Review

Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future

Lanfranco Conte[a], Alessandra Bendini[b,*], Enrico Valli[b], Paolo Lucci[a], Sabrina Moret[a], Alain Maquet[c], Florence Lacoste[d], Paul Brereton[e], Diego Luis García-González[f], Wenceslao Moreda[f], Tullia Gallina Toschi[b]

[a] Department of Agri-Food, Environmental and Animal Sciences, University of Udine, Via Sondrio 2/a, 33100, Udine, Italy
[b] Department of Agricultural and Food Sciences, University of Bologna, Piazza Goidanich 60, 47521, Cesena, FC, Italy
[c] European Commission, Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Retieseweg 111, 2440, Geel, Belgium
[d] ITERG, Institut des Corps Gras, rue Monge, 33610, Canejan, France
[e] Institute for Global Food Security, Queen's University Belfast, BT9 5BN, United Kingdom
[f] Department of Characterization and Quality of Lipids, Instituto de la Grasa, CSIC, Campus of Universidad Pablo de Olavide, Building 46, 41013, Seville, Spain

ARTICLE INFO

ABSTRACT

Background: The physical, chemical and organoleptic characteristics of olive oil (OO) are regulated by the European Union (EU) by Reg. (EEC) 2568/91 as amended, which also establishes methods for their analysis.

Despite the fact that the OO sector is highly regulated, it is acknowledged that there are still problems; fats and oils, including OOs, are ranked third, after meat and meat products and fish and fish products, in the 2016 EU Food Fraud report on non-compliances per product category.

For this reason, EU legislation, among the most advanced in the field, continuously chases after the emerging frauds. The process of proposing new methods or reviewing those current is constantly in progress, to ensure the robustness and the clarity required by official standardised procedures.

Scope and approach: This review will identify current gaps in EU legislation and discuss drawbacks of existing analytical methods with respect to OO. Suggestions for replacement of specific steps within the present EU methods with more efficient analytical solutions to reduce time and/or solvent consumption will be proposed.

Key findings and conclusions: This review critiques existing regulatory methods and standards, highlights weaknesses and proposes possible solutions to safeguard the consumer and protect the OO market.

1. Introduction

1.1. Normative and standard sources for olive oil quality and purity: a global framework

OOs have to comply with different rules and standards depending on where they are traded: three of the most important standards are those specified by the EU, the International Olive Council and the Codex Alimentarius. Within the EU, all OO legislation is comprised of regulations, i.e. mandatory rules. The early Regulation by EEC where olive oil has been mentioned was Reg (EEC) 136/66, a regulation for the establishment of a common organisation of the market of fats and oils that posed the basis for the descriptions and definitions of olive oils

and residue olive oils marketed within the Member States and third countries. It, however, just established descriptions and definitions of different types of olive oils and did not report a detailed list of analytical parameters and related analytical methods. Further Regulations were later published: Reg (EEC) 177/66, repealed by Reg (EEC) 618/72, subsequently repealed by Reg (EEC) 1058/77, which was finally repealed by Reg (EEC) 2568/91. This latter is the cornerstone of all EU legislation on OO, establishing four important issues:

- the parameters that can be used to check for OO quality and purity, also indicating that no other parameters can be used for this purpose when an official control is carried out;
- limits for each parameter and commercial category of OO;

- descriptions of analytical methods that have to be used to assess if a sample of OO fits the limits (specification) of the commercial category for all the parameters in the regulation;
- only methods reported within this regulation, as amended, can be used for official control.

The EU Regulations are valid within the EU area, while outside this, International Olive Council (IOC) standards apply. The IOC, formerly the International Olive Oil Council, established in 1963 a "Trade standard for olive oils and olive pomace oils" that is a reference for any country which is a member of IOC. Member countries are obliged to apply it in the frame of international trade and, in the meantime, are encouraged to approximate their legislation to IOC Trade standard. After EEC turned to EU, the latter became a member of IOC (while, earlier, it was an observer and single countries were members), so that EU must harmonize its regulation to IOC Trade Standard. If OOs are considered in a worldwide scenario, different rules must be considered: in such a case, edible OOs, as most of foods, undergo to the standard developed by Codex Alimentarius whose Commission has at now more than 180 Members made up of 188 Member Countries and 1 Member Organisation (The European Union) (i.e. within the frame of FAO-OMS), whose mission is to facilitate the international trade of foods and to reach harmonisation, that is a very hard mission due to the high number of countries involved. The OO standard is CODEX STAN 33–1981, reviewed in 2017. The Codex standard has a unique structure: some essential purity and quality characteristics are fixed as mandatory, while for a number of further characteristics adoption by a member is voluntary.

1.2. Standard establishment procedure

Fig. 1a, b and c summarise the basic procedure for standard elaboration in EU, IOC and Codex, respectively. The ultimate approval by Codex is made by a plenary session that is held alternatively in Rome and Geneva; the process of revision usually takes about four years.

1.3. Mandatory and voluntary standards

EU Regulations are mandatory within the EU area and must be applied without any further procedures by member countries; however, outside of the EU, the same regulations cannot be applied. The IOC is in charge of developing standards that can also be applied outside of the EU, by a large number of countries as for the Codex Standard. Unlike EU Regulations, the use of these standards is not strictly mandatory: if an EU operator does not respect an EU regulation, he can undergo a penalty, while the use of IOC and Codex standards is not mandatory. These latter two standards are adopted based on a consensus, so that it should be expected that anyone who signs it would respect it; however, the IOC and Codex are not in a position to apply penalties if a member does not apply their standards.

1.4. Analytical methods: drawbacks, inappropriateness, eventual normative failures and suggestions for improvement

Analytical methods adopted within EU Regulations and IOC Standards have often originated from methods developed previously in individual Member States. These methods underwent some updates, taking account of improvements in analytical instrumentation (e.g. replacement of packed columns with capillary columns in gas chromatography), to considerations about solvent toxicity or through the possibility to improve the method. Table 1 reports a list of official methods, their current drawbacks and possible improvements.

1.4.1. Determination of the peroxide value (PV)

PV is probably one of the oldest analytical tests used for quality evaluation of fats and oils and dates to the "chemistry of indexes" (a term used to identify a number of tests developed before separative techniques, mainly gas chromatography, were widely used). PV is related to only the primary oxidation products therefore it does not give an exhaustive representation of the oxidative status of an oil (e.g. secondary compounds are also formed during the oxidative process). Briefly, the method (Reg. (EEC) 2568/91) is based on a redox reaction between peroxide and an iodine ion, the latter being oxidised to molecular iodine. In the original method the oil is dissolved in a mixture of chloroform (that dissolves the oil) and acetic acid (to provide the acid reaction medium necessary for the redox reaction to take place). Successively, a saturated solution of potassium iodine is added and in the original method the reaction takes place for 5 min in the dark. Later, ISO (ISO, 2003) amended the test by substituting the toxic chloroform with isooctane and limiting the reaction time to 1 min. The main drawback when isooctane is used, instead of chloroform, is the fact that the addition of water produces an inversion of phases with the titratable one remaining at the bottom of the Erlenmeyer flask, necessitating the need for very efficient mixing to be applied in order for accurate titration to be carried out. For this drawback, the IOC, also, after some collaborative tests, returned to the use of chloroform as solvent. A further problem is that PV evaluation is strongly influenced by the amount of sample used for determination; thus it is particularly important to take care of the right amount of sample to be weighted (depending on the expected peroxide value, as recommended in the Annex III of the Reg. (EEC) 2568/91).

### 1.4.2. Determination of the ethyl esters of fatty acids (FAEE)

Oil quality depends by both agricultural aspects and manufacturing practices. The content of FAEE is related to low quality fruits that may have undergone fermentative processes in the case of ethanol, while hydrolytic processes linked to pectin esterase activity releases methanol. Since fermentation processes are thought to be only related to FAEE, the parameter was changed in 2013 to these compounds with a limit of 40 mg/kg, which was then reduced to 35 mg/kg after discussion within the IOC expert chemist group. Low quality OO with slight organoleptic defects may be subjected to illegal practices, such as neutralisation and/or soft deodorisation at low temperature, to conceal their negative attributes. This practice is difficult to detect and several methods have been proposed, although most have produced unreliable results due to the fact that different technologies are used, leading to the formation of a variety of different marker compounds. In an attempt to solve this problem, the determination of the content of FAAE was proposed (Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo, & Moreda, 2008). It is believed that the soft deodorisation used by fraudsters does not remove FAEE, thus remaining as indirect markers of soft deodorization, when this process is applied on oils sensory defected for fermentative reasons (Biedermann, Bongartz, Mariani, & Grob, 2008). On the other hand, not all oils subjected to soft deodorisation (e.g. rancid oils) have a high amount of FAEE, so the FAEEs are obviously markers for some and not all the oils subjected to soft deodorization (Gómez-Coca, Fernandes, Pérez-Camino, & Moreda, 2016).

The method was adopted by IOC in 2010 (IOC, 2010, pp. 1–17) and by the EU (amending Reg (EEC) 2568/91). The method has been modified several times: for example, as reported in the IOC website, a reduction of the amount of silica, as well as the use of *n*-hexane to eliminate the hydrocarbon fraction, was proposed. The latter is an important issue, since hydrocarbons can elute in the same region of the chromatogram as FAEE, and the purity of solvent is a key point in the analysis. As stated before, the amount of FAEE was thought to be stable over time, but this was shown to not be true since the amount of FAEE increased over time (Gómez-Coca et al., 2016), (Mariani & Bellan, 2013). Possible suggestions to improve the official procedure concern two main issues:
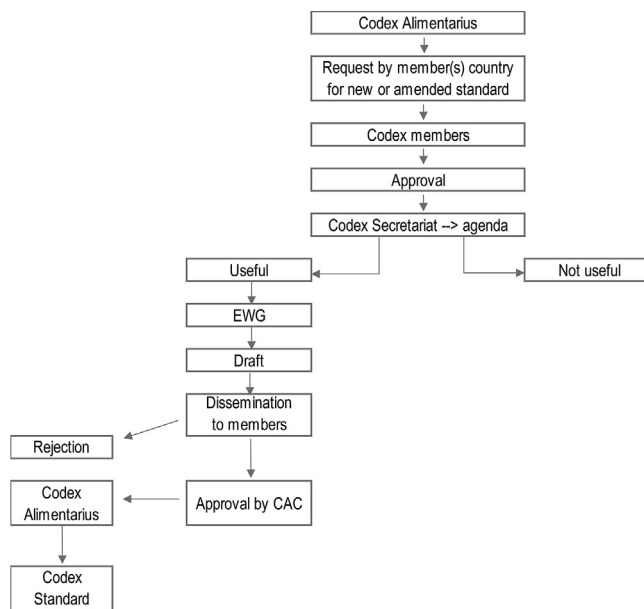
i) the separation of the fraction containing ethyl esters, by a preparative liquid chromatography on a hydrated silica gel column,

L. Conte, et al.
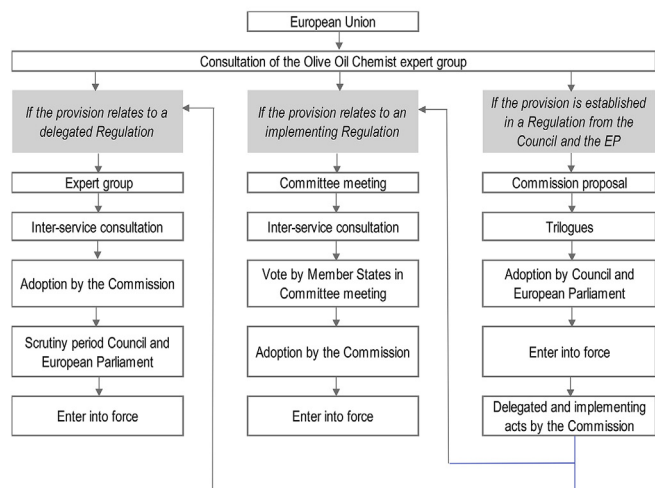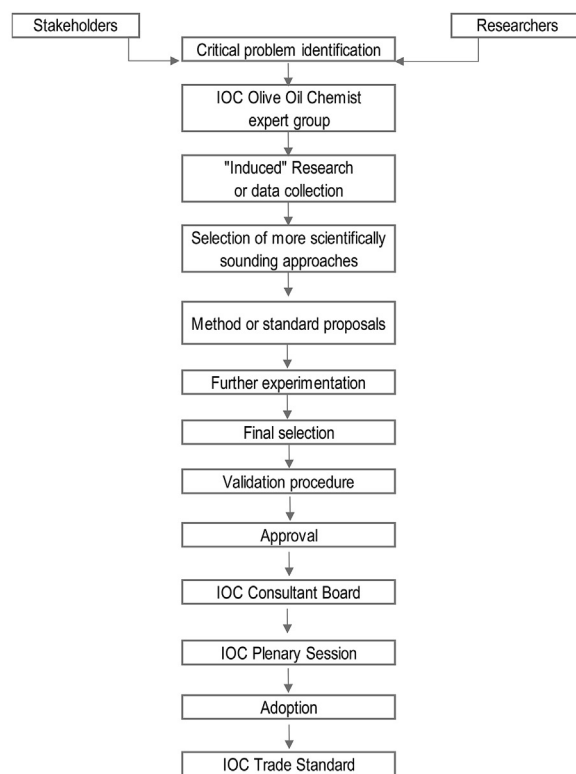
Fig. 1. a. Flow chart of standards development procedures within EU. b. Flow chart of standards development procedures within IOC. c. Flow chart of standards development procedures within Codex Alimentarius.

that is particularly laborious and time consuming and requires large volumes of solvents (about 70 mL of n-hexane and 220 mL of a n-hexane/diethyl ether mixture per replicate) and quantity of silica gel (around 15 g per replicate)

ii) the use of an on-column injector for the final GC analysis, that is not a widespread system in the laboratories all over the world due to its very limited used in analytical methods for the quality control.

For the first task, an alternative could be the fractionation by SPE or HPLC to reduce solvents and speed the preparative step, while for the second a PTV (Programmed Temperature Vaporization) could be considered a promising alternative, as one of the most versatile sample introduction devices for GC. A revision of the actual official method for the determination of the ethyl esters of fatty acids in virgin olive oils could take into consideration these ways of improvements.

Table 1
Methods for quality and purity evaluation of olive oils applied in official controls, related drawbacks and possible improvements.

	Purpose	Method	Drawbacks	Possible improvements
Quality assessment	Quality of raw matter (fruits)	Acid Basic titration Reg (EU) 2016/1227 Annex II	Use of toxic phenolphthalein.	Possibility to substitute phenolphthalein with alkali blue 6B or thymolphthalein.
	Oxidation status	Peroxide value Reg (EEC) 2568/1991 Annex III	Use of toxic solvent (chloroform).	Already experimented replacement of chloroform with isooctane: poor results depending on problem of solvent miscibility. ISO validates a potentiometric end point determination. Measurement of optical density of an emulsion between the oil sample and ferrous ion oxidation with xylenol orange reagent.
	Oxidation status	UV Absorption Reg (EU) 2015/1833 Annex IX	None.	Indication to check the response of the photocell and photomultiplier (wavelength and absorbance scales) by reference materials.
	Lower quality olive oils	Ethyl esters Reg (EU) 1348/2013 Annex XX	Sample preparation time lengthy. Need to use an on-column injector for GC analysis.	Shorten the time of the preparative step by proposing HPLC or SPE as an alternative to the traditional liquid chromatography. Use of PTV injector instead of the on-column injector.
	Quality grade of virgin olive oils	Sensory assessment Reg. (EU) 1348/2013 Annex XII	Low number of samples assessed per day, accuracy.	Reduce the number of samples to be assessed by panellists thanks to predictive models built using instrumental techniques based on the volatile compounds analysis. Formulate new reference materials for improving the training of assessors.
Purity assessment	Presence of extraneous oils	Fatty acids composition Reg.(EU) 2015/1833 Annex X	Not effective in case of blends with specific oils (e.g. high oleic sunflower), lack of sensitivity for some other blends.	None.
	Presence of extraneous oils	Sterols composition Reg. (EU) 1348/2013 Annex V	Sample preparation time lengthy.	Save time: i) Unsaponifiable fractionation by HPLC instead of TLC ii) Microwave Assisted Saponification (MAS) Improvement of information on free and esterified sterols
	Presence of extraneous oils	Δ ECN42 –TAGs Analysis Reg. (EC) 2472/1997 Annex XVIII	ECN42 instead of LLL depending on poor separation due to isocratic elution that is mandatory because of refractive index detector.	Improve HPLC separation of LLL e.g. by UHPLC-CAD. GC TAGs analysis suitable to detect small amounts of selected oils (HOSO, palm olein).
	Presence of extraneous oils	Global method–TAGs Analysis Reg. (EU) 1348/2013 Annex XX bis	False positive results?	Improvement of method performances.
	Presence of refined oils	Stigmastadienes Reg. (EEC) 656/1995 Annex XVII	Sample preparation time lengthy.	Direct analysis even if concentration is < 4 mg/kg.
	Presence of esterified oils	2 glyceril monopalmitate Reg. (EC) 702/2007 Annex VII	Need to use an on-column injector for GC analysis.	Use of PTV injector instead of the on-column injector.
	Presence of pomace oils	Waxes determination Reg. (EEC) 183/93 Annex IV	Sample preparation time lengthy. Need to use an on-column injector for GC analysis. GLC analysis: poor separation of selected compounds (e.g. C40).	Shorten the time of the preparative step by proposing SPE as an alternative to the traditional liquid chromatography. Use of PTV injector instead of the on-column injector.

1.4.3. Sterol composition

Since genetic improvement of edible seed oils deeply modifies their fatty acid composition, attention has been given to sterol composition, and determination of these compounds has become a powerful tool to assess their purity. The official EU method for determining sterols implements the IOC method by the use of GC capillary column instead of packed column. The method involves a saponification step with KOH solution in methanol, followed by liquid-liquid extraction with diethyl ether, clean-up of the unsaponifiable matter by thin layer chromatography (TLC), preparation of trimethylsilyl derivatives and GC-FID analysis. As reviewed by Panagiotopoulou and Tsimidou (Panagiotopoulou & Tsimidou, 2002) different approaches, mainly based on the use of solid phase extraction, have been investigated to speed up sample preparation. The ISO 12228:2009 (included in Codex standard) purposed a method circumvents solvent-extraction, replacing it with solid-phase extraction (SPE) on aluminum oxide. However, a footnote reports that results obtained by this method can lead to different results from those obtained by liquid-liquid extraction: because of this, part 2 of the method (ISO, 2014) was later published, devoted to OO and olive pomace oils only. With the aim to save time, some modifications of the saponification method have also been proposed.

Moreda suggested to carry out unsaponifiable fractionation by HPLC instead of TLC (Cert, Moreda, & García-Moreno, 1997). Earlier results of this approach have been presented at the IOC Olive Chemists group and the method's characteristics seem very promising.

On-line HPLC-GC determination of sterols have improvements in terms of time and solvent, increased sensitivity, avoiding sample contamination, but require dedicated instrumentation and skilled operators. In 1993, a method of this kind was proposed by Biedermann et al. (Biedermann, Grob, & Mariani, 1993). By optimising HPLC conditions, it was possible to separately analyse the individual classes of minor compounds, or to send them to the GC as a single fraction. Determination of sterols and other minor components of the oil were later achieved by employing HPLC-GC techniques with PTV based interface (Toledano, Cortés, Andini, Vázquez, & Villén, 2012). The use of reverse phase HPLC allowed to avoid the backflush of the LC column for eliminating any retained lipids, that is a step requested when using the normal phase. More recently, a fully automated method (Nestola & Schmidt, 2016), including a saponification and extraction step (performed by the autosampler), followed by injection into the LC-GC without previous derivatisation, was developed. When applied to OOs, the quantitative results were fully comparable with the ISO method.

Besides being time consuming, the official method (ISO, 2014) only evaluates the whole sterol composition, with no possibility to distinguish between free and esterified ones, despite the fact that such information can be useful to distinguish oils that present slight differences in whole sterol composition. A number of papers have highlighted that sterols in plant and vegetable oils extracted from both seeds and fruits may be in a free or esterified form and that the ratio between the two forms is not constant, and varies for different oils (Grob, Lanfranchi, & Mariani, 1989). Mariani et al. (Mariani, Bellan, Lestini, & Aparicio, 2006) proposed the evaluation of free and esterified sterols to highlight the presence of hazelnut oil in OO.

1.4.4. Determination of stigmastadienes

In 1975, Niewiadomski elucidated the structures of hydrocarbons deriving from the dehydration of sterols. Later, in 1989, Lanzon et al. (Lanzon, Cert, & Albi, 1989) proposed the use of the determination of these compounds to detect oils that had been refined. Cert et al. (Cert, Lanzon, Carelli, Albi, & Amelotti, 1994) identified stigmasta-3,5-diene, derived from β-sitosterols as the main compound of interest, and in 1995 it was adopted within the trade standards (Reg (EEC) 656/95, IOC, 2001b); the limit was fixed at 0.15 mg/kg for edible virgin OOs and 0.50 mg/kg for lampante OOs. Later the limit was amended to 0.10 and then to 0.05 mg/kg for edible virgin oils. IOC split the method into two parts: when the expected concentration is between 0.01 and 4.0 mg/kg, saponification of 20 g of oil is carried out, followed by LC on silica column to isolate the sterene fraction, while when the concentration is more than 4 mg/kg (refined oils), direct fractionation of the oils on silver ion silica is carried out.

One of the suggested purposes of this analytical determination was the possibility to check for the presence of seed oils depleted in sterol concentration in refined OO. The starting point was the consideration that the ratio between selected sterols should also be maintained in the sterene fraction. However, the dehydration reaction takes place at different speeds depending on whether it involves free or esterified sterols, the latter being slower. Attempts to improve the method e.g. by saving time were published by Grob et al. (Grob, Artho, & Mariani, 1992; Grob, Biedermann, Artho, & Schmid, 1994), Biedermann et al. (Biedermann, Grob, & Bronz, 1995) and Amelio et al. (Amelio, Rizzo,& Varazini, 1998).

1.4.5. Waxes composition and triterpenic dialcohols content

The analytical determination of fatty acid and sterol composition made it possible to detect the presence of seed oils (when sterols were not removed). Another possible fraud is the blend between pomace oils and refined OOs, so that no problems with the presence of fatty acids *trans*-isomers and stigmastadienes occur.

Since early '70s, erythrodiol and uvaol, two triterpenic dialcohols, whose concentration is very high in pomace as they are mainly present in the fruit skin, have been used as potential markers of pomace oil. A limit of 4.5% (calculated on the sum of sterols and erythrodiol plus uvaol) was established for any non-pomace OO. Due to the fact that fraudsters started to remove these compounds, the evaluation of the content of waxes, suitable to detect the presence of pomace oils, was proposed by Mariani and Fedeli (Mariani & Fedeli, 1986). The scientific basis was that waxes concentration is about tenfold in solvent extracted oils with respect to pressure extracted oils and that any attempt of remove them would lead to a significant loss of oil. The method was adopted as the EU and IOC official one in 1997. It had been widely and successfully applied for a number of years, and was also approved as the ISO method. In 2007 Ceci and Carelli (Ceci & Carelli, 2007) noted that when applying the method to selected authentic Argentinian extra virgin OO anomalous results were produced. It was discovered that the main problem was the measurement of the area of C40 peak, as this is split into two peaks that are not well resolved, one of which (the smaller) comprises the linear esters C40, while the other one is phytil behenate. A satisfactory separation of the two peaks, mainly when high concentrations of phytil behenate are present, may be problematic for less skilled laboratories, so that the proposed and adopted solution was to delete the C40 peak area in the sum for extra virgin and virgin oils.

Some improvements in the method need to be found, on one hand in enhancing the separation of GC peaks (e.g. by using a slightly more polar stationary phase), and on the other hand by reducing the need for the large volumes of solvents that are currently used. Nota et al. (Nota et al., 1999) applied a silica SPE, and Amelio et al. (Amelio, Rizzo, & Varazini, 1998) were able to separate waxes by a single HPLC run. LC-GC also remains a suitable approach.

1.4.6. Triacylglycerol analysis

Triacylglycerols (TAGs) consist of a glycerol moiety with each hydroxyl group esterified to a fatty acid (FAs). Twenty TAGs have been identified and independently quantified in olive oil, but only five are present in significant proportions (León-Camacho, Morales, & Aparicio, 2013).

The analytical evaluation of TAGs is carried out with different purposes:

1. To evaluate the botanical origin of the oil: the pathway for biosynthesis of TAGs, the so-called "Kennedy pathway", is the same in seeds and fruits. The flux of the pathway and, thus, the final amount of TAG synthesized, is mainly under the control of the first step of FA synthesis in about 27–31% in seeds, while in the case of fruits it is about 57% in olive and 61–65% in palm. Therefore, based on this knowledge, it is possible to check if an oil is extracted from fruits or from seeds.
2. To discriminate TAGs from plant biosynthesis from those of chemical synthesis, the latter obtained by a reaction between FAs (e.g. from oil refining) and glycerol (e.g. from biodiesel production): this is possible because the lysophosphatidic acyltransferase is selective to oleyl-CoA and has no activity to saturated fatty acids. The result is that for the 2-position of triacylglycerol molecule, no more than 1% of palmitic acid can be detected in most authentic vegetable oils (with the exception of palm oil), and does not depend on seed or fruit oils.

The International Olive Council trade standard (IOC, 2016), and Reg. (EEC) 2568/91 established methods for the control of the authenticity of OO; among them, the determination of FA composition in relationship with triglycerides seems to be very useful (Synouri, Frangiscos, Christopoulou, & Lazaraki, 1995). Within this context, trilinolein was identified as a possible and powerful marker for detecting the presence of other vegetable oils. However, the incomplete separation of trilinolein peak, and therefore the difficulty of accurate quantification using the refractive index, have prompted the scientific community to replace trilinolein content with the use of the equivalent carbon number (ECN) 42. The experimental and the calculated values of ECN42 are compared to assess the degree of correlation between FA composition and ECN42 TAGs. A limit of |0.20| as an absolute value in extra virgin OO had been established. An improvement of this method was obtained with the so-called global method (IOC, 2001a), which uses the same principle of the ECN42 but extended to some TAG of ECN44 and ECN46. With this method, the limit of detection of hazelnut oil in OO was set at 8–10%. The analysis of TAG to apply this method was also improved using propionitrile as HPLC eluting solvent (Moreda, Pérez-Camino, & Cert, 2003). Other approaches are being studied to compare reliability with the official method (Beccaria et al., 2016). Some drawbacks have arisen in terms of false positive results, with the result that in 2015 the method was deleted from the EU legislation.

In light of this, two main linked strategies for improving the TAGs analysis in OO can be considered:

1) the use of columns packed with sub-2 μm particles or with fused-core particle to avoid high backpressure; 2) the use of detectors that can accommodate gradients. Regarding the first point, it is well known that

reduction of particle size down to sub-2 μm (compared to conventional columns packed with 3 or 5 μm particles), as well as the use of columns packed with superficially porous particles, allows speeding up of the analytical process by a factor of 9 while maintaining similar efficiencies or a theoretical 3-fold increase in efficiency for a similar column length (Núñez, Gallart-Ayala, Martins, & Lucci, 2012). C18 phase, which is the most common, can provide satisfactory results in gradient elution even if other promising stationary phases such as C30 can also be explored to further improve the separation efficiency of TAGs. With regard to the second point, the use of universal detection in gradient elution is another crucial aspect. Two possible alternative methods to RI detection are represented by evaporative light scattering detection (ELSD) and charged aerosol detection (CAD). ELSD, however, provides a non-linear response, thus preventing accurate measurements of TAGs. On the contrary, CAD has been shown to provide linear response as well as homogenous response factors for the main TAGs in OO. Another option is analysis by HPLC-MS because MS can identify partially resolved HPLC peaks (Cozzolino & De Giulio, 2011), giving much more information on the position of the three FA molecules in TAGs. Although few ionisation techniques can be coupled to HPLC, the identification of positional isomers can be carried out by atmospheric pressure chemical ionisation (APCI) coupled to HPLC, whereas the identification of individual acyls cannot be done without electrospray ionisation (ESI) and reference materials. An interesting alternative is the use of atmospheric pressure photoionisation (APPI), which has been recently introduced for the ionisation of non-polar compounds that are insufficiently ionised by either APCI and ESI sources. In APPI, MS spectra TAG ions are present mainly as $[M+H]^+$. However, other fragment ions present in APPI-MS are those to acylium ion $[RCO]^+$ and $[RCO-H_2O]^+$ (Gómez-Ariza, Arias-Borrego, García-Barrera, & Beltran, 2006). This latter ion, which is absent in ESI mass spectra, provides valuable complementary information for identification of TAG molecular species. However, mass spectrometry is an expensive instrument that also requires skilled personnel. Therefore, it probably does not represent the best choice for routine TAG analysis.

Finally, GLC is scarcely applied today even though it also offers attractive possibilities as an efficient separation method, good quantitative recovery and reproducibility, adequate time for analysis and the availability of a flame ionisation detector (FID), a simple but universal linear response detector. However, the GLC technique is not free of problems such as, for example, the injection system and column deterioration. Selectivity in GLC depends on the length and chemical nature of the column stationary phase. For example, columns with phenyl-methyl-silicone phase can reach temperatures of about 360–370 °C for a long period and separate TAGs by carbon atom number; unsaturated positional isomers cannot be separated by this phase unless sample derivatisation followed by a reduction is previously performed (León-Camacho et al., 2013).

As stated above, the determination of FA composition in position 2 of TAG molecule gives very important information; this analysis applies enzymatic hydrolysis with pancreatic lipase, which is selective for positions 1 and 2, and in the original method the reaction mixture made by TAGs, DAGs, MAGs and FFA was fractionated on silica gel TLC plates, MAGs were recovered, submitted to transmethylation and finally GLC analysis of fatty acids was carried out (IOC, 2006). The method was very time consuming and cumbersome and several artefacts were detected, mainly depending on silica plate constituents. Thanks to the development of capillary GC, Motta et al. (Motta, Brianza, Stanga, & Amelotti, 1983) were able to skip all the procedures after hydrolysis and directly inject the reaction mixture after silylation; the method was adopted in Italy and later by the IOC and EU.

1.4.7. Method for organoleptic assessment of OO

A virgin OO, obtained from olives only by mechanical-physical processes (crushing, malaxation, centrifugation, filtration) and without additional refining, has a sensory profile strongly linked to the quality of raw material, namely olives. In fact, any damage to drupes and subsequent activity of microorganisms and enzymes, which can trigger triacylglycerol hydrolysis, FA oxidation, sugar fermentation and amino acid degradation, produces molecules that affect the compositional profile of the virgin OO obtained, mainly in terms of phenolic and volatile compounds (Angerosa et al., 2004; Cayuela, Gomez-Coca, Moreda, & Perez-Camino, 2015; García-González, Tena, & Aparicio, 2007; Morales, Luna, & Aparicio, 2005).

In the context of food regulation, there is no food other than virgin OO whose quality categories are defined with different international standards (e.g. Codex Alimentarius, International Olive Council, and European Union) including sensory assessment. The organoleptic assessment of virgin OOs by the "IOC Panel test" methodology (IOOC/T.20/Doc. no. 3, 1987) has been applied with a legal purpose since the early nineties in Europe (Reg. (EEC) 2568/91), and specifically to classify a sample within a commercial category. Over the years, it has undergone many revisions, as a result of a continuous study of its performance. In fact, since the most important result for sensory analysis of a virgin OO is to define its quality grade, identification of the main perceived defect and evaluation of its intensity, as well as of the fruity attribute, are the main outputs. Keeping in mind this objective, the methodology has been amended exhaustively starting from the Reg. (EEC) 2568/91 Annex XII:

i) The updated profile sheet requests evaluation of descriptors using a continuous 10-cm scale instead of the original score from 0 to 5; moreover, the median value related to the intensity of each attribute is calculated, thus replacing the overall score from 1 to 9 (introduced by amending Reg. (EC) 796/2002). The sensory data, collected by the panel leader, can be elaborated by an excel program that permits to calculate for each attribute: median value (indicates the 50th percentile of a distribution of numbers arranged in increasing order), the robust coefficient of variation % (useful for checking the reliability of the panel assessors), the 95% confidence intervals (represents the interval of variability of the test under operating conditions, assuming it is repeated many times) (introduced by amending Reg. (EC) 796/2002).

ii) More emphasis has been given to the list of defects (fusty/muddy sediment, musty-humid-earthy, winey-vinegary-acid-sour, rancid, frostbitten olives-wet wood, others), which is now present in the upper section of the profile sheet. Specifically, a unique negative attribute named "fusty/muddy sediment" has been inserted (by amending Reg. (EC) 640/2008) instead of the two separated ones. This merge was aimed to limit the variability of tasters in the identification of these two single defects; even if fusty and muddy are elicited by slightly different qualitative-quantitative volatile profiles (Angerosa et al., 2004; Aparicio, Morales, & García-Gonzalez, 2012; Bendini, Valli, Barbieri, & Gallina Toschi, 2012; Cayuela et al., 2015; Procida, Giomo, Cichelli, & Conte, 2005), because they are produced by specific microorganisms, but during different steps of olive processing ("fusty" when olives are stored in piles, while "muddy" when the olive oil is in contact with the sediment); these two defects were often confused by assessors, thus causing difficulties in the calculation of the main perceived defect.

iii) By the Reg. (EU) 1348/2013, the defect named "frostbitten olives-wet wood" already included among the "other negative attributes" according to Reg. (EC) 640/2008, has been moved to the list of the main defects and a IOC reference standard has been made available. This sensory defect, unusual until a few decades ago, has become a common defect today and little is known about its related volatile markers. The origin of this defect has been linked to the change in weather conditions during the autumn-winter period that can cause extracellular or intracellular ice formation in olives by a gradual drop of temperature or a rapid freezing repeated in several freeze–thaw cycles, respectively. The final effect due to the forming of ice crystals is cell dehydration and parenchyma destruction as well

as an increased contact between enzymes and their respective substrates that bring about the formation of several volatile compounds (Morello, Motilva, Ramo, & Romero, 2003; Romero, García-Gonzá;lez, Aparicio-Ruiz, & Morales, 2017). The identification and quantification of the most important markers of this defect would be highly desirable to understand better its origin and to define accurately its sensory notes. A recent study has highlighted the sensory importance of some volatile compounds, such as some esters with ripe fruity attributes, typically found in samples with this defect (Romero, García-Gonzá;lez, Aparicio-Ruiz, & Morales, 2017).

iv) The list of positive attributes has been restricted only to fruity, bitter and pungent, with the possibility of using a specific optional terminology for labelling purposes according to the frequency (green or ripe fruitiness, in Reg. (EC) 640/2008), and by specifying the intensity of these attributes (e.g. robust, medium, delicate, well balanced, mild oil in the Reg. (EC) 640/2008, amended by Reg. (EU) 1227/2016).

v) The organoleptic assessment is both a qualitative and quantitative method, since its application results in the classification of samples based on the median of the predominant defect and the presence or not of the fruity attribute. Consequently, tasters must be supervised for correct classification of samples and for correct recognition of the intensities of perceived attributes. With this aim in mind, it is strictly recommended that the IOC member laboratories performing sensory analysis of virgin OO apply the guidelines for the accomplishment of the Norm ISO 17025 requirements (COI/T.28/Doc. No 1, 2007 – revised in 2017), in order to work in compliance with the characteristics of a quality system (drawing up, implementing and maintaining of procedures, identifying possible minor, major or critical nonconformities) and also with the laboratory organisation and technical conditions of analysis requirements (COI/T.20/Doc. No 15/Rev. 8, 2015). In agreement with these guidelines, accredited laboratories should demonstrate that they obtain equivalent results within defined limits in terms of precision (repeatability and reproducibility) by calibration and testing activities (inter-laboratory tests), adopting the correct methodology to monitor the panel proficiency. The periodic participation of laboratories in proficiency tests (recommended at least once a year) permits detection of possible systematic errors and to check the validity of the entire quality system. Since current reference materials are "natural" virgin olive oils selected for being representative of a single sensory defect, they can be slight different year by year in sensory properties and intensity of the defect. On the contrary, a perfect reproducibility of each defect would be extremely useful to align all the panels. The availability of certified reference materials (e.g. samples from inter-laboratory tests conducted by the IOC or other accredited suppliers), having intensity ranges for specific attributes that cover different classes of virgin OO, can assure correct training of sensory assessors, and is useful to determine the trueness of the evaluation carried out by tasters (closeness to the accepted reference value is a measure of accuracy). It is particularly important to improve the sensory skills of the panel through the adoption of new formulated reference materials (RMs) built with a specific mixture of sensory relevant volatile molecules and appropriately combined in defined concentrations, also considering their odour thresholds.

In consistency with decisions taken at IOC level, the Reg. (EU) 1348/2013 recommends the number of oils to be assessed by the sensory panels, fixing a maximum number of four samples at each session. Moreover, a maximum of three sessions per day is specified, to leave enough time between a session and another, thus avoiding the contrast effect that could be produced by immediately tasting sequences of samples. These specifications strongly limit the number of samples (namely 12) that can be assessed by one panel per day; the establishment of instrumental methods for a rapid screening could represent a

solution for supporting the sensory panels (particularly for the large private industries) in the discrimination of sample far away from the boundaries (EVOO/VO and VO/LO). Actually, the need to support organoleptic analysis was also reported in a specific call of the Horizon 2020 EU program (H2020-SFS-14a-2014) and is one of the main objectives of the OLEUM project (Horizon 2020; Grant Agreement No. 635690). With this purpose, some encouraging examples of analytical instrumental techniques, of which many are based on the determination of volatile markers, have been proposed in the literature. These, thanks to the application of appropriate multivariate analysis tools, try to find relationships by targeted and untargeted approaches between instrumental signals (fingerprints) and sensory quality attributes (Aparicio, Morales, & García-González, 2012; Borràs et al., 2016; Lerma-García et al., 2010; Morales, Aparicio-Ruiz, & Aparicio, 2013; Morales et al., 2005; Procida et al., 2005; Sinelli, Cerretani, Di Egidio, Bendini, & Casiraghi, 2010; Vichi, Romero, Tous, López Tamames, & Buxaderas, 2008). Due to its rapidity, one of the most promising screening method under testing to support the sensory analysis is the flash gas-chromatography electronic nose) used in tandem with chemometrics (Melucci et al., 2016).

The volatile compounds, as molecules strongly dependent on the OOs sensory profiles, should be considered as relevant quality markers for OOs; the determination of these compounds could support the sensory analysis, especially within the so-called "boundary zones" between virgin OOs designations (e.g. extra virgin OO vs. virgin OO). In particular, during the last years researchers are working hard for the setting up of robust analytical methods for evaluating the quali-quantitative profiles of volatile compounds in OOs (Fortini, Migliorini, Cherubini, Cecchi, & Calamai, 2017; Romero, García-González, Aparicio-Ruiz, & Morales, 2015); further research efforts should be done in focusing on a low number of volatile compounds, previously selected as relevant markers of the sensory defects, to be determined by possibly using less expensive instruments, such as SPME-GC-FID.

1.5. Normative failure and inappropriateness

Despite the fact that OO is highly regulated, some critical aspects of the OO sector as i) the lack of proper analytical methods for identification of specific frauds ii) the lack of a defined method for specific markers, remain. There is therefore an urgent need to resolve these gaps and limitations in regulatory methods and frameworks and to identify appropriate analytical solutions for specific fraud and marker detection, as well as to provide relevant information required from international markets. Specific cases of the above-mentioned lacks are discussed in the following paragraphs: the assessment of the amount of olive oil in mixtures with seed oils and the assessment of deodorised oils as examples of i) whereas the methods to assess polyphenol health claims, to estimate OO freshness and to verify geographical origin as examples of ii).

1.5.1. Health claims related to selected polyphenol content

Consumers are cautious about the nutritional and health claims provided on food labelling, which are expected to assist them in making purchase decisions. To increase confidence in the market and ensure a high level of consumer protection, the European Food Safety Authority (EFSA) works for the approval of clear, accurate and corroborated nutritional and health claims (EFSA, 2011). Substantiation of a nutritional or health claim is often a time-demanding procedure that involves approval of several evaluation steps. For example, a health claim for "olive oil polyphenols" was made only very recently after many years of discussion (Reg. EC 432/2012). The health claim stated: "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress." The claim may be used only for OO that contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of OO. Furthermore, information is given to the consumer that "the beneficial effect is obtained with a daily intake of 20 g of olive

oil". This health claim presents some weaknesses regarding terminology interpretation and analytical methodology. The term "olive oil polyphenols" is not entirely clear and accurate, considering that only fresh virgin OO of high quality contains considerable amounts of oleuropein/ligstroside aglycons and derivatives. "Olive oil" is a generic term for all types of oils extracted by mechanical means from olives. Moreover, the term "polyphenols", probably derived decades ago from terminology used for wine phenolic compounds, does not match the basic structure of the secoiridoids present in virgin OO for which the claim was assigned (i.e., hydroxytyrosol and its derivative, e.g. oleuropein complex and tyrosol). Beyond concerns for ambiguous interpretation of the terminology, there is a lack of a standardised analytical methods that allow quantitative determination of unequivocally identified individual phenolic compounds belonging to the group of hydroxytyrosol/tyrosol and its derivatives. The latter comprises more than 10 identified compounds. This lack has an impact on the reliability of the lower limit set (5 mg/20 g oil) for the health claim.

A candidate protocol could be that recommended by the International Olive Council (IOC, 2009). However, difficulties in complete separation of all types of phenolic compounds in a single chromatographic run and limitations in the choice of standards for accurate quantification in the UV region or using other detection means, repeatedly discussed over the past 20 years, does not support its adoption for standardisation. To address such a challenge, any experienced analytical chemist would reach simplify the analytical protocol. Simplification in this case would involve hydrolysis of the bound forms of hydroxytyrosol and tyrosol, and quantification of their total free forms (Mulinacci et al., 2006; Purcaro, Codony, Pizzale, Mariani, & Conte, 2014; Romero & Brenes, 2012).

1.5.2. Estimate (predict) an appropriate "best before date"

According to the Reg. (EU) 1169/2011, the label of any food must report the "use by" date or the "best before" date; this regulation also very clearly describes the differences between these two phrases: the "best before" date is used in the case of foods that can undergo chemical, physical or sensory modifications without any prejudice for consumers' health, while the "use by" is used for foods whose modifications involve an health risk for consumers health.

Despite this mandatory rule, no method had been validated and no shared method is available to calculate the best before date for foods. From a scientific and technological point of view, these dates are called "shelf life" (McGinn, 1982) which is a very important aspect for managing companies (Stone & Sidel, 2004). As a preliminary step, the difference between "freshness" evaluation that estimates *ex post* the (residue) quality of an oil and "shelf life" that try to predict *ex-ante* the behaviour of the quality of the oil during its commercial life must be very clear.

The OO shelf life can be described as the period of time during which, in correct storing conditions, no off-flavours or defects arise and any quality parameters remain inside the limit established for the category the oil belongs to (Guillaume & Ravetti, 2016).

Although there are many studies describing the effects of selected environmental factors affecting OO quality in the scientific literature, no studies are available dealing with information that can be used by companies to predict shelf life, even bearing in mind the time and costs of the test. Del Nobile et al. (Del Nobile, Bove, La Notte, & Sacchi, 2003) developed a model by studying the effects of material and dimension of packaging on oxidative degradation. The model, however, did not consider light and temperature changes during storage. This aspect had been considered by Coutelieris and Kanavouras (Coutelieris & Kanavouras, 2005) who evaluated improvement in the concentration of hexanal to estimate the loss of oil quality by the activation energy of oxidative reactions. Later, the same authors (Coutelieris & Kanavouras, 2006; Kanavouras & Coutelieris, 2006), used the same approach to study additional aspects of the degradation of oil during storage. Mancebo-Campos et al. (Mancebo-Campos, Fregapane, & Salvador,

2008) developed a kinetic study to estimate the potential shelf life of OO, while Aparicio-Ruiz et al. (Aparicio-Ruiz, Aparicio, & García-González, 2014) used the degradation of pyropheophytines as a marker of OO ageing. An empiric model had been proposed by Guillaume and Ravetti (Guillaume & Ravetti, 2016), which uses induction time, 1,2-diacylglycerols, pyropheophytin A, and free FAs to predict the shelf life. On the other hand, Tena et al. (Tena, Aparicio, & García-González, 2017), also demonstrated the importance of light at moderate conditions during storage when measuring hydroperoxides by infrared spectroscopy and the utility of spectroscopy in this task.

The availability of a method that is reliable, fast and relativity inexpensive is a priority for companies in order to avoid legal problems related to oil degradation once its trade begins.

1.5.3. Assessment of the amount of olive oil in mixtures with seed oils

Olive oils, of any edible category, can be mixed with seed oils. When the presence of olive oils is mentioned in the labelling, outside of the list of ingredients, by words, images or graphic representations, the following trade description has to appear on that blend: "Blend of vegetable oils (or the specific names of the vegetable oils concerned) and olive oil', directly followed by the percentage of olive oil in the blend. The presence of olive oil may only be highlighted by images or graphics on the labelling of the mixtures referred, in the case that it accounts for more than 50% (Reg. (EU) 29/2012). Since the year of its publication, four amendments have been made to this regulation. All modifications are important, but perhaps the most significant is related to the possibility for Member States to prohibit the production in their territory of blends of olive oil and other vegetable oils for internal consumption. However, they may not prohibit the marketing in their territory of such blends coming from other countries, as well as the production in their territory of such blends for marketing in another Member State or for exportation. It is noteworthy that there is no mention about the values of the analytical parameters that these oils should comply with to ensure that the blend contains the percentage of OO that is established in the regulation, meaning, there is no analytical protocol to ensure the percentage of oils in the admixture. Moreover, it cannot be confirmed if OO is present or not in the blend. It is important to keep in mind that blends can be made with any type of OO; for this reason, the analytical parameters of virgin OO such as volatile compounds, triterpenic acids, or polyphenols, which are not present in the refined ones, should be discarded and work should be focused on compounds that remain after a refining process. There are several compounds at high enough concentration in OO (e.g. TAGs profile, sum of saturated aliphatic hydrocarbons, β/γ-tocopherol ratio, total sterol amount) that can be detected after blending if the blend is more than 50%.

1.5.4. Assessment of deodorised oils

The origin of soft deodorised olive oils and evaluation of ethyl esters as possible markers for their detection has been already discussed in a previous paragraph. Nowadays, it seems that more reliable analytical approaches have not been found; other parameters have been proposed such as diacylglycerols (DAGs) and pyropheophytin (PPP), but none is used since they are not unequivocal and also change during aging of oils. The high content of PPP or DAGs could mean the oil was either subjected to soft deodorisation or that the oil is old or was poorly stored. Possible interesting results could be obtained studying in-depth the differences between experimental DAG content and theoretical DAG content (this latter calculated from free acidity) of genuine OO and corresponding samples subjected to the soft deodorization process.

1.5.5. Verification of geographical origin of olive oils

The behaviour of consumers when purchasing foods is oriented towards a greater preference for products whose geographical origin is declared since this information clearly increases their confidence. The importance of geographical declaration on the label mainly concerns extra virgin OO since consumers perceive information on the

provenance as an additional warranty of their quality and authenticity. For this reason, the European Union implements a quality system of geographical indications, such as Protected Designation of Origin (PDO) (EU, 2012). In addition to the PDO information on the label, the consumer can also demand information on the provenance in the case of non-PDO oils. However, the complex regulation on PDO and labelling does not specify an analytical procedure to verify the information reported on the label. This fact has raised the interest of analysts and researchers to develop a reliable method for authentication purposes. After extensive research on the chemical characterisation of OOs from different locations, sometimes even from the same cultivar, there are now sufficient chemical and mathematical backgrounds to suggest that the chemical compositions of oils are partially associated with their provenance. A recent review (Valli et al., 2016) summarises the most interesting and innovative solutions (e.g. using optical techniques, measurement of electrical characteristics, instruments equipped with electronic chemical sensors) with a potential and realistic application for the development of rapid, easy-to-use, environmentally friendly instruments to be used in monitoring of the geographical origin of virgin OO.

The methodologies that have been proposed are based on chemical fingerprint of the oils by chromatographic analysis, spectroscopic methods and genetic studies (Aparicio & García-González, 2013; Gallina Toschi, Bendini, Lozano-Sánchez, Segura-Carretero, & Conte, 2013). Concerning the former approaches, Forina and Tiscornia (Forina & Tiscornia, 1982) were among the first researchers to apply FA composition to discriminate the geographical origin of extra virgin OOs from several areas of Italy. Later, a pioneering study called SEXIA (Spanish acronym for Expert System for Identification of Oils) was developed in the 1990s in addition to later research on the same topic (Aparicio & Alonso, 1994; Aparicio, Alonso, & Morales, 1994; García-González, Luna, Morales, & Aparicio, 2009). In the same years, the FA and the TAG profiles were used to discriminate Greek and French samples, respectively (Bajoub et al., 2016; Ollivier, Artaud, Pinatel, Durbec, & Guérère, 2003; Tsimidou & Karakostas, 1993; Tsimidou, Macrae, & Wilson, 1987). Furthermore, some studies have focused on the influence of the geographic area of origin on the positional distribution of FAs in the structure of triacylglycerols (Damiani et al., 1997; Vichi, Pizzale, & Conte, 2007).

Differences in volatile compounds has also been attributed to geographical origin (García-González, Romero, & Aparicio, 2010). Melucci et al. (Melucci et al., 2016) highlighted the potential of flash gas chromatography E-nose for rapid control of the compliance of information on geographic origin declared in the label ("100% Italian" vs "non-100% Italian" as specific case of oils originating from one Member State) using non-targeted chromatographic signals of the volatile fraction of virgin OOs as variables for multivariate analysis using more than 250 samples. An interesting investigation based on the simultaneous analysis of mono- and sesquiterpene compounds in virgin OO by HS-SPME-GC-MS (Vichi, Guadayol, Caixach, Lopez-Tamames, & Buxaderas, 2006) showed that this fraction may be used to distinguish samples from different cultivars grown in different geographical areas). The profile in terpenic hydrocarbons strictly depends on the variety and growing conditions of the olive trees and is not influenced by technological factors.

To better understand the variability that depends on geographical origin, it is desirable to identify the factors (e.g. temperature) that cause alterations in specific compounds, so that samples can be classified (Aparicio & García-González, 2013).

In addition to chromatographic methods, spectroscopic techniques can be proposed as a rapid alternative to obtain chemical profiling of oils without laborious lab work. Some of the techniques applied are inductively coupled plasma (ICP) coupled to atomic emission spectrometry or mass spectrometry, and atomic absorption spectrometry (AAS) to determine the presence of chemical elements (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015; Benincasa, Lewis, Perri,

Sindona, & Tagarelli, 2007). Multi-isotope ratio analysis (^{2}H/^{1}H or D/H, ^{13}C/^{12}C, ^{18}O/^{16}O, ^{15}N/^{14}N, ^{34}S/^{32}S, ^{87}Sr/^{86}Sr) has been also applied to geographical origin studies of olive oils (Camin et al., 2017).

A different strategic approach to verify geographical origins of OO is based on the genomics of olives and OOs. Thus, the molecular markers characterising cultivars have also been applied to 'olive oil fingerprinting' (Banilas & Hatzopoulos, 2013).

Regardless of the strategy followed for geographical identification, all approaches require a large database. The representativeness of oils selected for such a database according to market reality and genuineness in terms of geographical provenance is of paramount importance to develop a reliable method. This database would allow building an 'olive oil map' including both chromatographic and spectroscopic information from the most relevant cultivars and all approved PDOs (Aparicio & García-González, 2013).

2. Conclusions and future trends

Despite the OO analysis remains a cornerstone in terms of diagnostic possibilities of fraud in the field of food analytics and the constant and substantial efforts towards the development of new procedures to be used for assessment of OO quality and authenticity, some specific and proper analytical solutions (e.g. detection of selected blends of OOs with other vegetable oils, of soft-deodorised OOs, methods for supporting the organoleptic assessment of OOs, etc.) have not yet been found. It is therefore urgent to identify and/or improve analytical solutions that are able to detect both common and emerging frauds and to provide all the information required by the international market.

This review has highlighted weaknesses in the regulatory framework as well as some critical points of existing analytical methods adopted in OO quality and purity control. Suggestions for replacement of specific steps of analytical protocols, especially with more advanced analytical solutions to reduce time or solvent consumption, have also been proposed.

Once the weaknesses of the regulatory framework and the lack of proper analytical methodologies has been overcome, the next step should be an extensive and significant work towards global harmonisation of parameters, limits and analytical protocols in order to establish a worldwide-system of fraud protection, providing a unique framework that is unaffected by misunderstanding and misconceptions. Such action will definitely ensure fair trade as well as the safety and consumer protection of the entire OO sector.

References

Amelio, M., Rizzo, R., & Varazini, F. (1998). Separation of stigmasta-3,5-diene, squalene isomers, and wax esters from olive oils by single high-performance liquid chromatography run. *Journal of the American Oil Chemists Society, 75*, 527–530.

ARTICLE IN PRESS

L. Conte, et al.   Trends in Food Science & Technology xxx (xxxx) xxx–xxx

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., & Montedoro, G. F. (2004). Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. *Journal of Chromatography A, 1054*, 17–31.

Aparicio-Ruiz, R., Aparicio, R., & García-González, D. L. (2014). Does "best before" date embody extra-virgin olive oil freshness? *Journal of Agricultural and Food Chemistry, 62*, 554–556.

Aparicio, R., & Alonso, V. (1994). Characterization of virgin olive oil by SEXIA expert system. *Progress in Lipid Research, 33*, 29–38.

Aparicio, R., Alonso, V., & Morales, M. T. (1994). Detailed and exhaustive study of the authentication of European virgin olive oils by SEXIA expert system. *Grasas Y Aceites, 45*, 241–252.

Aparicio, R., & García-González, D. L. (2013). Olive oil characterization and traceability. In R. Aparicio, & J. Harwood (Eds.). *Handbook of olive oil* (pp. 431–478). New York: Springer Science.

Aparicio, R., Morales, M. T., & Garcıa-Gonzalez, D. L. (2012). Towards new analyses of aroma and volatiles to understand sensory perception of olive oil. *European Journal of Lipid Science and Technology, 114*, 1114–1125.

Bajoub, A., Medina-Rodriguez, S., Hurtado-Fernandez, H., Ajal, E., Ouazzani, N., Fernandez-Gutiérrez, A., et al. (2016). A first approach towards the development of geographical origin tracing models for North Moroccan olive oils based on tria-cylglycerols profiles. *European Journal of Lipid Science and Technology, 118*, 1223–1235.

Banilas, G., & Hatzopoulos, P. (2013). Genetics and molecular biology of olives. In R. Aparicio, & J. Harwood (Eds.). *Handbook of olive oil* (pp. 129–161). New York: Springer Science.

Beccaria, M., Moret, M., Purcaro, G., Pizzale, L., Cotroneo, A., Dugo, P., et al. (2016). Reliability of the ΔECN42 limit and global method for extra virgin olive oil purity assessment using different analytical approaches. *Food Chemistry, 190*, 216–225.

Beltrán, M., Sánchez-Astudillo, M., Aparicio, R., & García-González, D. L. (2015). Geographical traceability of virgin olive oils from south-western Spain by their multi-elemental composition. *Food Chemistry, 169*, 350–357.

Bendini, A., Valli, E., Barbieri, S., & Gallina Toschi, T. (2012). Sensory analysis of virgin olive oil. In D. Boskou (Ed.). *Constituents, quality, health properties and bioconversions* (pp. 109–130). Croatia: InTech.

Benincasa, C., Lewis, J., Perri, E., Sindona, G., & Tagarelli, A. (2007). Determination of trace element in Italian virgin olive oils and their characterization according to geographical origin by statistical analysis. *Analytica Chimica Acta, 585*, 366–370.

Biedermann, M., Bongartz, A., Mariani, C., & Grob, K. (2008). Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. *European Food Research and Technology, 228*, 65–74.

Biedermann, M., Grob, K., & Bronz, M. (1995). Determination of stigmastadiene in edible oils by HPLC–HPLC–UV. *Rivista Italiana Delle Sostanze Grasse, 72*, 397–401.

Biedermann, M., Grob, K., & Mariani, C. (1993). Transesterification and on-line LC–GC for determining the sum of free and esterified sterols in edible oils and fats. *Lipid/Fett, 95*, 127–133.

Borràs, E., Ferré, J., Boqué, R., Mestres, M., Aceña, L., Calvo, A., et al. (2016). Prediction of olive oil sensory descriptors using instrumental data fusion and partial least squares (PLS) regression. *Talanta, 155*, 116–123.

Camin, F., Boner, M., Bontempo, L., Fauhl-Hassek, C., Kelly, S. D., Riedl, J., et al. (2017). Stable isotope techniques for verifying the declared geographical origin of food in legal cases. *Trends in Food Science & Technology, 61*, 176–187.

Cayuela, J. A., Gomez-Coca, R. B., Moreda, W., & Perez-Camino, M. C. (2015). Sensory defects of virgin olive oil from a microbiological perspective. *Trends in Food Science & Technology, 43*, 227–235.

Ceci, L. N., & Carelli, A. A. (2007). Characterization of monovarietal argentinian olive oils from new productive zones. *Journal of the American Oil Chemists Society, 84*, 1125–1136.

Cert, A., Lanzon, A., Carelli, A., Albi, T., & Amelotti, G. (1994). Formation of stigmasta-3,5 diene in vegetable oils. *Food Chemistry, 49*, 287–293.

Cert, A., Moreda, W., & García-Moreno, J. (1997). Determination of sterols and triterpenic dialcohols in olive oils using HPLC separation and GC analysis. Standardization of the analytical method. *Grasas Y Aceites, 48*, 207–218.

Codex Alimentarius Commission (2003). *Codex standard for olive oils and olive pomace oils Codex stan 33-1981. Adopted in 1981. Revised in 1989.* 2015, 2017. Amended in 2009, 2013.

Coutelieris, F., & Kanavouras, A. (2005). Use of the activation energy concept to estimate the quality reduction of packaged olive oil. *Journal of the American Oil Chemists Society, 82*, 119–123.

Coutelieris, F. A., & Kanavouras, A. (2006). Experimental and theoretical investigation of packaged olive oil: Development of a quality indicator based on mathematical pre-dictions. *Journal of Food Engineering, 73*, 85–92.

Cozzolino, R., & De Giulio, B. (2011). Application of ESI and MALDI- TOF MS for tria-cylglycerols analysis in edible oils. *European Journal of Lipid Science and Technology, 113*, 160–167.

Damiani, P., Cossignani, L., Simonetti, M. S., Campisi, B., Favretto, L., & Gabrielli-Favretto, L. (1997). Stereospecific analysis of the triacylglycerol fraction and linear discriminant analysis in a climatic differentiation of Umbrian extra-virgin olive oils. *Journal of Chromatography A, 758*, 109–116.

Del Nobile, M. A., Bove, S., La Notte, E., & Sacchi, R. (2003). Influence of packaging geometry and material properties on the oxidation kinetics of bottled virgin olive oil. *Journal of Food Engineering, 57*, 189–197.

EFSA Panel on Dietetic Products Nutrition and Allergens (2011). Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638 1639, 1696 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to

the upper respiratory tract health" (ID 3468), "can help to maintain a normal func-tion of gastrointestinal tract" (3779), and "contributes to body defences against ex-ternal agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No. 1924/2006. *EFSA Journal, 9*, 2033–2058.

European Commission Regulation 1169/2011 on the provision of food information to consumers. Official Journal of the European Communities 2011, October 25, L 304, 18−63.

European Commission Regulation 136/66 of the Council of 22 September 1966 on the establishment of a common organisation of the market in oils and fats.

European Commission Regulation 183/93 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, Official Journal of the European Communities, 1993, January 29, L 22, 58-68.

European Commission Regulation 2472/97 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and Council Regulation (EEC) No 2658/91 on the tariff and statistical no-menclature and on the Common Customs Tariff, Official Journal of the European Communities, 1997, December 11, L 341, 1-18.

European Commission Regulation 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, and subsequent amendments. Official Journal of European Community 1991, July 11, L248, 1–102.

European Commission Regulation 29/2012 on marketing standards for olive oil. Official Journal of the European Union, 2012, January 13, 12-21.

European Commission Regulation 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. Official Journal of the European Communities, 2012, May 16, L 136, 1−40.

European commission regulation 640/2008. Amending regulation No 2568/91/EEC. Official Journal of the European Communities 2008, July 4, L178, 11-16.

European Commission Regulation 656/95 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis and Council Regulation (EEC) No 2658/87 on the tariff and statistical no-menclature and the Common Customs Tariff, Official Journal of the European Communities, 1995, March 28, L 69, 1-12.

European Commission Regulation 702/2007 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, Official Journal of the European Communities, 2007, June 21, L 161, 11-27.

European commission regulation 796/2002. Amending regulation No 2568/91/EEC. Official Journal of the European Communities 2002, May 6, L128, 8-28.

European Commission. EU Programmes Horizon 2020. (2013, December 11) https://ec. europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/ sfs-14a-2014.html H2020-SFS-14a-2014, Authentication of olive oil topic,.

European Commission implementing Regulation 1348/2013 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the re-levant methods of analysis. Official Journal of the European Union 2013, December 16, L 338, 31-67.

European Commission implementing Regulation 2015/1833 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the re-levant methods of analysis. Official Journal of the European Union 2015, October 12, L 266, 29-52.

European Commission implementing Regulation 2016/1227 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the re-levant methods of analysis. Official Journal of the European Union 2016, July 27, L 202, 7-13.

Forina, M., & Tiscornia, E. (1982). Pattern recognition methods in the prediction of Italian olive oils origin by the fatty acids content. *Annali di Chimica, 72*, 143–155.

Fortini, M., Migliorini, M., Cherubini, C., Cecchi, L., & Calamai, L. (2017). Multiple in-ternal standard normalization for improving HS-SPME-GC-MS quantitation in virgin olive oil volatile organic compounds (VOO-VOCs) profile. *Talanta, 165*, 641–652.

Gallina Toschi, T., Bendini, A., Lozano-Sánchez, J., Segura-Carretero, A., & Conte, L. (2013). Misdescription of edible oils: Flowcharts of analytical choices in a forensic view. *European Journal of Lipid Science and Technology, 115*, 1205–1223.

García-González, D. L., Luna, G., Morales, M. T., & Aparicio, R. (2009). Stepwise geo-graphical traceability of virgin olive oils by chemical profiles using artificial neural network models. *European Journal of Lipid Science and Technology, 111*, 1003–1013.

García-González, D. L., Romero, N., & Aparicio, R. (2010). Comparative study of virgin olive oil quality from single varieties cultivated in Chile and Spain. *Journal of Agricultural and Food Chemistry, 58*, 12899–12905.

García-González, D. L., Tena, N., & Aparicio, R. (2007). Characterization of olive paste volatiles to predict the sensory quality of virgin olive oil. *European Journal of Lipid Science and Technology, 109*, 663–672.

Gómez-Ariza, J. L., Arias-Borrego, A., García-Barrera, T., & Beltran, R. (2006). Comparative study of electrospray and photospray ionization sources coupled to quadrupole time-of-flight mass spectrometer for olive oil authentication. *Talanta, 70*, 859–865.

Gómez-Coca, R. B., Fernandes, G. D., Pérez-Camino, M. C., & Moreda, W. (2016). Fatty acid ethyl esters (FAEE) in extra virgin olive oil: A case study of a quality parameter. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 66*, 378–383.

Grob, K., Artho, A., & Mariani, C. (1992). Determination of raffination of edible oils and fats by olefinic degradation products of sterols and squalene, using coupled LC–GC. *Fat Science Technology, 10*, 394–400.

Grob, K., Biedermann, M., Artho, A., & Schmid, J. P. (1994). LC, GC and MS of sterol dehydration products. *Rivista Italiana Delle Sostanze Grasse, 72*, 533–538.

Grob, K., Lanfranchi, M., & Mariani, C. (1989). Determination of free and esterified sterols and wax esters on oils and fats by coupled liquid chromatography gas chromato-graphy. *Journal of Cromatography, 471*, 397–398.

Guillaume, C., & Ravetti, L. (2016). Shelf-life prediction of extra virgin olive oils using an empirical model based on standard quality tests. *Journal of Chemistry,* 1–7.

International Olive Council (2009, November). *COI/T.20/Doc. no. 29. Determination of biophenols in olive oil by HPLC.*

International Olive Council (2001a). *Determination of the difference between actual and theoretical content of triacylglycerols with ECN 42. COI/T.20/Doc. No. 20 Rev. 3. Madrid, Spain.*

International Olive Council (2001b). *COI/T.20/Doc. no. 16/Rev. 1 Method of analysis determination of sterenes in refined vegetable oils. Madrid, Spain.*

International Olive Council (2006). *T.20/Doc No. 23. Method for the determination of the percentage of 2-glyceryl-monopalmitate, Madrid, Spain.*

International Olive Council (2010). *Determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography. COI/T. 20/NC No 28/ Rev. 1.* 1–17.

International Olive Council IOC/T.15/Doc No 3/Rev 11 (July 2016). *Trade standard applying to olive oils and olive pomace oils.*

International Olive Council IOC/T.20/Doc No 15/Rev 8 (November 2015). *Sensory analysis of olive oil method for the organoleptic assessment of virgin olive oil.*

International Olive Council IOC/T.28/Doc No 1/Rev 1 (May 2017). *Guidelines for accomplishment of the requirements of the norm ISO 17025 by the laboratories of sensory analysis of virgin olive oil.*

International Olive Oil Council (1987). *Sensory analysis of olive oil method for the organoleptic assessment of virgin olive oil. IOOC/T.20/Doc. no. 3.*

International Standard Organization (2003). *Animal and vegetable fats and oils — determination of peroxide value — iodometric (visual) endpoint determination – ISO 3960:2003.*

International Standard Organization (2014). *Determination of individual and total sterols contents, Gas chromatographic method, Part 2: Olive oils and olive pomace oils ISO 12228-2:2014.*

Kanavouras, A., & Coutelieris, F. A. (2006). Shelf-life predictions for packaged olive oil based on simulations. *Food Chemistry, 96,* 48–55.

Lanzon, A., Cert, A., & Albi, T. (1989). Detection of refined olive oil in virgin olive oil. *Grasas Y Aceites, 40,* 385–388.

León-Camacho, M., Morales, M. T., & Aparicio, R. (2013). Chromatographic methodologies: Compounds for olive oil traceability issues. In R. Aparicio, & J. L. Harwood (Eds.). *Handbook of olive oil: Analysis and properties* (pp. 163–218). (2nd ed.). New York: Springer.

Lerma-García, M. J., Cerretani, L., Cevoli, C., Simó-Alfonso, E. F., Bendini, A., & Gallina Toschi, T. (2010). Use of electronic nose to determine defect percentage in oils. Comparison with sensory panel results. *Sensors and Actuators B, 147,* 283–289.

Mancebo-Campos, V., Fregapane, G., & Salvador, D. M. (2008). Kinetic study for the development of an accelerated oxidative stability test to estimate virgin olive oil potential shelf life. *European Journal of Lipid Science and Technology, 110,* 969–976.

Mariani, C., & Bellan, G. (2013). On the possible increase of alkyl esters in extra virgin olive oil. Note 2. *Rivista Italiana Delle Sostanze Grasse, 90,* 211–217.

Mariani, C., Bellan, G., Lestini, E., & Aparicio, R. (2006). The detection of the presence of hazelnut oil in olive by free and esterified sterols. *European Food Science and Technology, 223,* 655–661.

Mariani, C., & Fedeli, E. (1986). Individuazione di oli di estrazione in quelli di pressione. Nota I. *Rivista Italiana Delle Sostanze Grasse, 63,* 3–17.

McGinn, C. J. P. (1982). Evaluation of shelf-life. Part 2 *IFST Proceedings, 15*(3), 153–161 (IFST, London).

Melucci, D., Bendini, A., Tesini, F., Barbieri, S., Zappi, A., Vichi, S., et al. (2016). Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics. *Food Chemistry, 204,* 263–273.

Morales, M. T., Aparicio-Ruiz, R., & Aparicio, R. (2013). Chromatographic methodologies: Compounds for olive oil traceability issues. In R. Aparicio, & J. Harwood (Eds.). *Handbook of olive oil. Analysis and properties* (pp. 261–309). (Edition). New York: Springer 2.

Morales, M. T., Luna, G., & Aparicio, R. (2005). Comparative study of virgin olive oil sensory defects. *Food Chemistry, 91,* 293–301.

Moreda, W., Pérez-Camino, M. C., & Cert, A. (2003). Improved method for the determination of triacylglycerols in olive oils by high performance liquid chromatography. *Grasas Y Aceites, 54,* 175–179.

Morello, J. R., Motilva, M. J., Ramo, T., & Romero, M. P. (2003). Effect of freeze injuries in olive fruit on virgin olive oil composition. *Food Chemistry, 81,* 547–553.

Motta, L., Brianza, M., Stanga, F., & Amelotti, G. (1983). Analisi gascromatografica dei gliceridi parziali dopo lipolisi con lipasi pancreatica. *Rivista Italiana Delle Sostanze Grasse, 60,* 625–630.

Mulinacci, N., Giaccherini, C., Ieri, F., Innocenti, M., Romani, A., & Vincieri, F. F. (2006). Evaluation of lignans and free and linked hydroxy-tyrosol and tyrosol in extra virgin olive oil after hydrolysis processes. *Journal of the Science of Food and Agriculture, 86,*

757–764.

Nestola, M., & Schmidt, T. C. (2016). Fully automated determination of the sterol composition and total content in edible oils and fats by online liquid chromatography-gas chromatography-flame ionization detection. *Journal of Chromatography A, 1463,* 136–143.

Niewiadomski, H. (1975). The sterol hydrocarbons in edible oils. *Nahrung, 19,* 525–536.

Nota, G., Naviglio, D., Romano, R., Sabia, V., Spagna, Musso, S., & Improta, C. (1999). Determination of the wax ester content in olive oils. Improvement in the method proposed by EEC Regulation 183/93. *Journal of Agricultural and Food Chemistry, 47,* 202–205.

Núñez, O., Gallart-Ayala, H., Martins, C. P., & Lucci, P. (2012). New trends in fast liquid chromatography for food and environmental analysis. *Journal of Chromatography A, 1228,* 298–323.

Ollivier, D., Artaud, J., Pinatel, C., Durbec, J. P., & Guérère, M. (2003). Triacylglycerol and fatty acid compositions of French virgin olive oils. Characterization by chemometrics. *Journal of Agricultural and Food Chemistry, 51,* 5723–5731.

Panagiotopoulou, P. M., & Tsimidou, M. (2002). Solid phase extraction: Applications to the chromatographic analysis of vegetable oils and fats. *Grasas Y Aceites, 84*(53), 84–95.

Pérez-Camino, M. C., Cert, A., Romero-Segura, A., Cert-Trujillo, R., & Moreda, W. (2008). Alkyl esters of fatty acids a useful tool to detect soft deodorized olive oils. *Journal of Agricultural and Food Chemistry, 56,* 6740–6744.

Procida, G., Giomo, A., Cichelli, A., & Conte, L. S. (2005). Study of volatile compounds of defective virgin olive oils and sensory evaluation: A chemometric approach. *Journal of the Science of Food and Agriculture, 85,* 2175–2183.

Purcaro, G., Codony, R., Pizzale, L., Mariani, C., & Conte, L. (2014). Evaluation of total hydroxytyrosol and tyrosol in extra virgin olive oils. *European Journal of Lipid Science and Technology, 116,* 805–811.

Romero, N., & Brenes, M. (2012). Analysis of total contents of hydroxytyrosol and tyrosol in olive oils. *Journal of Agricultural and Food Chemistry, 60,* 9017–9022.

Romero, I., García-González, D. L., Aparicio-Ruiz, R., & Morales, M. T. (2015). Validation of SPME-GCMS method for the analysis of virgin olive oil volatiles responsible for sensory defects. *Talanta, 134,* 394–401.

Romero, I., García-González, D. L., Aparicio-Ruiz, R., & Morales, M. T. (2017). Study of volatile compounds of virgin olive oils with 'frostbitten olives' sensory defect. *Journal of Agricultural and Food Chemistry, 65,* 4314–4320.

Sinelli, N., Cerretani, L., Di Egidio, V., Bendini, A., & Casiraghi, E. (2010). Application of near (NIR) infrared and mid (MIR) infrared spectroscopy as a rapid tool to classify extra virgin olive oil on the basis of fruity attribute intensity. *Food Research International, 43,* 369–375.

Stone, H., & Sidel, J. L. (2004). *Sensory evaluation practices.* London: Elsevier Academic Press.

Synouri, S., Frangiscos, S. E., Christopoulou, E., & Lazaraki, E. M. (1995). Influence of certain factors on the composition of olive– pomace oil. *Rivista Italiana Sostanze Grasse, 72,* 483–491.

Tena, N., Aparicio, R., & García-González, D. L. (2017). Virgin olive oil stability study by mesh cell-FTIR spectroscopy. *Talanta, 167,* 453–461.

Toledano, R. M., Cortés, J. M., Andini, J. C., Vázquez, A., & Villén, J. (2012). On-line derivatization with on-line coupled normal phase liquid chromatography–gas chromatography using the through oven transfer adsorption desorption interface: Application to the analysis of total sterols in edible oils. *Journal of Chromatography A, 1256,* 191–196.

Tsimidou, M., & Karakostas, K. (1993). Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. *Journal of the Science of Food and Agriculture, 62,* 253–257.

Tsimidou, M., Macrae, R., & Wilson, I. (1987). Authentication of virgin olive oils using principal components analysis of triglyceride and fatty acid profiles: Part 1. Classification of Greek olive oils. *Food Chemistry, 25,* 227–239.

Valli, E., Bendini, A., Berardinelli, A., Ragni, L., Riccò, B., Grossi, M., et al. (2016). Rapid and innovative instrumental approaches for quality and authenticity of olive oils. *European Journal of Lipid Science and Technology, 118,* 1601–1619.

Vichi, S., Guadayol, J. M., Caixach, J., Lopez-Tamames, E., & Buxaderas, S. (2006). Monoterpene and sesquiterpene hydrocarbons of virgin olive oil by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry. *Journal of Chromatography A, 1125,* 117–123.

Vichi, S., Pizzale, L., & Conte, L. (2007). Stereospecific distribution of fatty acids in triacylglycerols of olive oils. *European Journal of Lipid Science and Technology, 109,* 72–78.

Vichi, S. N. J., Romero, A., Tous, J., López Tamames, E., & Buxaderas, S. (2008). Determination of volatile phenols in virgin olive oils and their sensory significance. *Journal of Chromatography A, 1211,* 1–7.