX enzymes (Schwingshack et al., 2015).

Finally, the phenolic fraction of olive oils exhibited chemopreventive effects against several kinds of cancer (Acquaviva et al., 2012; Pampaloni et al., 2014; Reboredo-Rodríguez et al., 2018), while secoiridoids addition to cancer therapy (in combination with other chemotherapeutic drugs) determined synergistic effects in reducing the proliferation of tumor cells (Emma et al., 2021). Numerous biological effects against breast cancer have also been recently attributed to oleocanthal (Elnagar et al., 2011; Emma et al., 2021). This molecule has been evaluated as potential therapeutic option in the treatments of hormone-dependent breast cancer (Ayoub et al., 2017), and reported to attenuate cell proliferation and tumor growth in several cancer models (Akl et al., 2014).

All these healthy properties justified the interest to preserve at most the phenolic content in EVOOs, but bio-phenols and secoiridoids are relatively labile molecules, and their content in EVOOs is strongly conditioned by the production workflow (Amirante et al., 2008a, 2008b, 2010a, 2010b; Bellumori et al., 2019; Clodoveo et al., 2017; Di Stefano & Melilli, 2020). To date, the best practices to obtain quality EVOOs are individuated in the early harvesting of the drupes, product prompt working, process water reduction and in the avoidance to heat the crushed olives pasta. Following these good practices also positively influences other parameters (peroxide value, acidity, K232 and K270 indexes) required for the

classification of olive oils as EVOO and Virgin Olive Oils (VOO) (Bellumori et al., 2019). This classification has been conceived in 1991 but is insufficient to describe the qualitative differences of the EVOOs in the market. Therefore, a complete and accurate determination of the bio-phenols, could be a useful tool to evaluate the best products, evidencing both the "healthiest" and the "highest quality" EVOOs (Roselli et al., 2017). To date, however, the olive-oil industry has not exploited such opportunity, being still unclear the most appropriate bioactive compounds to determine, to achieve this goal. It is also lacking a consensus on the analytical protocols to apply (Celano et al., 2018).

Taking into account the growing interest in the determination of such substances and the actual importance of the topic, in this review we pursue the aim to collect the most recent mass spectrometry based analytical determinations of bio-phenols in EVOOs. In particular were evaluated the sample treatment, methods sensitivity and selectivity required to overcome the actual limits of the International Olive Council (IOC) method. For this reason it was encompassed the most recent literature reporting mainly papers published from 2015 up to June 2021, citing also some older works when some peculiarities or a relevant approach, was relieved. Notably, just some older articles in literature were

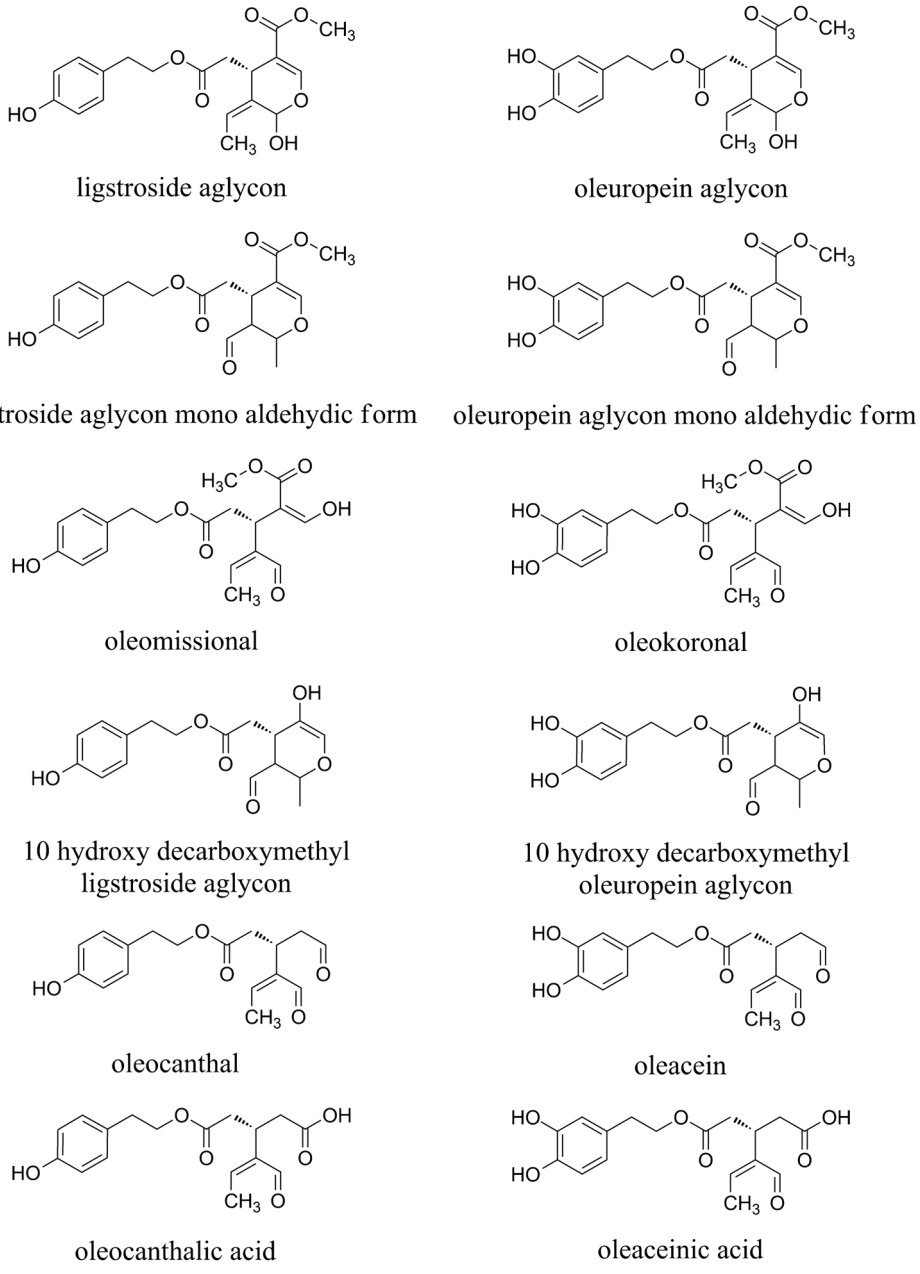


FIGURE 1 Chemical structures of secoiridoids

making use of APCI source for the determination of the bio-phenols content in EVOO (Caruso et al., 2000; McDonald et al., 2001), whereas the most recent works took advantage of the ESI source, preferably in negative ion mode, or of more exotic real time/ambient ionization sources.

1.1 | EVOO bio-phenols classification

The large variety of bio-phenols found in EVOO is different in chemical structures and concentrations (0.02–600 mg/kg) depending on several factors including variety, geographic origin, agricultural techniques, maturity of the olive fruit at harvest, and processing.

The most important bio-phenol classes in olive oils are represented by phenolic alcohols, secoiridoid derivatives, flavonoids, lignans, and phenylpropanoids. (Belščak-Cvitanovic et al., 2018; Celano et al., 2018; Di Nunzio et al., 2018; Keys et al., 1995; Grosso et al., 2014; Larrauri et al., 2016; López-Fernández et al., 2020; Ouni et al., 2011; Servili et al., 2009).

Hydroxytyrosol (3,4-dihydroxyphenylethanol: HTYR) and tyrosol (*p*-hydroxyphenylethanol: TYR) concentrations are usually low in fresh oils but increase during oil storage due to the hydrolysis of secoiridoids.

1.1.1 | Secoiridoids in olive oils

Secoiridoids (Figure 1) are a group of compounds that are usually glycosidically bound, produced from the secondary metabolism of terpenes. The secoiridoids, found exclusively in family of Oleaceae that includes *Olea europaea* L., are compounds characterized by the presence of elenolic acid in its glycosidic or aglyconic form. Oleuropein and ligstroside are the main secoiridoids in olive fruits (Marković et al., 2019; Pérez et al., 2005; Servili et al., 2009).

During crushing and malaxation for the production of olive oil, oleuropein and ligstroside (Figure 2) come into contact with endogenous β-glucosidases and are transformed to the corresponding oleuropein and ligstroside aglycons (3,4-DHPEA-EA and p-HPEA-EA, respectively).

Really oleuropein aglycon and ligstroside aglycones correspond to many isomers, due to the occurrence of keto-enol tautomerism, that in many cases are not well described (Scheme 1). These two aglyconic forms (p-HPEA-EA and 3,4-DHPEA-EA) are particularly unstable and have only been observed under very specific conditions (Christophoridou & Dais, 2009). Within the olive oil matrix or generally in a nonaqueous medium, the aglycones do not exist and are transformed mainly into several closed and stable monoaldehydic forms (Limiroli et al., 1995) or alternatively into the open ring dialdehydic forms.

Dialdehydic forms derived from oleuropein aglycon and ligstroside aglycon were called oleocanthal or p-HPEA-EDA, and oleacein or 3,4-DHPEA-EDA, respectively (Karkoula et al., 2012, 2014; Servili et al., 2009) (Figure 1). The closed ring forms were also described in different forms of steroisomers (Perez-Trujillo et al., 2010).

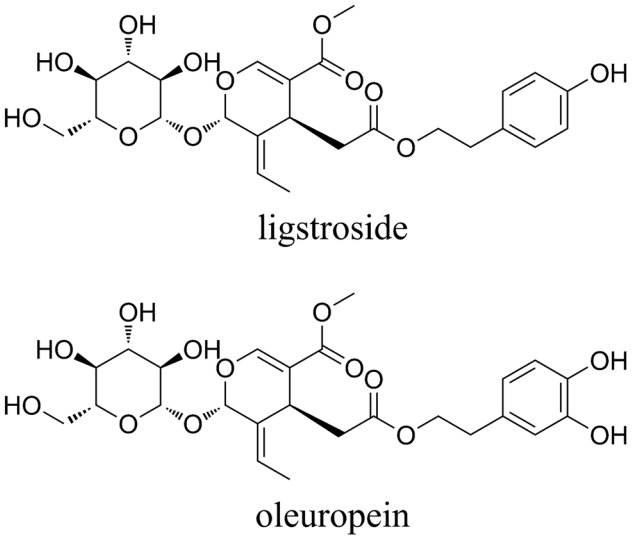
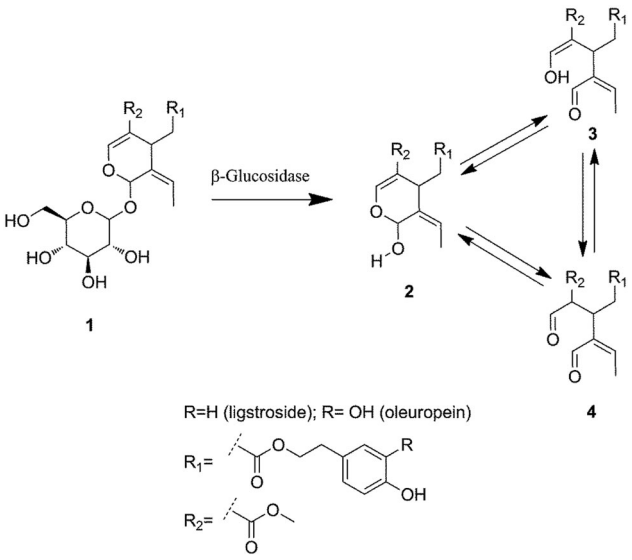


FIGURE 2 Chemical structures of ligstroside and oleuropein



SCHEME 1 Simple isoforms of oleuropein and ligstroside (1); oleuropein or ligstroside aglycons hemiacetal forms (2), oleuropein or ligstroside aglycons in mono aldehydic forms (3), oleuropein or ligstroside aglycons in di aldehydic forms (4)

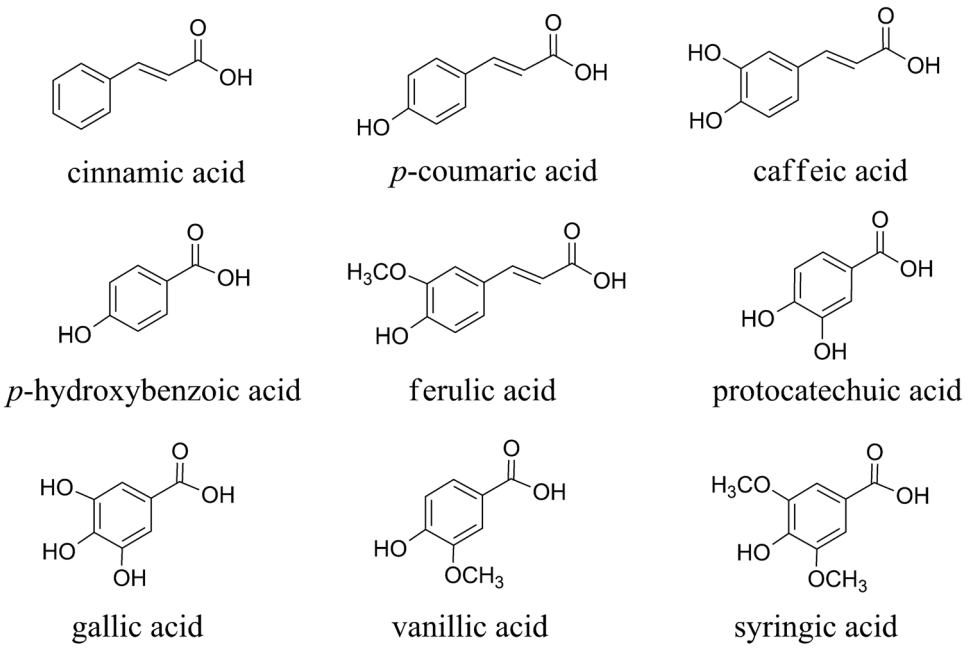


FIGURE 3 Chemical structures of phenolic acids

EVOO can also contain other derivatives of oleuropein and ligstroside aglycon, named oleomissional and oleokoronal, whose structures have recently been completely elucidated by NMR by Diamantakos et al. (2015). Oxidation products of oleocanthal and oleacein were found in fresh oils in very low concentrations. During the storage time, levels of oleaceinic acid and oleocanthalic acid increase, while the oleacein and oleocanthal concentrations are reduced (Di Stefano & Melilli, 2020).

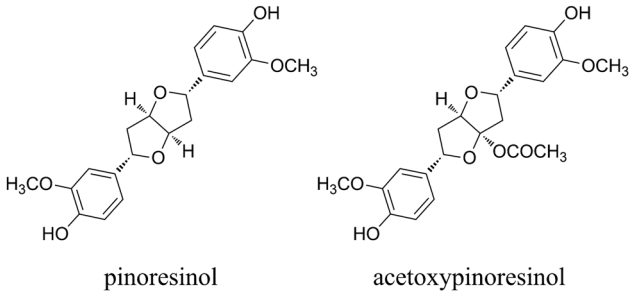


FIGURE 4 Chemical structures of lignans

1.1.2 | Others biophenols in olive oils

Phenolic acids can be divided into two groups: those derived from hydroxybenzoic and those derived from hydroxycinnamic acids. The first group includes several compounds that have been found in very low concentration in EVOOs: *p*-hydroxybenzoic, gallic, protocatechuic, syringic, and vanillic acids. The second group includes *p*-hydroxycinnamic, *p*-coumaric, caffeic, and ferulic acids (Figure 3).

Other phenolic compounds that have been identified in olive oils are lignans (Figure 4) such as pinoresinol and 1-acetoxypinoresinol. These compounds, very abundant in EVOOs, possess biological and pharmacological properties (Brenes et al., 2000) other than their antioxidant properties (Charlton et al., 1998), indeed, for lignans have been reported similarities in structure with estradiol or tamoxifen suggesting possible activity also against breast cancer (Welshons et al., 1987).

Flavonoids are largely planar molecules with a common structure, based on 2-phenyl-benzogamma-pyrane, derived from fruits, vegetables, and other plant components. Luteolin, diosmetin, and apigenin (Ben Brahim et al., 2017; Olmo-García et al., 2019) were found in EVOO (Figure 5) as a minor fraction (total content below 5%). They play an important role on health. The long-term consumption of foods rich in flavonoids offer health benefits, that is, neuroinflammation decrease and attenuation of oxidative stress, once their play a pivotal role on pathways that are responsible for neuronal proliferation and survival, thus attenuating the Alzheimer's associated symptoms (Bakoyiannis et al., 2019).

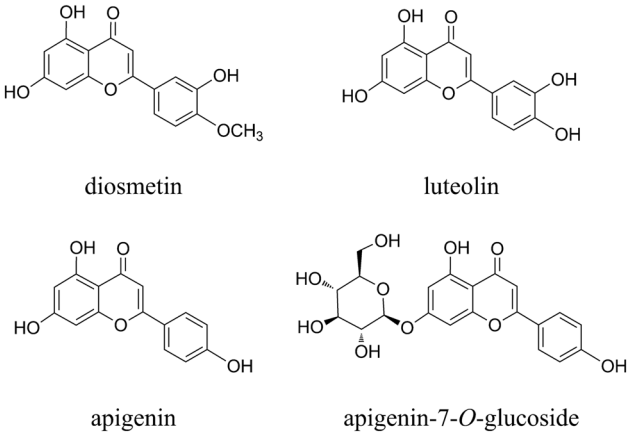


FIGURE 5 Chemical structures of flavonoids

1.2 | Earlier analytical approaches

Considered the importance of bio-phenols for defining healthy and organoleptic characteristic of EVOOs, since 1972 (Montedoro, 1972) a large number of quali-quantitative analytical methods have been proposed for their determination. Many of these methods are based on the absorption capacity in the visible or UV zone of bio-phenols and some older assays, still in use, tend toward a coarse quantification of the phenolic components through a colorimetric approach. A widely used quantitative determination of total phenols in VOO, is based on the reaction of the Folin-Ciocalteu reagent. This reacts with the hydroxy groups of phenolic compounds generating a blue chromophore (constituted by a phosphotungstic-phosphomolybdenum complex) with a maximum absorbance at 700–750 nm. The absorbance, measured at 700 nm, is proportional to the total amount of phenolic groups in the samples and is easily quantified by means of UV/VIS colorimeters. This assay is very fast, simple, applicable in routine and low-cost (Blekas et al., 2002), but its major disadvantage is the low specificity.

Other more sophisticated approaches have, over time (Carrasco-Pancorbo et al., 2005), tried to evaluate and quantify the individual bio-phenol components. In particular the increasing need for the profiling and identification of individual phenolic compounds led to the replacement of the unspecific colorimetric methods by more specific chromatographic determinations. Paper chromatography had a limited application for the separation of phenolic compounds of VOO (Vazquez-Roncero et al., 1974a, 1974b). However, using this approach up 20 phenolic compounds were separated (Ragazzi & Veronese, 1973).

The qualitative and quantitative determinations of the phenolic compounds present in EVOOs has also been accomplished by capillary gas chromatography (GC) of the unmodified molecules or, more often, of some their derivatives (Forcadell et al., 1987; Janer del Valle & Vazquez-Roncero, 1980; Solinas & Cichelli, 1982). The GC systems are historically coupled to flame ionization detector (FID) or to Electron Ionization Mass Spectrometer (EI-MS). This latter approach allows to achieve chromatographic separation and the EI-MS spectrum of each component, showing the m/z ratio of the ionized molecules and the fragment ions generated during this hard ionization process. GC/MS and the more sophisticated GC/MS/MS systems lead to an improvement in the identification of phenolic compounds (Angerosa et al., 1996; Saitta et al., 2002). The GC analysis requires a sample derivatization step to overcome the limited volatility of most phenols. This is a critical step, as it not always leads to a complete transformation of the native substances in their corresponding derivatives, causing the elution of multiple peaks arising from the same parent compound. Further, the necessity to volatilize the analytes could lead to their partial or total thermal degradation. For these reasons, during the years, alternative chromatographic techniques as HPLC have been developed and exploited. The first experiment to isolate and separate bio-phenols adopted normal chromatography (Graciani-Costante & Vazquez-Roncero, 1980). Far better results in terms of reproducibility of retention time and separation of the most polar compounds were obtained by reversed phase chromatography (Cortesi et al., 1981; Graciani-Costante & Vazquez-Roncero, 1980). The detection of phenolic compound was left to the UV detectors, set to measure the absorption at 225, 240, or 280 nm. Later, the availability of photodiode array detectors (DAD), capable to simultaneously detect the absorbance at various wavelengths, allowed to more properly follow the UV absorbance of some phenolic compounds that show several absorption maxima, leading to an easier identification (Esti et al., 1998; Montedoro et al., 1992; Pirisi et al., 1997).

1.3 | Limits of current low-cost polyphenol determination

The reduction of the analysis costs is always one of the key factors in the choice of an analytical method. The cost–benefit ratio represents the guideline for the definition of the instrumental approach. Therefore, for bio-phenols determination, the official method of the IOC relies on instruments that could be accessible to most of analysis laboratories, including corporate ones. The separation of bio-phenolic components is obtained by coupling HPLC with UV detection at 280 nm. Sample

preparations require an extraction of bio-phenols from olive oil obtained by means of a methanol solution using syringic acid as the internal standard (COI/T.20/Doc. No 29/Rev.1 2017).

It is evident that this last approach is far from defining, in an accurate and complete way, the composition of the bio-phenolic fraction. Rather, it returns values that are expressed in terms of tyrosol, used as external standard, to quantify all the bio-phenols.

In effect, the considerable structural complexity of some bio-phenols and the high number of derivatives implied a substantial lack of some commercial standards (mostly for secoiridoids), that are essential for an accurate qualitative and quantitative determination of these compounds. Therefore, are still less common literature examples in which the quantitation essays have been performed using isotopically labeled or pure reference compounds (De Nino et al., 2005; Di Donna et al., 2011; Mazzotti et al., 2012; Olmo-García et al., 2019). On the other hand, is widespread in the laboratory practice, the use of obsolete and inaccurate determinations such as the Folin-Ciocalteau assay, that often tend to large under estimations (about twofold) of the amount of bio-phenols (Ricciutelli et al., 2017).

Semiquantitative determination of secoiridoids is far more common and can be performed by comparing the peak areas for each metabolite to the area of a structurally similar compound. The accuracy of these data, however, relies on the assumption that the response factor of each derivative is comparable with the response factor of the reference compound that is far to be true (Boussahel et al., 2020). In fact, even very refined and specific separation techniques can lead to remarkable inaccuracies in the quantitative determination in the absence of pure standards (Luque-Muñoz et al., 2019; Olmo-García et al., 2019).

Finally, matrix effects may influence the analytical response, leading to either suppression or enhancement phenomena of analytical signals (Arena et al., 2020; Luque-Muñoz et al., 2019), even leading to an impairment of linearity, accuracy, and repeatability.

If all these factors are combined with the chemical instability of these antioxidants, which tend to degrade and change their concentration with the aging of the oil, it is clear that the determination of bio-phenols in EVOOs remains a still a challenging topic.

2 | RECENT TRENDS IN ANALYTICAL TECHNIQUES

As aforesaid the bio-phenols class is quite ample and they are important, even if quantitatively minor components, of olive oils. It should also be considered that bio-phenols are present in several forms as aglycons and easily undergo to several equilibria also during the chromatographic runs. The existence of these equilibria could lead to modifications of the original structure, increasing the complexity of these active substances' mixtures. The will to resolve this complexity requires a state of the art instrumentation, constituted by the coupling of HPLC or UPLC, even adopting comprehensive bidimensional chromatography, with soft ionization and MS detection (Agozzino et al., 2010; Indelicato et al., 2017; Trombetta et al., 2017). This approach is capable to separate the constituents of the olive oils based on elution time(s) and mass/charge ratio. The resulting, very informative, LC-MS traces theoretically, should lead to an independent quantification of each single component. However, the presence of several isomers and of their derivatives complicate the overall bio-phenols pattern, sometimes leading to partially resolved chromatographic peaks and co-elution of substances with identical nominal or accurate mass. This aspect, together with the lack of a large basis of pure secoiridoids standards, still make complicate the correct identification and quantitation of the important constituents. It is possible to overcome some of these limitations by adopting multiple stage analyzer instruments (for MS/MS experiments) or adopting instrumentation equipped with at least one high-resolution sector. MS/MS approaches exploit the selectivity of multiple stage instrumentation to evidence structure-specific fragmentation pathways, using three differing approaches: multiple reaction monitoring (MRM), precursor ion scan and neutral loss. The first is the most widely adopted, as it guarantees the highest sensitivity, retaining a very good selectivity.

High-resolution sectors, more recently adopted for the determination of bio-phenols in EVOOs, are based on only two technological approaches, time of flight (TOF) and Fourier transform orbital traps (FT-OT) that lead to quite differing resolution powers (DeHoffman & Stroobant, 2007). In the modern TOF instruments the presence of reflection grid (reflector) allows the refocussing of the ions traveling in the flight tube and bearing the same mass, leading to a resolution of up to 50,000 full width at half maximum (FWHM). In the FT-OT the ions are instead trapped in spindle-shaped electrostatic field. By applying the Fourier transform to the complex waveform of the image current, generated on the surface of the outer electrode, the ion masses can be accurately determined. The actual instrumentation based on FT-OT reached a resolution of 1,000,000 FWHM.

2.1 | EVOOs sample handling for exhaustive bio-phenols extraction

Extraction of phenolic compounds from oil is generally a prerequisite for any in-depth analysis scheme. The extraction steps are generally performed to obtain a sample extract enriched in phenolic compounds with a reduced content of interfering substances.

A large number of procedures for the preparation of the bio-phenolic fraction of EVOO use two basic extraction techniques, liquid-liquid extraction (LLE) or solid phase extraction (SPE). Several authors have proposed various enrichment systems that differ not only in the solvent and/or solid phase cartridges, but also in the amount of sample taken for the analysis, solvent volumes, and further details.

In the most common approach adopted, the polar fraction of olive oil is extracted from the oil by means of a methanol/water mixture (with different water content: ranging from 0% up to 40%) (Cortesi & Fedeli, 1983; Montedoro et al., 1992; Solinas, 1987; Tsimidou et al., 1996). Montedoro et al. (1992) examined many of the extractive methods for phenolic compounds in EVOO, comparing various solvents mixtures, with different volumes and mixing percentages. Accordingly, the best results could be obtained using two 20 ml aliquots of methanol/water (80:20, v/v) to extract a sample of 100 g of olive oil (Montedoro & Cantarelli, 1969; Vazquez Roncero et al., 1978). However, the results reported by Angerosa et al. (1995) are in contrast with those that suggest the use of methanol/water mixtures. Indeed, they observed an incomplete recovery of some components and the formation of an emulsion between the oil and the methanol/water layer. These findings led them to choose pure methanol as the extraction solvent.

Cortesi et al. (1995) proposed a further extraction method to recover the polar fraction of olive oil using a tetrahydrofuran/water (80:20 v/v) mixture. This approach allowed higher recoveries in terms of HTYR and TYR with respect to methanol/water (60:40 v/v) solvent mixtures.

Recently an innovative method through direct introduction and LC-IT-MS analysis has been proposed by Olmo-García et al. (2019). In this study a procedure that involves the simple dilution of 1 g of olive oil with 5 ml of acetone was developed, followed by filtration through nylon syringe filter and injection into the instrument (LC-MS). The principal advantages of this method are the recovery of all phenolic compounds without the extraction, the avoidance of artificial isomers creation and the elimination of partially oxidated bio-phenols. The method has been fully validated and proved to be very reliable for quantitation purposes.

Kıvrak and Kıvrak (2020) reported an ultrasonic-assisted extraction of phenolic compounds from EVOO samples, taking advantage of the immiscible solvent mixture: acetonitrile and n-hexane. The samples are vortexed for 2 min and then extracted in an ultrasonic bath. It follows a centrifugation step, which eases the two-layer separation. The acetonitrile extracts are then washed with petroleum ether, evaporated to dryness and the residue dissolved in water: methanol mixture (60:40, v/v), before the final introduction in the HPLC-MS system.

2.2 | MS based approaches for bio-phenol determination and the comparison with UV detection

Looking at the sheer EFSA numbers, the sensitivity for the determination of bio-phenols in oils should not be a problem. However, it should be also considered the presence of many bio-phenol isomers, that inevitably leads to a distribution of these amounts on a very large number of molecules, each one with a far lower concentration level. This implies that some sensitivity problems can also be observed with traditionally sensitive techniques such as LC-MS. Among bio-phenols the class that most suffer for sensitivity problems is that of the anthocyanins, which represents a minor fraction often neglected due to an insufficient instrumental sensitivity.

As with other MS-based analytical approaches, even for the determination of bio-phenols, the use of multi-sector ion beam instruments (other conditions being equal) guarantees the best sensitivity. In fact, considering TYR and HTYR as an example, the sensitivity levels (expressed as LOD) are in the order of few ppm for the typical analyses carried out through single sector devices (Monasterio et al., 2017; Olmo-García et al., 2017; Pedan et al., 2019). LOD values are instead one or two orders of magnitude lower (Bartella et al., 2019, 2020) using multiple sector MS/MS instruments. Recent high-resolution equipment comes very close to these letter levels of sensitivity (Arena et al., 2020; Bajoub et al., 2020; Dini et al., 2020; Kanakis et al., 2013). In both approaches (MS/MS or HRMS), the increase in sensitivity is achieved by reducing the background noise due to interfering ions passing through the analyzer that are finally perceived by the detector. In one case the fragmentation process, in the collision cell, leads to specific fragments formation. This allows a greater selectivity and therefore a better isolation capacity of the analyte, based on the intensity the analytical signal of these specific fragments.

In the case of HRMS analysis (Xian et al., 2012), the noise reduction occurs by drastically shrinking the

analyzed mass interval, leading to the definition of a very narrow mass range (in the order of a few ppm) with respect to the exact mass of the analyte, excluding a series of interferers of similar but not identical mass.

In both cases, the coupling of a cutting-edge separative system such as UPLCs equipped with high-efficiency chromatographic columns also leads to an improvement in sensitivity. In fact, high-resolution chromatographic systems, if properly exploited, lead to a reduction in the width of the chromatographic peaks, to a corresponding increase in their height, improving the S/N ratio.

Still excellent levels of sensitivity, comparable to the best approaches based on MS/MS or HRMS, can also be obtained using much less expensive DAD type detectors. The tradeoff, however, require to give up on the selectivity and information level that could be obtained from a mass spectrometry apparatus. Obviously, opportunely combining a mass spectrometer, cascaded with a DAD detector, both HPLC (DAD and MS) traces can be obtained, and in the end, the analytically less complex and/or more sensitive signal can be chosen for quantitative analysis. This, moderately more expensive, approach, is quite common in the case of bio-phenol determinations in olive oil (Bellumori et al., 2019; Caprioli et al., 2019; Ricciutelli et al., 2017) or also in other matrices.

Based on these aspects in the literature we can find two categories of applications.

Some authors are mainly focused on the characterization of the olive oil matrix and exploit the selectivity of mass spectrometry to obtain as much information as possible on the type and amount of bio-phenols (Ammar et al., 2017; Bajoub et al., 2015b, 2016b; Ben Brahim et al., 2017; Kıvrak & Kıvrak, 2020).

Other authors try to relate and overcome the UV determination typical of the IOC method with a more accurate or faster mass spectrometry-based approach. The goal in some cases is to correlate the overall or individual quantities of bio-phenols with the results of less expensive methods such as LC-DAD or even the determination of Folin-Ciocalteau (Olmo-García et al., 2019). In some papers, an accurate evaluation of the matrix effect on quantitative determinations of bio-phenols (Arena et al., 2020) is performed. In other ones, the aim is to overcome the current official methods to achieve a faster execution of the analyses (Bartella et al., 2020) or a greater reliability for bio-phenols and their metabolites recognition (Kelebek et al., 2017; Kotsiou & Tasioula-Margari, 2016; Sánchez de Medina et al., 2017).

2.3 | Low-resolution MS and MS/MS applications

Phenols are acidic substances, and therefore is not surprising that most of the works, carried out in mass spectrometry, take advantage of the ESI ionization in negative ion mode. In this case the loss of a proton during the ionization process generates the negatively charged ions. Furthermore, the presence in their molecules of one or more electronegative atoms, as oxygen, increase the response factor and consequently the sensitivity operating in negative ion mode.

For ESI negative ion mode is often required to reduce voltages to avoid the electron emission from the tip and the induction of discharges (Wampler et al., 1993), thus reducing ionization efficiency. On the other hand, ESI negative ionization is reported to reduce matrix effect in several cases (Indelicato et al., 2013, Oldekop et al., 2017). As a matter of fact, the large majority of the latest mass spectrometry related publications on bio-phenols have been conducted in ESI negative ion mode.

Different determination techniques, based on several quantitative approaches with external calibrations or, more rarely, with the use of internal standards, and different mass analyzers such as ion trap and triple quadrupole (QQQ), have been developed.

The proper chromatographic separation of bio-phenols is mandatory to obtain reliable quantitative data. Ricciutelli et al. (2017) evaluated several reversed phase columns to determine the best separation that was achieved using a Synergy Polar (Phenomenex) reversed phase column adopting water and methanol/isopropanol (9:1) as mobile phases. In this paper mass spectrometry adoption is limited to bio-phenols identification obtained from the ESI (negative ion mode) MS spectra, using an Ion Trap instrument. On the other hand, an interesting comparison with IOC and FC method has been conducted to determine relative responses in terms of total bio-phenols and in terms of HTYR concentration. It was found a good correlation between quantitative data arisen from this HPLC-DAD-MS approach and the IOC method. On the contrary the FC assay was found to underestimate the concentration in all samples analyzed by a factor of 2.6 or 2.3 with respect to IOC and Ricciutelli approach, respectively.

Olmo-García et al. (2019) compared four different methods to determine the total bio-phenol content in the EVOOs polar fraction. The terms of comparison were the IOC method, the FC assay and the hydrolysis approach followed by HPLC-DAD determination. It has been shown that the total phenolic content is higher

(two-threefold expressing data in mg/kg) if the quantitation is performed measuring and summing up the individual phenol's concentration, using an LC-MS/MS approach. The LC-MS/MS analysis was carried out on an ion trap instrument and the MS spectra were recorded, in negative ion mode. An external calibration curve obtained using standards solutions prepared in acetonitrile/water (50:50, v/v) allowed the quantification of individual compounds in the concentration range 0.1–300 mg/L. The authors managed to provide the most of the available reference compounds and only when the corresponding pure standard was not available, the most similar molecule was used as reference. Hydroxytyrosol acetate, elenolic acid methylester, hydroxy decarboxymethyl oleuropein aglycon, acetoxypinoresinol, de-oxy elenolic acid and diosmetin were therefore quantified adopting, as reference structurally related compounds (HTYR, elenolic acid, decarboxymethyl oleuropein aglycon, pinoresinol, elenolic acid and luteolin). Quantification of secoiridoids was also performed using, for oleuropein derivatives, a HTYR calibration curve, and for ligstroside derivatives a TYR calibration curve.

In this study the hydrolysis of the glycosylated phenols has been considered a reasonable compromise for the determination of HTYR and TYR, required for the attribution of the health claims on the EVOOs. Eighteen compounds (Table 1) have also been evaluated and their detection (LOD) and the quantification (LOQ) limits have been determined (Olmo-García et al., 2019).

Bartella et al. (2020) proposed an isotopic dilution approach for the quantification of TYR and HTYR. This method meets the requirement of the recent EU Regulation regarding health claims, with a fast sample preparation (30 min), and a rapid (13 min) LC-MS/MS analysis. They carried out the quantitation using deuterated internal standards D_2-TYR and D_2-HTYR to determine the unconjugated (free) TYR and HTYR as well as "total" value of these substances, obtained after the hydrolysis of the secoiridoidic fraction. This was achieved by means of a microwave apparatus.

The determination of free and total TYR and HTYR is based on a negative ion mode ESI/MRM detection. The selected transitions are those due to the loss of CH_2OH radical (m/z 137 > m/z 106) and formaldehyde (m/z 153 > m/z 123) from the negative quasi molecular ions $[M-H]^-$ of TYR and HTYR, respectively. Those transitions, which are the most abundant, have been used for the assay. To validate the analysis some other gas phase reactions were considered. In particular, the ions due to formal loss of a molecule of water from the $[M-H]^-$ ion of TYR and the loss of a CH_2OH radical from the $[M-H]^-$ ion of HTYR were chosen as validation ions.

Among the secoiridoidic fraction, oleacein and oleocanthal are prone to the formation of artifacts during the extraction phases or the LC-MS run. This problem has been studied by Sánchez de Medina et al. (2017). In this study the suitability of LC–MS/MS for absolute quantitation of oleocanthal and oleacein in VOO was evaluated. In particular, two extractions procedures were compared, one based on acetonitrile and the other based on methanol:water mixtures. Two differing chromatographic gradients (using methanol or acetonitrile, respectively, as organic eluents) were also evaluated. The results showed that during the chromatographic run, based on a methanol gradient, the conversion of these secoiridoids in their corresponding hemiacetal, takes place. However, the conversion rate is very low, and for this reason the chromatographic separation using methanol as organic phase is still a viable option. Further, the comparison of extraction procedures showed that no significant differences could be observed (at p-value < 0.05) between the percentages of extracted secoiridoids. To accomplish to this study two different mass spectrometric approaches have been adopted: (i) the qualitative determination of oleocanthal and oleacein and their hemiacetals identification have been performed on a LC–QTOF instrument, that allowed to determine quasi molecular and product ions with a high mass accuracy (5 ppm); (ii) the quantitative determinations have been performed on a more sensitive LC–QQQ MS/MS instrument using MRM detection in negative ion mode (Table 1). Kotsiou and Tasioula-Margari (2016) adopted a mass spectrometry-based approach to follow the phenolic fraction of 42 Greek EVOOs during a storage period of 24 months. In this study, quantitative variations of the phenolic compounds and their degradation products were monitored by HPLC-DAD, while HPLC-MS was used for their identification. The data showed wide variability in phenolic compounds content (ranging between 251 and 926 mg/kg) that correlated with the oil production system, olive variety, and maturity of the drupes. In all 42 samples the phenolic fraction of EVOO consisted mainly of secoiridoids that exceeded 90% of the whole biophenol fraction. After 18 months in the dark glass bottles, the degree of reduction in total phenolic compounds was up to 25%, while after 24 months did not exceed 31%. The ESI mass spectra allowed to individuate degradation products and it was ascertained that the hydrolysis and oxidation processes are more effective on the more polar secoiridoids. These oxidative processes lead to a pronounced decrease of oleocanthal and oleacein causing, at the same time, an increase in HTYR and TYR content. Other compounds as lignans and flavones present at a much smaller concentrations do not suffer appreciably of concentration decreases during the storage.

TABLE 1 Bio-phenols reported in literature, recognized by LC-MS and LC-MS/MS, ionization mode and their diagnostic ions

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, $[M-H]^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
Simple phenols					
TYR	ESI-IT	137(*)			Olmo-García et al. (2019)
	ESI-IT	137(*)		137 > 119	Bajoub et al. (2015b)
				137 > 106	
	ESI-MS (Q)	137			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	137	137 > 106	137 > 119	Bartella et al. (2020)
	ESI-QQQ	137	137 > 119	137 > 115	Kelebek et al. (2017)
	ESI-QQQ	137	137 > 106	137 > 115	Ben Brahim et al. (2017)
d_2-TYR	ESI-QQQ	139	139 > 106		Bartella et al. (2020)
Tyrosol acetate	ESI-QQQ	195	195 > 135		Ben Brahim et al. (2017)
HTYR	ESI-IT	153 (*)			Olmo-García et al. (2019)
	ESI-IT	153 (*)		153 > 123	Bajoub et al. (2015b)
	ESI-MS (Q)	153			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	153	153 > 123	153 > 122	Bartella et al. (2020)
	ESI-QQQ	153	153 > 123	153 > 108	Miho et al. (2018)
	ESI-QQQ	153	153 > 123	153 > 115	Kelebek et al. (2017)
	ESI-QQQ	153	153 > 123	153 > 115	Ben Brahim et al. (2017)
	ESI-QQQ	153	153 > 109	153 > 69	Arena et al. (2020)
d_2-HTYR	ESI-QQQ	155	155 > 123	155 > 122	Bartella et al. (2020)
Hydroxytyrosol acetate	ESI-IT	195 (*)			Olmo-García et al. (2019)
	ESI-QQQ	195	195 > 159	195 > 115	Kelebek et al. (2017)
	ESI-MS (Q)	195			Kotsiou and Tasioula-Margari (2016)
Secoiridoids					
p-HPEA-EDA oleocanthal	ESI-IT	303 (*)			Olmo-García et al. (2019)

(Continues)

TABLE 1 (Continued)

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, [M-H]$^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
	ESI-IT	303 (*)		303 > 284 303 > 165	Bajoub et al. (2015b)
	ESI-QQQ	303	303 > 137	303 > 59	Sánchez de Medina et al. (2017)
		303		303 > 119	
		303		303 > 139	
	ESI-QQQ	303	303 > 285	303 > 115	Ben Brahim et al. (2017)
	ESI-QQQ	303	303 > 59	303 > 115	Kelebek et al. (2017)
	ESI-QQQ	303	303 > 59	303 > 137	Miho et al. (2018)
Methyl hemiacetal of oleocanthal	ESI-QQQ	335	335 > 137	335 > 59 335 > 139	Sánchez de Medina et al. (2017)
Dimethyl acetal of oleocanthal	ESI-QQQ	349	349 > 137	349 > 59 349 > 139	Sánchez de Medina et al. (2017)
3,4-DHPEA-EDA oleacein	ESI-IT	319 (*)			Olmo-García et al. (2019)
	ESI-QQQ	319	319 > 123	319 > 59; 319 > 137; 319 > 139	Sánchez de Medina et al. (2017)
	ESI-QQQ	319	319 > 195	319 > 123	Ben Brahim et al. (2017)
	ESI-QQQ	319	319 > 59	319 >135	Kelebek et al. (2017)
	ESI-QQQ	319	319 > 59	319 > 139	Miho et al. (2018)
Methyl hemiacetal of oleacein	ESI-QQQ	351	351 > 123	351 > 59 351 > 139	Sànchez de Medina et al. (2017)
Dimethyl acetal of oleacein	ESI-QQQ	365	365 > 123	365 > 59 365 > 139	Sánchez de Medina et al. (2017)
Oleuropein	ESI-QQQ		539 > 377	539 > 307	Arena et al. (2020)
Oleuropein-aglycon	ESI-IT	377 (*)			Olmo-García et al. (2019)

TABLE 1 (Continued)

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, [M-H]$^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
3,4 DHPEA-EA	ESI-IT	377 (*)		377 > 307	Bajoub et al. (2015a, 2015b)
				377 > 275	
	ESI-MS (Q)	377			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	377	377 > 275	377 > 115	Ben Brahim et al. (2017)
	ESI-IT	377	377 > 275	377 > 115	Kelebek et al. (2017)
	ESI-QQQ	377	377 > 275	377 > 307	Miho et al. (2018)
Oleuropein-aglycon mono aldehydic form	ESI-MS (Q)	377			Kotsiou and Tasioula-Margari (2016)
Oxidized form of Oleuropein-aglycon mono aldehydic form	ESI-MS (Q)	393			Kotsiou and Tasioula-Margari (2016)
Ligstroside-aglycon	ESI-IT	361 (*)			Olmo-García et al. (2019)
Mono aldehydic form	ESI-IT	361 (*)		361 > 291	Bajoub et al. (2015b)
				361 > 259	
	ESI-QQQ		361 > 912	361 > 115	Kelebek et al. (2017)
	ESI-QQQ		361 > 291	361 > 101	Miho et al. (2018)
Ligstroside-aglycon di aldehydic form	ESI-MS (Q)	361			Kotsiou and Tasioula-Margari (2016)
Dehydro oleuropein aglycon	ESI-IT	375(*)		375 > 307	Bajoub et al. (2015a, 2015b)
				375 > 275	
Methyl oleuropein aglycon	ESI-IT	391 (*)		391 > 321	Bajoub et al. (2015a, 2015b)
				391 > 275	
Decarboxymethyl oleuropein aglycon	ESI-MS (Q)	319			Kotsiou and Tasioula-Margari (2016)
	ESI-IT	319(*)		319 > 195	Bajoub et al. (2015a, 2015b)
				319 > 165	

(Continues)

TABLE 1 (Continued)

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, $[M\text{-}H]^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
10-Hydroxy decarboxymethyl oleuropein aglycon hydroxylated form of oleacein	ESI-IT	335 (*)			Olmo-García et al. (2019)
	ESI-MS (Q)	335			Kotsiou and Tasioula-Margari (2016)
Methyl decarboxymethyl oleuropein aglycon	ESI-IT	333 (*)			Bajoub et al. (2015b)
Oxidized form of decarboxymethyl oleuropein aglycon	ESI-MS (Q)	319			Kotsiou and Tasioula-Margari (2016)
Oxidized of decarboxymethyl ligstroside aglycon	ESI-MS (Q)	319			Kotsiou and Tasioula-Margari (2016)
Oxidized form of aldehydic form of decarboxymethyl ligstroside aglycon	ESI-MS (Q)	377			Kotsiou and Tasioula-Margari (2016)
Phenolic acids					
Vanillin	ESI-QQQ	151		151 > 136	Kıvrak and Kıvrak (2020)
				151 > 92	
				151 > 108	
Vanillic acid	ESI-MS (Q)	167			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	167	167 > 123	167 > 90	Kelebek et al. (2017)
	ESI-QQQ	167		167 > 123	Kıvrak and Kıvrak (2020)
				167 > 108	
				167 > 152	
p-Coumaric acid	ESI-MS (Q)	163			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	163	163 > 119	163 > 90	Ben Brahim et al. (2017)
	ESI-QQQ	163	163 > 119	163 > 90	Kelebek et al. (2017)
	ESI-IT	163 (*)		163 > 119	Bajoub et al. (2015b)
	ESI-QQQ	163		163 > 119	Kıvrak and Kıvrak (2020)
				163 > 93	
				163 > 117	

TABLE 1 (Continued)

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, [M-H]$^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
Ferulic acid	ESI-MS (Q)	193			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	193	193 > 134	193 > 115	Ben Brahim et al. (2017)
	ESI-QQQ	193	193 > 134	193 > 90	Kelebek et al. (2017)
	ESI-QQQ	193		193 > 134	Kıvrak and Kıvrak (2020)
				193 > 149	
				193 > 178	
Gallic acid	ESI-QQQ	169	169 > 125	169 > 73	Arena et al. (2020)
	ESI-QQQ	169		169 > 125	Kıvrak and Kıvrak (2020)
				169 > 97	
				169 > 107	
Protocatechuic acid	ESI-QQQ	153	153 > 109	153 > 115	Ben Brahim et al. (2017)
Caffeic acid	ESI-QQQ	179	179 > 135	179 > 90	Kelebek et al. (2017)
	ESI-QQQ		179 > 135	179 > 115	Ben Brahim et al. (2017)
Lignans					
Pinoresinol	ESI-IT	357 (*)			Olmo-García et al. (2019)
	ESI-MS (Q)	357			Kotsiou and Tasioula-Margari (2016)
Acetoxy pinoresinol	ESI-IT	415 (*)			Olmo-García et al. (2019)
		415 (*)		415 > 325	Bajoub et al. (2015b)
	ESI-MS (Q)	415		415 > 295	Kotsiou and Tasioula-Margari (2016)
Flavonoids					
Diosmetin	ESI-IT	299 (*)			Olmo-García et al. (2019)
	ESI-QQQ	299	299 > 284	299 > 135	Ben Brahim et al. (2017)
	ESI-QQQ	299	299 > 284	299 > 135	Kelebek et al. (2017)

(Continues)

TABLE 1 (Continued)

Negative ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, $[M\text{-}H]^-$ m/z**	**Transitions used for quantitation**	**Transitions used for confirmation**	**References**
Luteolin	ESI-IT	285 (*)			Olmo-García et al. (2019)
		285		285 > 151	Bajoub et al. (2015b)
				285 > 165	
	ESI-MS (Q)	285			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	285	285 > 133	285 > 135	Ben Brahim et al. (2017)
	ESI-QQQ	285	285 > 133	285 > 175	Arena et al. (2020)
	ESI-QQQ	285	285 > 133	285 > 175	Miho et al. (2018)
	ESI-QQQ	285	285 > 133	285 > 135	Kelebek et al. (2017)
	ESI-QQQ			285 > 133	Kıvrak and Kıvrak (2020)
	ESI-QQQ			285 > 107	
				285 > 151	
Apigenin	ESI-IT	269 (*)			Olmo-García et al. (2019)
		269 (*)		269 > 151	Bajoub et al. (2015b)
	ESI-MS (Q)	269			Kotsiou and Tasioula-Margari (2016)
	ESI-QQQ	269	269 > 123	269 > 135	Ben Brahim et al. (2017)
	ESI-QQQ	269	269 > 117	269 > 150	Arena et al. (2020)
	ESI-QQQ	269	269 > 117	269 > 135	Kelebek et al. (2017)
		269	269 > 117	269 > 151	Miho et al. (2018)
		269		269 > 117	Kıvrak and Kıvrak (2020)
				269 > 107	
				269 > 149	

Positive ion mode					
Compound	**Instrumental setup**	**Precursor (*)used for quantitation, $[M+H]^+$ m/z**	**Transitions used for quantitation**	**Major fragments**	**References**
Simple phenols					
TYR	ESI-MS (Q)	139		121; 103	Pedan et al. (2019)
HTYR	ESI-MS (Q)	155		137; 119	Pedan et al. (2019)
Hydroxytyrosol acetate	ESI-MS (Q)	197		137; 119	Pedan et al. (2019)
Secoiridoids					
p-HPEA-EDA oleocanthal	ESI-MS (Q)	305		121	Pedan et al. (2019)
Oleuropein	ESI-MS (Q)	541		137; 360	Pedan et al. (2019)
Phenolic acids					
Vanillic acid	ESI-MS (Q)	169		125;110	Pedan et al. (2019)
Hydroxy benzoic acid	ESI-MS (Q)	139			Pedan et al. (2019)
p-Coumaric acid	ESI-MS (Q)	165		145; 147; 119	Pedan et al. (2019)
Ferulic acid	ESI-MS (Q)	195		177	Pedan et al. (2019)
Lignans					
Pinoresinol	ESI-MS (Q)	359		341; 175	Pedan et al. (2019)
Flavonoids					
Luteolin	ESI-MS (Q)	287			Pedan et al. (2019)
Apigenin	ESI-MS (Q)	271			Pedan et al. (2019)
Luteolin-7-*O*-glucoside	ESI-MS (Q)	449			Pedan et al. (2019)
Apigenin-7-*O*-glucoside	ESI-MS (Q)	433		271	Pedan et al. (2019)

The monitoring conducted evidenced that for EVOOs the phenolic compounds fraction should be higher than 350 mg/kg to meet EFSA's health claim (250 mg/kg) throughout a 24 months storage.

Bajoub et al. (2016b) determined 32 bio-phenolic compounds and, for the first time the quinic acid in 203 Moroccan olive oil samples, collected during two consecutive crop seasons. For the identification and assignation of phenolic compounds retention times and accurate m/z values obtained by HRMS (using a Q-TOF) and MS data (obtained from an IT-MS) were compared with the retention times and mass spectra of the corresponding pure standards, tested under the same conditions. In this paper it was emphasized the higher accuracy of quantification attainable only by the specificity of MS-based studies. For each identified compound, the calibration curves were obtained by LC-IT/MS analysis. Linearity ranges for calibration curves, limits of detection (LOD) and quantification (LOQ) were determined. In this study and in many others, the use of 3,4-dihydroxyphenylacetic acid (DOPAC) as an internal standard for accurate quantification is made (Bajoub et al., 2015b, 2016a, 2016b; Olmo-García et al., 2017).

Kelebek et al. (2017) evaluated and compared different Turkish EVOOs obtained at two different harvesting periods (early and late harvest dates). The analysis was carried out on a HPLC-DAD/MS instrument by MRM operating in negative ion mode. The calibration curves of each bio-phenol present in olive oils were obtained by means of standard materials. The authors summarized the previous MS results reported by Sánchez de Medina et al. (2017) and describe the common transitions and losses for each compound classes. The quasi-molecular ions of TYR and HTYR showed the losses of water or formaldehyde, respectively. In MS/MS experiments, the loss of 44 amu corresponding to CO_2 molecule is frequent for phenolic acids. The authors also evidenced a common neutral loss of 70 amu corresponding to C_4H_6O moiety that was adopted to confirm the presence of ligstrosides and their derivatives.

Finally, flavonoids present in polar fraction of olive oil (luteolin, apigenin and diosmetin) were identified and quantified by MRM mode with the transitions of m/z 285 > m/z 133, m/z 269 > m/z 117 and m/z 299 > m/z 284 corresponding to CH_3 loss.

Ammar et al. (2017) evaluated the chemical composition of Tunisian monocultivar EVOOs extracted after the addition of different amounts (0% and 3%) of olive leaves. The MRM approach revealed 14 phenolic compounds quantified by HPLC-DAD-ESI-MS/MS using an external calibration and individually optimized MRM transitions. In all the studied olive oil samples, oleuropein aglycon and ligstroside aglycon were the most abundant compounds. In addition, EVOO extracted with 3% of olive leaves presented higher amounts of individual phenolic compounds. The same analytical approach was adopted by Amanpour et al. (2019) evidencing once again that olive maturity indices had a significant impact on the phenols content.

Miho et al. (2018), using a LC–MS/MS (MRM) method, compared 80 oil samples of differing selected cultivars. They evaluated their phenolic composition, and it was found that secoiridoidic derivatives showed a high variability. Adopting a multivariate statistical approach, the authors were able to correlate bio-phenols composition to the differing cultivars investigated.

In a similar analytical approach, Kıvrak and Kıvrak (2020) characterized the phenolic compositions of Turkish EVOOs obtained from Delice and Memecik olives. EVOOs were extracted by means of ultrasounds using a mixture of acetonitrile and hexane, that are not miscible. The polar extracts, constituted by the acetonitrile layer, after the sample treatment previously described by Bajoub et al. (2015b), were analyzed by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). This approach allowed to extract and determine a total of 32 phenolic compounds in EVOOs, showing high quantity of luteolin and abundant amounts of *p*-coumaric acid, vanillic acid, and caffeic acid.

Ben Brahim et al. (2017) and coauthors, studying four rare Tunisian cultivars, correlated their bio-phenolic and tocopherols composition content to sensory analysis, oxidative stability, antioxidant activity. The phenolic fractions, identified and quantified using LC-DAD-ESI-MS/MS in MRM operating in negative ion mode, showed qualitative and quantitative differences in the composition, monitoring a total of 13 phenolic compounds. These authors used an external standard calibration approach, building seven calibration curves for protocatechuic acid, TYR, caffeic acid, *p*-coumaric acid, luteolin, apigenin, and diosmetin. For the substances lacking an appropriate reference material, the authors report that the closest in structural terms was chosen as reference. They do not specify the reference substance adopted for quantitation of secoiridoids, though. Differing bio-phenol patterns were observed comparing differing cultivars harvested each at three sampling times. On the contrary to the agronomical traits, during maturation, the quantitation data show a general decrease of total phenolic fraction and of antioxidant compounds, like tocopherols. In this study for the first time, diosmetin was found in Tunisian olive oils. In addition, since bio-phenolic compounds has an important impact on the oils organoleptic properties,

three positive descriptors were perceived by a sensory evaluation conducted by a panel test. The bitterness and pungency notes were attributed to HTYR and oleacein. Further a clear difference between cultivars was observed for the oleuropein aglycon content which is the main secoiridoidic component that has been found in the studied Tunisian oil.

Another recent work correlates phenolic compounds, with the sensory properties of EVOOs (Pedan et al., 2019). Oleocanthal and oleacein were the most abundant phenolic compounds, with average amounts of 77.9 and 41.8 mg/kg, respectively. It was found that the bitter taste sensation and the total phenolic content were correlated with HTYR and oleuropein aglycon. The study also showed that all the detected HTYR level in EVOOs were too low to provide the minimum intake of 5 mg of HTYR per serving of olive oil, which is required to have an antioxidant effect in a balanced diet. Besides, just four out of hundred EVOOs could provide total phenolic compounds (determined by FC method) higher than 250 mg/kg of bio-phenols. In this article the ESI-MS was conducted in positive ion mode, and allowed the proper recognition of bio-phenols and secoiridoids, but the final quantitation was performed by means of DAD absorbance data. The LC-MS approach was essential for the individual determination, of the 19 bio-phenols and in particular oleoside 11-methylester, that allowed its association with the aroma descriptors "freshly cut grass", "leaves," and "nuts."

Arena et al. (2020) evaluated the matrix-related signal suppression phenomena comparing LC and LC x LC separations both coupled to MS (selective ion monitoring, SIM) and MS/MS (MRM) detection systems. Phenolic compounds were quantified by SIM in the negative ionization mode. The MRM transitions employed in the MS/MS experiments were selected using the most intense product ions obtained from the corresponding standards materials, optimizing collision energies for each compound, in the range 16–35 eV. Matrix effect was evaluated comparing the slopes (A) of the calibration curves obtained from standard dissolved in pure solvent and the slopes (B) of the corresponding curves obtained from matrix matched calibration curves. The matrix effect (%) was determined as $\frac{A}{B} \times 100$. A value of 100% means a lack of matrix effects, values less than 100% indicate matrix signal suppression, whereas values higher than 100% indicate matrix signal enhancements. The adoption of a two dimensions chromatographic separation grants a significant reduction of matrix effect for most of analyzed compounds.

2.4 | High-resolution MS and MS/MS applications

As evidenced so far, low-resolution mass spectrometry and mostly tandem mass spectrometry approaches have been more widely adopted for bio-phenols quantitative determination, and this is mostly due to the lower price of the instrumentation (for MS) or to the high sensitivity of MS/MS approach, that now rival DAD results. However, the number of isomers present in this limited fraction of olive oils, is high enough to command the adoption of the more selective mass spectrometry approaches as high-resolution mass spectrometry (HRMS). As aforesaid HRMS analysis is characterized by a high selectivity. This is most beneficial for the identification of single bio-phenols, but also increase the overall sensitivity of the MS acquisition, enormously reducing the noise due to matrix. HRMS provide accurate m/z values which greatly help to reduce the number of nominal mass isobar candidates allowing the determination of the ion's elemental composition. This leads to a significant simplification of the understanding of molecules. Also, for HRMS approaches, ESI negative ion mode is by far the most adopted ionization polarity. For bio-phenol identification and quantification purposes the *quasi*-molecular ion has been monitored together with the most common losses that in negative ion mode are water and CO_2 losses. In positive ion mode the protonated *quasi*-molecular ions and the sodium cationated ones have been mostly monitored.

Cifuentes (2012) and Ibanez et al. (2015) adopted HRMS and HRMS/MS approach, in positive and negative ESI mode, to enable the detection and identification of the widest range of phenolic compounds in olive oil on the basis of agronomic and productive factors such as variety, geographical origin, irrigation, maturity, environmental conditions as well as the date of harvest, processing and conservation of the olives.

Since the first studies based on liquid chromatography coupled to UV detection (Montedoro et al., 1993), strong evidence of the presence of several structural isomers among secoiridoids have been reported. The chromatograms of polar fraction of EVOO samples are usually characterized by complex peaks, attributable to the presence of many isomers and derivatives of oleuropein and ligstroside aglycons (p-HPEA-EA and 3,4-DHPEA-EA respectively).

These two aglycones, corresponding to unstable cyclic hemiacetals, are suitable substrates to undergo an intramolecular rearrangement. giving the oleuropein aglycon monoaldheydic form and ligstroside aglycon monoaldheydic form (Figure 2). Both derivatives,

through an enzymatic and/or chemical degradation process, lose the carboxymethyl fraction on C10, to give decarboxymethyl oleuropein aglycone and decarboxymethyl ligstroside aglycon.

Monoaldehydic forms with closed ring through a keto-enol tautomerism spontaneously transform into more stable open dialdehyde structures called oleocanthal or p-HPEA-EDA, and oleacein or 3,4-DHPEA-EDA (Fu et al., 2009a). Some of the described reactions are reported in (Scheme 1).

The same complex scenario is repeated in the case of the other two important secoiridoids contained in olive oil, oleacein and oleocanthal (Boussahel et al., 2020; De La Torre-Carbot et al., 2005; Grilo et al., 2020, 2021; Obied et al., 2007).

Due to these equilibria that take place in the condensed phase, bio-phenols chromatograms are always very complex and difficult to interpret. It is also to point out that most of the hypothesized structures have not been neither isolated or elucidated. Despite numerous attempts to correlate chromatographic characteristics with the structural properties of isomeric secoiridoids in olive oil, the goal has not yet been reached. Therefore, the HRMS/MS data (Table 2) were critical to progress in secoiridoids' structural clarification.

Several reports have demonstrated that HRMS instruments can be used as a single MS platform in qualitative and quantitative approaches. When it comes to quantitative analysis HRMS has two major advantages compared to tandem MS: first, in HRMS full scan data are recorded. Together to quantitative information of the target analyte, qualitative information about other compounds in sample monitoring can be obtained, even in retrospect. In addition, HRMS method development could be simpler as MS/MS experiments are often unnecessary

2.5 | High throughput: Ambient mass spectrometry (AMS) applications

AMS was introduced by Cooks' group in 2004 (Takáts et al., 2004). In this approach the sample ionization can be performed in open air and requires no or little sample pretreatments. Since then, several AMS techniques have been developed, each based on different desorption/ionization mechanisms and allowing the direct analysis of molecules in a wide range of molecular weights and polarities.

According to the ionization mechanism, AMS methods can be roughly grouped into different classes:

a) spray- or jet-based ionization techniques: desorption electrospray (DESI), desorption atmospheric pressure photoionization (DAPPI), easy ambient sonic-spray ionization (EASI);
b) electric discharge-based ionization techniques: direct analysis in real time (DART); desorption atmospheric pressure chemical ionization (DAPCI), low temperature plasma (LTP) AMS, dielectric barrier discharge ionization (DBDI);
c) Paper spray (PS), where ion species are generated by applying a high voltage to a triangle wetted with small volumes of analyte solution.

Of course, only some of these ambient ionization techniques have been applied to the determination of bio-phenols in olive oils, but these expanding approaches always granted a minimal sample preparation but with few tradeoffs, admittedly. For qualitative/fingerprinting matrices determinations AMS proved to be very feasible. Among the first class, EASI adopts a super-sonic spray ionization (Hirabayashi et al., 1995) to create minuscule charged droplets. The ionization is caused by the statistical imbalance distribution of cations and anions. A dense stream of the super-sonic charged droplets is originated applying a high-pressure N_2 or air flow, running coaxially with a silica capillary which delivers the chosen solvent (methanol). The charged droplets pick up from the surface the analyte causing its ionization. This technique has been used for the quality control and certification of geographical origin of olive oils (Riccio et al., 2011), and to provide characteristic profiles and their chemotaxonomic markers. Among other substances, several bio-phenols and secoiridoids have been determined. The approach involved the adoption of the AMS EASI source coupled with a tandem HRMS system operating in negative ion mode and applying to the incoming ions a moderate (15–30 eV) collision energy to generate reliable MS/MS spectra.

In LTP-MS method (Figure 6) a low temperature plasma is generated using a low-flow helium carrier gas (discharge gas) flowing in a glass tube within a grounded electrode (Harper et al., 2008). To a copper tape surrounding the outer part of the glass tube, a high voltage (3 kV) and a radiofrequency (2,5 kHz) are applied, generating a dielectric barrier plasma. The plasma is capable to desorb and gently ionize the analytes of interest from matrices, that is, olive oil. LTP-MS analysis on raw olive oils have been performed to identify and quantify the main phenolic compounds (phenolic acids and phenolic alcohols). The profiles of different olive oils (acquired in negative ion mode) have been reported by Lara-Ortega et al. (2018), evidencing

TABLE 2 Bio-phenols reported in literature, recognized by HRMS and HRMS/MS, ionization mode and their diagnostic ions

Compound	Negative ions	Positive ions	Instrumental setup	References
	Diagnostic ion (m/z)	Diagnostic ion (m/z)		
Simple phenols				
Tyrosol (p-HPEA) $C_8H_{10}O_2$	$[C_8H_9O_2]^-$ (137.060)		ESI-Q-OT	Celano et al. (2018)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Gilbert-López et al. (2014)
				Loubiri et al. (2017)
				Negro et al. (2019)
	$[C_8H_7O]^-$ (119.050)		ESI-Q-OT	Dini et al. (2020), Di Stefano and Melilli (2020)
Hydroxytyrosol (3,4 DHPEA) $C_8H_{10}O_3$	$[C_8H_9O_3]^-$ (153.056)		ESI-Q-OT	Boussahel et al. (2020)
				Celano et al. (2018)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
			ESI-TOF	Nikou et al. (2020)
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Gilbert-López et al. (2014)
	$[C_7H_7O_2]^-$ (123.045)		ESI-Q-OT	Loubiri et al. (2017)
				Dini et al. (2020)
			ESI-TOF	Nikou et al. (2020)
		$[C_8H_{11}O_3]^+$ (155.070)	ESI-Q-OT	Bajoub et al. (2016a, 2016b)
				Celano et al. (2018)
		$[C_8H_{10}O_3Na]^+$ (177.052)	ESI-Q-OT	Dini et al. (2020)
				Celano et al. (2018)
				Dini et al. (2020)

(Continues)

TABLE 2 (Continued)

Compound	Negative ions: Diagnostic ion (*m*/*z*)	Positive ions: Diagnostic ion (*m*/*z*)	Instrumental setup	References
Secoiridoids				
p-HPEA-EDA oleocanthal	$[C_{17}H_{19}O_5]^-$ (303.123)		ESI-Q-OT	Boussahel et al. (2020)
				Celano et al. (2018)
$C_{17}H_{20}O_5$				Dini et al. (2020)
				Di Stefano and Melilli (2020)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016b)
				Fu et al. (2009b)
				Negro et al. (2019)
	$[C_{10}H_{11}O_3]^-$ (179.072)		ESI-Q-OT	Celano et al. (2018)
				Dini et al. (2020)
				Nikou et al. (2020)
		$[C_{17}H_{21}O_5]^+$ (305.1281)	ESI-Q-OT	Celano et al. (2018)
		$[C_{17}H_{21}O_5Na]^+$ (327.120)	ESI-Q-OT	Celano et al. (2018)
3,4-DHPEA-EDA oleaceinoleaecin	$[C_{17}H_{19}O_6]^-$ (319.118)		ESI-Q-OT	Celano et al. (2018)
				Dini et al. (2020)
$C_{17}H_{20}O_6$				Di Stefano and Melilli (2020)
				Kanakis et al. (2013)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016b)
				Fu et al. (2009b)
				Loubiri et al. (2017)
				Negro et al. (2019)
	$[C_{17}H_{17}O_5]^-$ (301.108)		ESI-Q-OT	Dini et al. (2020)
				Kanakis et al. (2013)
	$[C_{10}H_{11}O_4]^-$ (195.066)		ESI-Q-OT	Celano et al. (2018)
				Kanakis et al. (2013)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016b)

TABLE 2 (Continued)

Compound	Negative ions Diagnostic ion (*m*/*z*)	Positive ions Diagnostic ion (*m*/*z*)	Instrumental setup	References
				Fu et al. (2009b) Loubiri et al. (2017)
				Negro et al. (2019)
	$[C_9H_9O_3]^-$ (165.056)		ESI-Q-OT	Kanakis et al. (2013)
		$[C_{17}H_{20}O_6\ Na]^+$ (343.114)	ESI-Q-OT	Celano et al. (2018)
		$[C_{17}H_{21}O_6]^+$ (321.132)	ESI-Q-OT	Celano et al. (2018)
Oleuropein-aglycon	$[C_{19}H_{21}O_8]^-$ (377.1242)		ESI-Q-OT	Abbattista et al. (2013)
(3.4 DHPEA-EA) $C_{19}H_{22}O_8$				Celano at al. (2018)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
				Kanakis et al. (2013)
			ESI-TOF	Nikou et al. (2020)
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Gilbert-López et al. (2014)
				Loubiri et al. (2017)
			ESI-Q-OT	Negro et al. (2019)
	[C18H17O7]$^-$ (345.0978)			Celano et al. (2018)
				Dini et al. (2020)
			ESI-TOF	Kanakis et al. (2013)
				Bajoub et al. (2016b)
	$[C_{15}H_{15}O_7]^-$ (307.085)		ESI-Q-OT	Fu et al. (2009)
				Celano et al. (2018)
			ESI-TOF	Kanakis et al. (2013)
				Bajoub et al. (2016a)
	$[C_{15}H_{15}O_5]^-$ (275.089)		ESI-TOF	Fu et al. (2009a)
				Bajoub et al. (2016b)

(Continues)

TABLE 2 (Continued)

Compound	Negative ions	Positive ions	Instrumental setup	References
	Diagnostic ion (*m*/*z*)	Diagnostic ion (*m*/*z*)		
	$[C_{11}H_{13}O_6]^-$ (241.0716)		ESI-Q-OT	Fu et al. (2009a)
				Dini et al. (2020)
				Abbattista et al. (2013)
				Di Stefano and Melilli (2020)
			ESI-TOF	Kanakis et al. (2013)
				Nikou et al. (2020)
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Gilbert-López et al. (2014)
				Loubiri et al. (2017)
				Negro et al. (2019)
Oleuropein-aglycon mono aldehydicmonoaldeidic form $C_{19}H_{22}O_8$	$[C_{19}H_{22}O_8]^-$ (377.1242)		ESI-Q-OT	Nikou et al. (2020)
			ESI-TOF	Fu et al. (2009a)
				Negro et al. (2019)
	$[C_{18}H_{17}O_7]^-$ (345.098)		ESI-Q-OT	Kanakis et al. (2013)
			ESI-TOF	Fu et al. (2009a)
	$[C_{15}H_{15}O_7]^-$ (307.082)		ESI-Q-OT	Kanakis et al. (2013)
			ESI-TOF	Fu et al. (2009a)
	$[C_{18}H_{17}O_5]^-$ (275.092)		ESI-Q-OT	Kanakis et al. (2013)
			ESI-TOF	Fu et al. (2009a)
	$[C_{14}H_{11}O_6]^-$ (275.056)		ESI-Q-OT	Kanakis et al. (2013)
	$[C_{10}H_{11}O_4]^-$ (195.066)		ESI-Q-OT	Kanakis et al. (2013)
	$[C_8H_5O_4]^-$ (149.025)		ESI-Q-OT	Kanakis et al. (2013)
			ESI-TOF	Fu et al. (2009a)
	$[C_7H_5O_3]^-$ (121.031)		ESI-Q-OT	Kanakis et al. (2013)
Ligstroside -aglycon monoMono aldehydic form (p-HPEA-EA) $C_{19}H_{22}O_7$	$[C_{19}H_{21}O_7]^-$ (361.129)		ESI-Q-OT	Kanakis et al. (2013)

TABLE 2 (Continued)

Compound	Negative ions	Positive ions	Instrumental setup	References
	Diagnostic ion (m/z)	Diagnostic ion (m/z)		
	$[C_{15}H_{15}O_6]^-$ (291.087)		ESI-Q-OT	Kanakis et al. (2013)
	$[C_{15}H_{15}O_4]^-$ (259.097)		ESI-Q-OT	Kanakis et al. (2013)
Dehydro ligstroside aglycon $C_{19}H_{20}O_7$	$[C_{19}H_{20}O_7]^-$ (359.112)		ESI-TOF	Olmo-García et al. (2018)
Dehydro oleuropein aglycon $C_{19}H_{20}O_8$	$[C_{19}H_{19}O_8]^-$ (375.109)		ESI-TOF	Olmo-García et al. (2018)
Methyl oleuropein aglycon $C_{20}H_{24}O_8$	$[C_{20}H_{23}O_8]^-$ (391.139)		ESI-Q-OT	Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Negro et al. (2019)
				Olmo-García et al. (2018)
10-Hydroxy oleuropein aglycon $C_{19}H_{22}O_9$	$[C_{19}H_{21}O_9]^-$ (393.119)		ESI-Q-OT	Kanakis et al. (2013)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016a, 2016b)
				Negro et al. (2019)
10-Hydroxy decarboxymethyl ligstroside aglycon (hydroxylated form of oleocanthal) $C_{17}H_{20}O_6$	$[C_{17}H_{19}O_6]^-$ (319.117)		ESI-TOF	Bajoub et al. (2016b)
	$[C_9H_{11}O_5]^-$ (199.060)		ESI-TOF	Bajoub et al. (2016b)
10-Hydroxy decarboxymethyl oleuropein aglycon (hydroxylated form of oleacein) $C_{17}H_{20}O_7$	$[C_{17}H_{20}O_7]^-$ (335.114)		ESI-Q-OT	Kanakis et al. (2013)
				Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016a, 2016b)
				Negro et al. (2019)
	$[C_9H_{11}O_5]^-$ (199.061)		ESI-Q-OT	Kanakis et al. (2013)
			ESI-TOF	Bajoub et al. (2016b)

(Continues)

TABLE 2 (Continued)

Compound	Negative ions	Positive ions	Instrumental setup	References
	Diagnostic ion (*m*/*z*)	Diagnostic ion (*m*/*z*)		
Methyl decarboxymethyl oleuropein aglycon $C_{18}H_{22}O_6$	$[C_{18}H_{22}O_6]^-$ (333.134)		ESI-TOF	Bajoub et al. (2016a, 2016b)
Methyl hemiacetal di 3,4-DHPEA-EA $C_{20}H_{26}O_9$	$[C_{20}H_{25}O_9]^-$ (409.148)		ESI-Q-OT	Celano et al. (2018)
	$[C_{19}H_{21}O_8]^-$ (377.123)			
		$[C_{20}H_{27}O_9]^+$ (411.165)	ESI-Q-OT	Celano et al. (2018)
		$[C_{20}H_{26}O_9Na]^+$ (433.246)		
Methyl hemiacetal di p-HPEA-EA $C_{20}H_{26}O_8$	$[C_{20}H_{25}O_8]^-$ (393.154)		ESI-Q-OT	Celano et al. (2018)
	$[C_{19}H_{21}O_7]^-$ (361.128)			
		$[C_{20}H_{27}O_8]^+$ (395.169)	ESI-Q-OT	Celano et al. (2018)
		$[C_{20}H_{26}O_8Na]^+$ (417.152)		
Oleocanthalic acid $C_{17}H_{20}O_6$	$[C_{17}H_{19}O_6]^-$ (319.119)		ESI-Q-OT	Angelis et al. (2018)
				Nikou et al. (2020)
Oleaceinic acid $C_{17}H_{20}O_7$	$[C_{17}H_{19}O_7]^-$ (335.114)		ESI-Q-OT	Angelis et al. (2018)
Phenolic acids				
Vanillin $C_8H_8O_3$	$[C_8H_7O_3]^-$ (151.039)		ESI-Q-OT	Boussahel et al. (2020)
				Di Stefano and Melilli (2020)
			ESI-TOF	Bajoub et al. (2016b)

TABLE 2 (Continued)

Compound	Negative ions Diagnostic ion (*m*/*z*)	Positive ions Diagnostic ion (*m*/*z*)	Instrumental setup	References
				Loubiri et al. (2017)
				Negro et al. (2019)
Vanillic acid $C_8H_8O_4$	$[C_8H_7O_4]^-$ (167.035)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
	$[C_7H_4O_4]^-$ (152.012)		ESI-TOF	Di Stefano and Melilli (2020)
				Loubiri et al. (2017)
				Gilbert-López et al. (2014)
				Negro et al. (2019)
p-hydroxy benzoic acid $C_7H_6O_3$	$[C_7H_5O_3]^-$ (137.023)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
Cinnamic acid $C_9H_8O_2$	$[C_9H_7O_2]^-$ (147.045)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
	$[C_8H_7]^-$ (103.04501)		ESI-Q-OT	Dini et al. (2020)
p-Coumaric acid $C_9H_8O_3$	$[C_9H_7O_3]^-$ (163.040)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
			ESI-TOF	Gilbert-López et al. (2014)
	$[C_8H_7O_3]^-$ (119.050)		ESI-Q-OT	Dini et al. (2020)
			ESI-TOF	
Ferulic acid $C_{10}H_{10}O_4$	$[C_{10}H_9O_4]^-$ (193.05063)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
			ESI-TOF	Gilbert-López et al. (2014)
				Negro et al. (2019)
	$[C_9H_6O_4]^-$ (178.02685)		ESI-Q-OT	Dini et al. (2020)
Syringic acid $C_9H_{10}O_5$	$[C_9H_7O_2]^-$ (197.045)		ESI-Q-OT	Boussahel et al. (2020)
			ESI-TOF	Gilbert-López et al. (2014)

(Continues)

TABLE 2 (Continued)

Compound	Negative ions	Positive ions	Instrumental setup	References
	Diagnostic ion (*m*/*z*)	Diagnostic ion (*m*/*z*)		
Gallic acid $C_7H_6O_5$	$[C_7H_5O_5]^-$ (169.014)		ESI-Q-OT	Boussahel et al. (2020)
				Gilbert-López et al. (2014)
Protocatechuic acid $C_7H_6O_4$	$[C_7H_5O_4]^-$ (153.017)		ESI-Q-OT	Boussahel et al. (2020)
				Nikou et al. (2020)
Lignans				
Pinoresinol $C_{20}H_{22}O_6$	$[C_{20}H_{21}O_6]^-$ (357.133)		ESI-Q-OT	Boussahel et al. (2020)
				Celano et al. (2018)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
			ESI-TOF	
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Negro et al. (2019)
Acetoxy pinoresinol $C_{22}H_{20}O_8$	$[C_{22}H_{19}O_8]^-$ (415.139)		ESI-Q-OT	Boussahel et al. (2020)
				Celano et al. (2018)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
			ESI-TOF	Nikou et al. (2020)
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
		$[C_{22}H_{20}O_8Na]^+$ (439.136)	ESI-Q-OT	Negro et al. (2019)
				Celano et al. (2018)
Flavonoids				
Diosmetin $C_{16}H_{12}O_6$	$[C_{16}H_{11}O_6]^-$ (299.056)		ESI-Q-OT	Boussahel et al. (2020)
				Di Stefano and Melilli (2020)
Luteolin $C_{15}H_{10}O_6$	$[C_{15}H_9O_6]^-$ (285.0393)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)

TABLE 2 (Continued)

Compound	Negative ions Diagnostic ion (*m*/*z*)	Positive ions Diagnostic ion (*m*/*z*)	Instrumental setup	References
				Kanakis et al. (2013) Nikou et al. (2020)
			ESI-TOF	Bajoub et al. (2016a, 2016b)
				Gilbert-López et al. (2014)
				Loubiri et al. (2017)
				Negro et al. (2019)
	$[C_8H_5O_2]$ 133.02957		ESI-Q-OT	Dini et al. (2020)
Apigenin $C_{15}H_{10}O_5$	$[C_{15}H_9O_5]^-$ (269.046)		ESI-Q-OT	Boussahel et al. (2020)
				Dini et al. (2020)
				Di Stefano and Melilli (2020)
				Kanakis et al. (2013)
			ESI-TOF	Nikou et al. (2020)
				Bajoub et al. (2016a, 2016b)
				Fu et al. (2009b)
				Gilbert-López et al. (2014)
	$[C_{14}H_9O_3]^-$ 225.05592		ESI-Q-OT	Loubiri et al. (2017)
				Negro et al. (2019)
				Di Stefano and Melilli (2020)
				Dini et al. (2020)
	$[C_{21}H_9O_9]^-$ 431.09854		ESI-Q-OT	Boussahel et al. (2020)
Apigenin-7-glucoside $C_{21}H_{20}O_{10}$				Di Stefano and Melilli (2020)
				Kanakis et al. (2013)

differences in the relative intensity and distribution of the quasi-molecular ions. However, it was observed lower signal intensities, with respect to other ionization techniques (ESI, APCI or PS), leading to fewer information. Low sensitivity of LTP may be due to a relatively low vapor pressure of phenolic compounds. In other LTP based approaches, to gather the most of information MS/MS experiments have been preferred (García-Reyes et al., 2009) and the sample substrate has been heated at 150°C to increase the sensitivity. The data, acquired according to this method, showed the main phenolic acids: *p*-hydroxybenzoic acid, syringic acid, coumaric acid and ferulic acid. TYR and HTYR were also detected. The similarities between the MS/MS spectra of many compounds made impossible to rule out contributions from isomers. This problem is in common with several techniques that do not involve a prior analytical separation of the analytes.

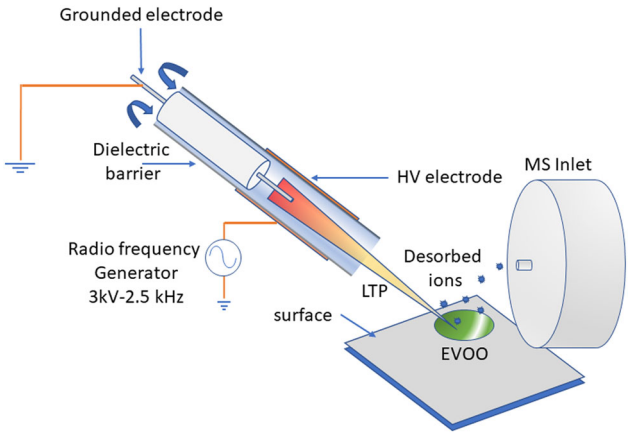


FIGURE 6 Schematic representation of a LTP source [Color figure can be viewed at wileyonlinelibrary.com]

In the basic DART source, a gas (nitrogen or helium) flows into tube constituted by several chambers. The gas is introduced into the first discharge chamber. An electrical potential of several kilovolts is applied between two electrodes and generates ions, electrons and vibrationally excited species. This plasma is forced to flow into the second chamber where a second perforated electrode is used to remove ions from the gas stream. The gas then flows through a third chamber that could be occasionally heated, and finally interacts with a third grid electrode that repels ions of opposite polarity. The (heated) plasma is directed toward the mass spectrometer sampling orifice; or reflected on the sample surface (Cody et al., 2005).

Farré et al. (2019) developed a DART-based method for the fingerprinting identification of bio-phenols in EVOO. The bio-phenols could be identified by DART-MS in 2 min, whereas the same results by LC-MS required 45 min of chromatographic run. To reduce source cross-contamination and enhance sensitivity, a sample pretreatment has been conducted: oil samples were simply diluted in hexane and extracted in a centrifuge tube using methanol–water (70:30) mixture. The treated sample is preferable to the direct analysis of EVOO since this matrix is very problematic due to the EVOO major components (95%–98%) constituted by triacylglycerols (TAGs). Indeed, TAGs presence interfere with minor components detection as the whole oil matrix is characterized by a boiling point >180°C and by high viscosity that reduce volatilization and increase the cross-contamination. The results were further processed adopting principal component analysis (PCA), allowing a classification of the EVOO samples, according to the olive variety.

Paper spray is a direct sampling ionization method for MS analysis. The samples are loaded onto a triangular-shaped paper, which is then moistened with a suitable solution that acts as a mobile phase. Alternatively, the sample can be transferred onto the paper support by dabbing it with the support itself. Paper is typically made from cellulose, that is a hydrophilic porous material capable to hold liquids of various nature (Liu et al., 2010). It allows a solvent-mediated analyte displacement along a dense network of microchannels. An intense electric field is generated applying a high electric potential between the paper triangle and the MS inlet. This induces solvated ions movement up to the edge of the paper triangle, where the liquid can form charged droplets induced by the high potential. Similarly to ESI, the charged droplets undergo subsequent desolvation processes and generate dry ions that finally enter the MS orifice. Several applications have been reported for PS to reveal small organic compounds, peptides, and proteins (Liu et al., 2010).

The adoption of PS for quantitative purposes shows some limitations: the peaks absolute intensity in MS spectra is not quite repeatable. Further, matrix effec

t negatively impacts single stage instrumentation, introducing strong noise and impairing sensitivity. To overcome these limits an appropriate internal standard and a multistage mass spectrometer such as a triple quadrupole should be used (Bartella et al., 2019, 2020; Di Donna et al., 2017; Taverna et al., 2016).

Lara-Ortega et al. (2018) adopted this technique for direct olive oil analyses. EVOO methanolic extracts, obtained from oils diluted in hexane, were spotted onto Whatman 42 filter paper wetted with a mixture of methanol: chloroform (9:1, v-v) with 0.1% NH_3. The PS spectra were recorded in negative ionization mode applying a voltage of 3.5 kV. Results obtained agree with ESI direct infusion of the methanolic extracts since the ionization mechanism is similar.

In a recent paper, Bartella et al. (2020) presented an innovative method to determine HTYR and TYR derivatives by means of PS tandem mass spectrometry (PS-MS/MS). To improve the accuracy of the method d_2-Hydroxytyrosol and d_2-Tyrosol were adopted (Figure 7). The method for the quantitative determination of free and total TYR and HTYR is divided in two steps. In the first step were quantified free TYR and HTYR: the EVOO samples were fortified with internal standard, diluted in hexane and loaded into normal phase SPE cartridges to remove triacylglycerols. The methanolic eluates were spotted and analyzed by PS.

In the second step, the esterified compounds, namely oleocanthal, oleacein, ligstroside aglycon and oleuropein aglycon, are hydrolyzed and quantified as TYR and HTYR. In this case the olive oil samples, enriched with deuterated standards, were extracted in a polar solvent

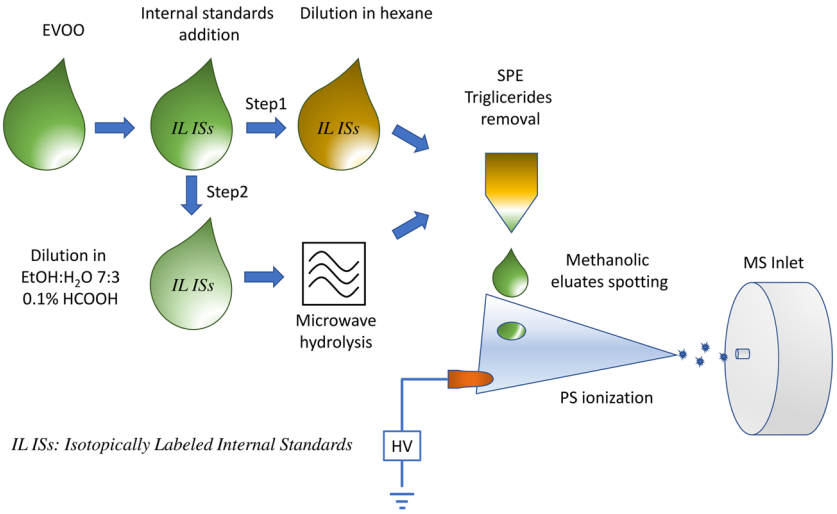


FIGURE 7 Workflow for the rapid and accurate determination of TYR and HTYR in EVOO samples by PS/MS [Color figure can be viewed at wileyonlinelibrary.com]

mixture (EtOH/H_2O (0,1% HCOOH) 7:3) and submitted to a microwave hydrolytic step. The hydrolysates were cleaned by reversed phase (C18) SPE cartridges, and the methanolic eluates were finally evaluated by PS-MS/MS. The adoption of PS allowed to reduce the whole analysis time, comprising the sample pretreatment and microwave hydrolysis, to no more than10 min.

Although AMS suffers from various difficulties for quantitative determinations, its speed and the use of derivatives marked as internal standards make it of increasing interest. Several recent publications report analytical determinations through AMS (Table 3).

2.6 | Ion mobility mass spectrometry (IM-MS)

IM-MS is increasingly wide spreading as an orthogonal separation method, to complement the more classical separation approaches as chromatography and electrophoresis. IM-MS owns the unique capacity to separate ionic species differing in size, shape and/or charge state, and it allows to determinate their cross-sections. All these features make IM-MS an elective technique to separate isomers with differing mobility and to investigate (even in complex mixtures) the structural organization of variously sized molecules up to supramolecular aggregates (Bongiorno et al., 2014, 2016; Hernández-Mesa et al., 2019; Uetrecht, et al., 2010).

Piñero et al. (2020) proposed a new analytical method coupling desolvating low-flow secondary electrospray ionization (D-LFSESI), differential mobility analysis (DMA), and mass spectrometry (MS) for the analysis of bio-phenols in olive oils.

D-LFSESI is a recently developed secondary ion ESI source that can ionize liquid and gaseous samples. In this case the liquid samples were delivered with a low flow to the DMA device, using electrospray fused-silica capillary with the following characteristics: 360 μm o.d., 50 μm i.d., 15 μm tip i.d., and 80 cm length. The DMA voltage (V DMA) was provided by the MS internal ionspray power supply. The MS software allowed a simultaneous scan of DMA and the MS parameters. The ions that successfully flow through the DMA device were finally analyzed according to their mass to charge ratio (m/z) in a triple quadrupole MS. One of the aims of this study was the determination of the polar compound extract of the three categories of olive oil (EVOO, VOO, and lampante olive oil). The authors observed significant differences in peak distributions and intensities in the DMA plots. These results, united to some statistical evaluation, led to a successful identification of edible and nonedible oils.

3 | CONCLUSIONS

This review summarizes the recent studies reported in literature that contributes to the identification and/or quantification of the specific bio-phenols characteristic of EVOOs through mass spectrometric methods.

This is of current interest as the amount and the variety of bio-phenols in olive oils can be used for the prediction of health benefits. In several articles the quantitative determinations of bio-phenols in EVOOs are carried out by LC separation using spectrophotomers (UV-VIS, DAD, Fluorimeters) as detectors and the International Olive Council still considers the coupling of HPLC with UV detection at 280 nm as the official approach. Often mass spectrometry is used together to these techniques to overcome their low selectivity and to obtain the identification of each analyte.

TABLE 3 Bio-phenols recognized in ambient mass spectrometry on the basis of the instrumental setup and their diagnostic ions

Compound	Instrumental source setup	Negative ion m/z[a]	CID product ions m/z[a]	References
Simple phenols				
Tyrosol (p-HPEA) $C_8H_{10}O_2$	EASI	137	119	Riccio et al. (2011)
	LTP	137	119, 109, 93	Lara-Ortega et al. (2018)
	DART	137.02418	–	Farré et al. (2019)
	PS	137	119, 93	Lara-Ortega et al. (2018)
Hydroxytyrosol D2 $C_8H_8D_2O_2$	PS	155	123	Bartella et al. (2018)
Hydroxytyrosol $C_8H_{10}O_3$	DART	153.0555	–	Farré et al. (2019)
	PS	153	123, 109	Lara-Ortega et al. (2018)
				Bartella et al. (2018)
Hydroxytyrosol	EASI	195	137, 153, 123	Riccio et al. (2011)
acetate	DART	195.06626	–	Farré et al. (2019)
	PS	195	163,165	Lara-Ortega et al. (2018)
Secoiridoids				
Oleuropein-aglycon $C_{19}H_{22}O_8$	EASI	377	275, 307, 333, 301	Riccio et al. (2011)
	LTP	377	345, 307, 275	Lara-Ortega et al. (2018)
	DART	377.12441	–	Farré et al. (2019)
	PS	377	345, 307, 275	Lara-Ortega et al. (2018)
Oleuropein-aglycon mono aldehydic form $C_{19}H_{22}O_8$	EASI	377	275, 307, 333, 301	Riccio et al. (2011)
	LTP	377	345, 307, 275	Lara-Ortega et al. (2018)
	DART	377.12441	–	Farré et al. (2019)
	PS	377	345, 307, 275	Lara-Ortega et al. (2018)
Ligstroside-aglycon mono aldehydic form $C_{19}H_{22}O_7$	EASI	361	291, 259, 101	Riccio et al. (2011)
	LTP	361	291, 259, 223	Lara-Ortega et al. (2018)
	DART	361.12952	–	Farré et al. (2019)
	PS	361	291, 259, 223	Lara-Ortega et al. (2018)

TABLE 3 (Continued)

Compound	Instrumental source setup	Negative ion m/z[a]	CID product ions m/z[a]	References
Dehydro oleuropein aglycon $C_{19}H_{20}O_8$	DART	375.10888	–	Farré et al. (2019)
Methyl oleuropein aglycon $C_{20}H_{24}O_8$	DART	391.13999	–	Farré et al. (2019)
10-Hydroxy oleuropein aglycon $C_{19}H_{22}O_9$	DART	393.11925	–	Farré et al. (2019)
Dihydroxy oleuropein aglycon $C_{19}H_{22}O_{10}$	DART	409.11413	–	Farré et al. (2019)
Keto oleuropean aglycon $C_{19}H_{19}O_9$	DART	391.10356		Farré et al. (2019)
10-Hydroxy decarboxymethyl ligstroside aglycon $C_{17}H_{20}O_6$	LTP	319	301, 195	Lara-Ortega et al. (2018)
	DART	319.11895	–	Farré et al. (2019)
	PS	319	301, 291, 275	Lara-Ortega et al. (2018)
Oleaceinic acid $C_{17}H_{20}O_7$	DART	335.11394	–	Farré et al. (2019)
Phenolic acids				
Vanillin $C_8H_8O_3$	DART	151.03987	–	Farré et al. (2019)
Vanillic acid $C_8H_8O_4$	DART	167.03484	–	Farré et al. (2019)
p-Hydroxy benzoic acid $C_7H_6O_3$	DART	137.02418	–	Farré et al. (2019)
Ferulic acid $C_{10}H_{10}O_4$	DART	193.05066	–	Farré et al. (2019)
Protocatechuic acid $C_7H_6O_4$	DART	153.01913	–	Farré et al. (2019)

(Continues)

TABLE 3 (Continued)

Compound	Instrumental source setup	Negative ion m/z[a]	CID product ions m/z[a]	References
Lignans				
Acetoxy pinoresinol $C_{22}H_{20}O_8$	EASI	415	–	Riccio et al. (2011)
Flavonoids	Not detected	–	–	–

[a]Decimal values indicate the adoption of HRMS analyzers.

However, mass spectrometry has been also used, and nowadays it is more and more used, as a self-sufficient and complete analytical tool for both identification and quantification of bio-phenols in EVOOs. As it has been previously discussed for bio-phenols determination some analytical problem, even adopting the advanced HPLC-MS systems stand still: isomers separation, lack of pure substances and, potentially, matrix suppression.

To overcome the first problem, the isomer separation, this cannot be achieved even adopting the most resolving mass analyzers, as, being the elemental composition identical, isomers are characterized by the same accurate mass. Therefore, isomer separation shall be obtained based on chemical or sterical properties of the molecules. 2D liquid chromatography proven to be a possible approach, capable at least to reduce matrix effects (Arena et al., 2020) for more accurate determination of selected bio-phenols. On the other hand, a fully orthogonal separation system could be entirely based on the differing steric hindrances of bio-phenol isomers, adopting an ion mobility-based separation.

A combination of LC and ion mobility technique could be potentially very efficient to accomplish this task, that could have beneficial effect on other analytical aspects as sensitivity and accuracy.

Matrix effect on bio-phenol determination has been evaluated with two differing approaches (Arena et al., 2020; Luque-Muñoz et al., 2019). In both cases it has been evidenced that the matrix effect has a low influence on bio-phenol recoveries, this effect ranging from 3% up to 13%. That shows that matrix effect is less than a hassle for the most common LC-MS (/MS) approaches.

Recently ambient mass spectrometry techniques, which allow to speed up analysis times as well as to significantly reduce sample handling, are being used more frequently. The trade-off to achieve a fast analysis does not allow to efficiently separate bio-phenols isomers. Pure bio-phenol standards are still difficult to purchase. For this reason, the concentration of several bio-phenols has been often referred to the available reference material adopting different response factor based on the molecular structures and the detectors used (Pirisi et al., 2000). In addition, some authors have proposed the purification procedures that allow to isolate pure secoiridoids to use as quantitation reference (Karkoula et al., 2012, 2014). Lastly some secoiridoid standards have become available for the purchase, even if at a very steep price.

In conclusion this review highlighted that the most recent trends individuate in the MS approach the most convincing one, mostly due to its intrinsic selectivity. However, the cost of the mass spectrometry instrumental apparatus, far pricier with respect to simple LC-DAD devices in both aspects: initial investment and maintenance costs limit the number of laboratories that can afford it. On the other hand, EVOOs characterization, that is also based on the individual bio-phenols identification and quantification, is gaining momentum. Even if some simple methods to report the health claims "on polyphenols' oil" have been developed (Reboredo-Rodríguez et al., 2016), it is uncertain if their application could be approved by EFSA. On the other hand, the accurate bio-phenol determination, that could be achieved by MS methods should allow to more deeply characterize high-quality EVOOs. Probably it will not take long for these approaches to join to the official IOC method. Taking into account the actual need of more precise and accurate bio-phenols determination those based on mass spectrometry are surely among the best candidates to face this challenge.

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Serena Indelicato received degree in Pharmaceutical Chemistry and Technology in 2003 Summa cum Laude at University of Palermo, Ph.D. in "Pharmaceutical Sciences" at the same University in 2009. Degrees Summa cum laude of Master of "Cosmetic Sciences and Technology" at the University of Ferrara (2005) and of Master of "Mass Spectrometry and Applications" at the University Palermo (2008). Actual position: analyst at CQRC lab in Ospedali Riuniti Villa Sofia Cervello, in Palermo. Research activity mainly devoted to study self-assembling process of surfactants either in solution or in gas-phase and to clinical and food applications of mass spectrometry-based analytical techniques.

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Leopoldo Ceraulo graduated in Chemistry at the University of Palermo. Actual position: retired. At the University of Palermo he covered the following positions: Assistant Professor of Organic Chemistry, Associate Professor of Medicinal Chemistry, Full Professor of Medicinal Chemistry, Full Professor of Organic Chemistry, Director of the University Network of Laboratories (UniNetLab), Coordinator of the Master "Mass Spectrometry and Applications". He has been President of the Division of Mass Spectrometry of the Italian Chemical Society

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