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Olive oil authentication: A comparative analysis of regulatory frameworks with especial emphasis on quality and authenticity indices, and recent analytical techniques developed for their assessment. A review

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ABSTRACT

Over the last decades, olive oil quality and authenticity control has become an issue of great importance to consumers, suppliers, retailers, and regulators in both traditional and emerging olive oil producing countries, mainly due to the increasing worldwide popularity and the trade globalization of this product. Thus, in order to ensure olive oil authentication, various national and international laws and regulations have been adopted, although some of them are actually causing an enormous debate about the risk that they can represent for the harmonization of international olive oil trade standards. Within this context, this review was designed to provide a critical overview and comparative analysis of selected regulatory frameworks for olive oil authentication, with special emphasis on the quality and purity criteria considered by these regulation systems, their thresholds and the analytical methods employed for monitoring them. To complete the general overview, recent analytical advances to overcome drawbacks and limitations of the official methods to evaluate olive oil quality and to determine possible adulterations were reviewed. Furthermore, the latest trends on analytical approaches to assess the olive oil geographical and varietal origin traceability were also examined.

Introduction

Olive oil is an economically important product in most of the Mediterranean countries, where its production has longstanding historical roots. Interest in this product has recently been accentuated to a larger extent, both inside and outside the Mediterranean region, by various studies that have focused on demonstrating its human health beneficial effects and its wide culinary applications (Boskou, 2011). Large amounts of olive oil are globally consumed every year; indeed, over 2.27 million tons were estimated to be consumed from the olive crop of 2015–2016 (International Olive Council (IOC), 2015). It is also important to highlight that the worldwide olive oil consumption has steadily risen, achieving an average annual growth rate of 2.7% between 1991 and 2012 (IOC, 2014).

In parallel to this quantitative expansion of olive oil consumption, there has been an intensification of the consumer interest in high-quality oil and some labeled olive oil categories, such as organic olive oil and oils with certified geographic indications or declared as monovarietal (Di Vita et al., 2013). Keeping in mind that consumers are willing to pay higher prices for these categories of olive oil, the price achieved in the market for these products is often remarkably high, which makes them prone to suffer adulteration and mislabeling practices (Garcia et al., 2013). For this reason, olive oil authentication issues are, actually, topics of prominent importance, not only for consumers, but also for suppliers, retailers, regulatory agencies, and administrative authorities. In this regard, it

seems interesting to mention the four-year EU project "OLEUM", founded by H2020 programme (http://cordis.europa.eu/project/rcn/204671_en.html), and coordinated by Prof. Tullia Gallina Toschi of the University of Bologna (Italy), which started on 1st September 2016. OLEUM project will focused on the development of new analytical methods as well as on the improvement of the existing protocols for detecting olive oil fraud and for assuring its quality, discussing legislative and harmonization aspects pursuing improvements to international regulations.

In a broad sense, the concept of "authentication" refers to the control of different kinds of fraudulent practices, including adulteration, mislabeling, and misleading origin, among others (Aparicio et al., 2013a; Gallina Toschi et al., 2013). Indeed, as illustrated by Figure 1, because of the large number of olive oil categories that can be produced, extra virgin olive oil (EVOO), which is the olive oil top grade, is more susceptible of adulteration practices, being the most common one the addition of other olive oils of lower commercial value and/or seed oils, such as sunflower, soybean and hazelnut oils (De Oliveira and Catharino, 2015). Furthermore, the guarantee of olive oil authentic and reliable geographical and varietal origins is another subject of concern for the olive oil sector (Dias et al., 2014).

Traditional strategies to control olive oil adulteration and guarantee its quality are relied on the analytical determination of
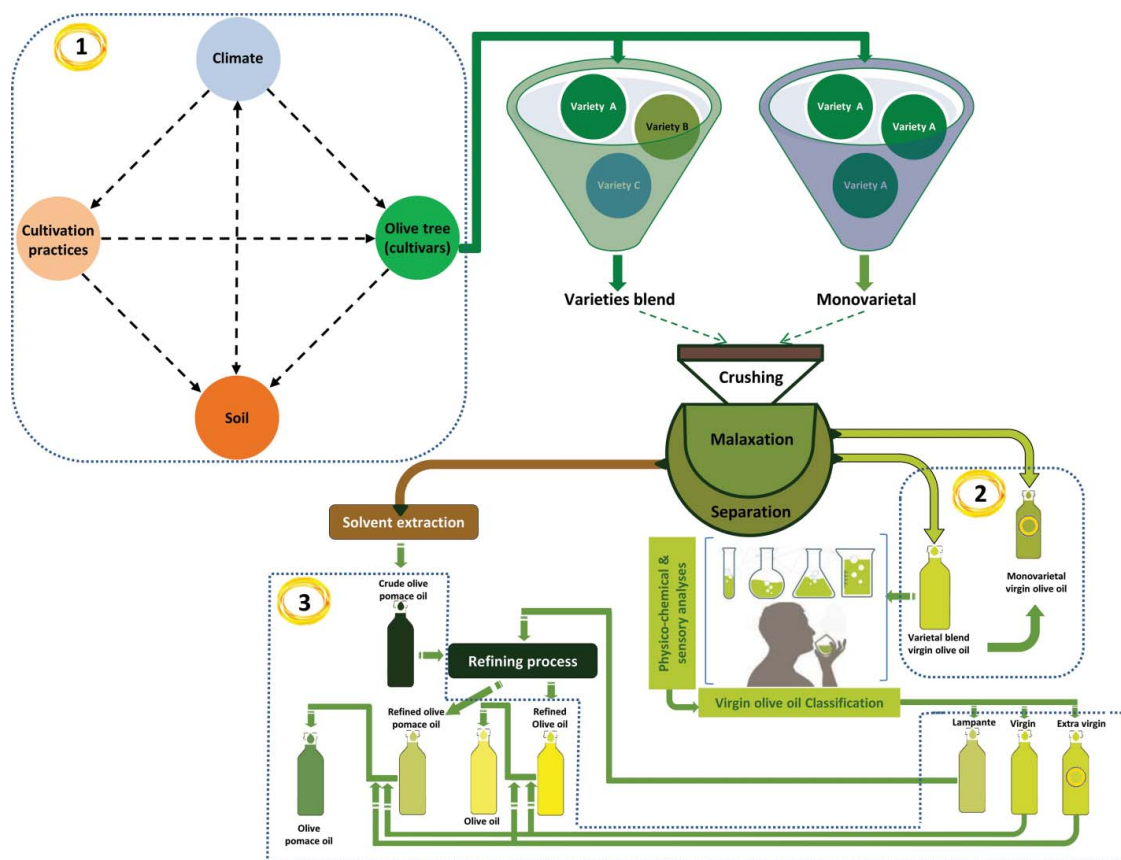

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Figure 1. Flow diagram of olive oils and olive-pomace oils categories production steps, stressing possible adulteration types ((1) adulteration of olive oils produced on specific territory/under geographical indication certification; (2) adulteration of monovarietal olive oils with varieties blend olive oils; and (3) addition/mixture of EVOO with other olive oils and olive-pomace oils).

various quality and purity parameters in the evaluated material and the subsequent comparison of the obtained value(s) with those established as thresholds by the standard regulations. In this context, olive oil authentication is governed by specific regulations that define standards and criteria for classifying it, and give a comprehensive description of the analytical methods for assessing its quality and testing its authenticity. Nowadays, extensive regulatory frameworks have been laid down by different national and international organizations, such as: United States Department of Agriculture (USDA) standards (USDA, 2010), Californian State regulations (California Department of Food and Agriculture, 2014), Australian standards (Standards Australia, 2011), European Commission standards (EEC, 1991), Codex Alimentarius (Codex) regulations (Codex, 1991) and IOC standards (IOC, 2016). Those from IOC have always been the most widely used for olive oil standards grading all over the world, since they are drawn up and updated on the basis of IOC olive oil records and databases of the countries which are members of this council, which covers the vast majority of the global olive oil production. Nevertheless, although considerable efforts are dedicated to continuously update and amend IOC regulations in order to make them evolve at the same rhythm as the constant analytical innovations as well as the sophisticated fraudulent practices, currently, there is a very active global debate about olive oil standards setting and the effectiveness of official analytical methods (Aparicio et al., 2013b).

The starting point for establishing threshold values of olive oil quality and purity criteria is to know in depth the regular and usual olive oil physico-chemical characteristics and composition; in other words, it is necessary to verify what the "normal values" are. However, it is important to be aware about the fact that these properties can greatly vary in oils coming from the same country (even more if they come from different countries), depending on various factors such as variety, pedoclimatic conditions, ripening, extraction system and storage conditions, among others (Dabbou et al., 2010). In this sense, considering the fact that not all IOC countries members have developed proper and comprehensive databases for their olive oil, and the spreading of olive tree (*Olea europaea* L.) cultivation and oil production outside the Mediterranean region (the historical region of cultivation of *Olea europaea* L.), some studies have reported that certain IOC regulation limits cannot be fulfilled by some olive oils produced in various regions or countries (Ceci and Carelli, 2007; Bajoub et al., 2015). Consequently, some olive oil producing countries are requesting the revision of certain limits fixed by the IOC standards. Moreover, the above-mentioned debate also includes olive oil quality criteria, as some emerging olive oil producing countries, especially Californian State, suggests to modify the threshold for some parameters, such as free fatty acids (FFAs) and peroxide values (PV) (California Department of Food and Agriculture, 2014), whereas other countries, like Australia, New Zealand, and Californian State consider in their standards the measurement of new quality parameters, such as pyropheophytins (PPPs) and the 1,2-diacylglycerols (1,2-DAGs) as indicators of olive oil freshness (Standards

Australia, 2011; California Department of Food and Agriculture, 2014).

When the official analytical methods for olive oil authenticity assessment are considered, it is necessary to face a number of challenges that can be broadly categorized into three key areas. The first is associated with the characteristics of conventional analytical methods used for the official control of olive oil quality and authenticity. Indeed, most of these methods are highly empirical, time consuming, require the use of organic solvents, generate wastes and their accuracy is strongly dependent on reproducing very literally the operating instructions of the standardized procedure (Dais and Hatzakis, 2013). The second is associated with some limitations that the conventional methods for olive oil adulteration control exhibit, such as their inability to identify the nature of the adulterant agent, their ineffectiveness at low adulteration levels, as well as their difficulties in the detection of some adulterants such as hazelnut oil, which present great similarities to olive oil regarding the triacylglycerols (TAGs) and fatty acid (FAs) composition (Zabaras, 2010). The third challenge is linked to the lack of a standardized workflow, which would allow monitoring olive oils labeled with a declaration of production within a specific region (geographic indications) or certified as monovarietal olive oils.

In order to overcome the aforementioned limitations, researchers in olive oil authentication field are continuously working for the development of more robust, efficient, sensitive, rapid and cost-effective analytical methodologies to guarantee the quality, authenticity, and geographic and varietal origins traceability of this valuable matrix, promoting the recent technological progress in the analytical field (Valli et al., 2016).

Thus, in view of all the stated above, the present review paper aims to give an overview on the current state-of-the-art of the most relevant regulatory standards for olive oil authentication, highlighting their differences and discussing their effectiveness, limitations, and the future perspectives of the analytical methods used to carry out the official controls. The paper is structured in two main parts: in the first one, the quality and authenticity indices—required for officially assessing the quality of olive oil and performing its adulteration control—are introduced and their legal thresholds are made explicit and discussed, comparing the values established by the most relevant national and international olive oil authentication legislations. The regulations reviewed herein, were selected on the basis of the importance of the contribution of the countries adopting these systems to olive oil worldwide trade. Furthermore, in this part of the paper, official analytical methods used for the determination of these parameters are outlined. The second part of this contribution focuses, however, on recent developments and applications of modern instrumental analytical techniques to ensure olive oil quality and authenticity, as well as the trends and advances on olive oil geographical and varietal origin traceability.

Olive oil regulatory frameworks: A comparative analysis

The international olive oil market can be considered, as one of the most worldwide regulated markets, in particular because of the existence, for a long time, of international standards (IOC and Codex standards), and European standards (EEC (No

2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis and subsequent modifications)) which regulate the European Union olive oil sector that represents more than 76% and 69% of olive oil production and consumption, respectively (IOC, 2014). However, the globalization of this sector, the emergence of new olive oil producing countries outside the Mediterranean area, and the rise of olive oil consumption in non-traditional olive oil markets, are among the factors that recently stimulated the interest in setting national standard regulations in some of these new producing countries. Some of these regulations are the following: the "United States standards for grades of olive oil and olive-pomace oil" adopted by the USDA in 2010 (USDA, 2010); the "Olive oils and olive-pomace oils Australian standards" adopted by Australian government in 2011 (Standards Australia, 2011), and, most recently, the "Grade and labeling standards for olive oil, refined-olive oil and olive-pomace oil" approved on 2014 by the Department of Food and Agriculture of the State of California (California Department of Food and Agriculture, 2014). However, from the beginning, the emergence of these regulatory standards is prompting a lively debate about their utility, the risk that can represent for the harmonization of the international olive oil trade standards and the need to consolidate efforts to bring major coherence and clarity of olive oil grading and authentication.

Nevertheless, in spite of the differences that can be observed between the above-mentioned olive oil regulations, their basic form remains quite similar. It consists of a description of olive oil grades, and a list of quality and purity criteria, highlighting their threshold values. Furthermore, references for food additives, contaminants, hygiene, and methods of sampling and analysis can be found in these legislations.

Olive oil legal designations and grades

In general, the above-mentioned olive oil regulatory standards gather the various types of oils that can be obtained through olive fruits extraction, on two main categories:

– **Olive oil**: representing the oil obtained solely from the fruit of the olive tree and excludes oils obtained using solvents or mixture of other type oils. It includes two main types of oils: virgin olive oils (also called "natural olive oils" in Australian regulations) which correspond to those oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration; and oils obtained from virgin olive oils by refining methods.

– **Olive-pomace oils**: comprising oils obtained by treating olive pomace (the solid by-product remaining after the mechanical extraction of olive oil) with solvents or other physical treatments, excluding the oils obtained by synthetic processes or by re-esterification processes and mixture with oils of other kinds.

Each one of these categories includes various oil grades, classified according to specific quality criteria fixed by each one of the previously mentioned olive oil regulatory standards (Table 1). Thus, the category of virgin olive oils is divided in two

Table 1. Comparative analysis of the threshold values of physico-chemical (Free fatty acid content (FFAs in % of oleic acid), Peroxide value (PV expressed in meq O_2/kg oil), Ultraviolet-specific extinction coefficients (K_{232}, K_{268} or K_{270}, and ΔK), Free fatty acids ethyl esters content (FAEEs in mg/kg), Pyropheophytins content (PPPs in %) and 1,2-diacylglycerol (1,2- DAGs in %)) and sensory (median of olive oil fruitiness (MeF) and defects (MeD)) quality parameters fixed by the different reviewed olive oil regulatory systems.

				Physico-chemical quality parameters								Sensory evaluation	
				FFAs	PV	K232	K268 or K270	ΔK	FAEEs	PPPs	1,2-DAGs	MeD	MeF
Olive oils	Virgin olive oils	EVOO	IOC	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ /0.01/	≤ 35	N/C	N/C	0	> 0.0
			Codex	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ /0.01/	N/C	N/C	N/C	0	> 0.0
			EU	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ /0.01/	≤ 35	N/C	N/C	0	> 0.0
			USDA	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ /0.01/	N/C	N/C	N/C	0	> 0.0
			AUS	≤ 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ /0.01/	N/C	≤ 17	≥ 35	0	> 0.0
			CAF	≤ 0.5	≤ 15.0	≤ 2.40	≤ 0.22	≤ /0.01/	N/C	N/C	N/C	0	> 0.0
		VOO	IOC	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/A	N/C	N/C	0 < Me ≤ 3.5	> 0.0
			Codex	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/C	N/C	N/C	0 < Me ≤ 2.5	> 0.0
			EU	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/A	N/C	N/C	0 < Me ≤ 3.5	> 0.0
			USDA	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/C	N/C	N/A	0 < Me ≤ 2.5	> 0.0
			AUS	≤ 2.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/C	N/C	N/A	0 < Me ≤ 2.5	> 0.0
			CAF	≤ 1.0	≤ 20.0	≤ 2.60	≤ 0.25	≤ /0.01/	N/C	N/C	N/A	0 < Me ≤ 2.5	> 0.0
		OVOO	IOC	≤ 3.3	≤ 20.0	N/A	≤ 0.30	≤ /0.01/	N/A	N/C	N/C	3.5 <Med ≤ 6.0	N/A
			Codex	≤ 3.3	≤ 20.0	N/A	≤ 0.30	≤ /0.01/	N/C	N/C	N/C	2.5 < Me ≤ 6.0	N/A
		LVOO	IOC	> 3.3	N/A	N/A	N/A	N/A	N/A	N/C	N/C	Me > 6.0	N/A
			EU	> 2.0	N/A	N/A	N/A	N/A	N/A	N/C	N/C	Me > 3.5	N/A
			USDA	> 2.0	N/A	N/A	N/A	N/A	N/A	N/C	N/C	Me > 2.5	N/A
			AUS	> 2.0	> 20.0	>2.60	>0.25	>/0.01/	N/C	N/C	N/A	Me > 2.5	N/A
			CAF	> 1.0	> 20.0	>2.60	>0.25	≤ /0.01/	N/C	N/C	N/A	Me > 2.5	N/A
	ROO		IOC	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/A	N/C	N/C	N/A	N/A
			Codex	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/C	N/C	N/C	N/A	N/A
			EU	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/A	N/C	N/C	N/A	N/A
			USDA	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/C	N/C	N/C	N/A	N/A
			AUS	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/C	N/C	N/A	≤ 2.5	N/A
			CAF	≤ 0.3	≤ 5.0	N/A	≤ 1.10	≤ /0.16/	N/C	N/C	N/A	≤ 2.5	N/A
	OO		IOC	≤ 1.0	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/A	N/C	N/C	N/A	N/A
			Codex	≤ 1.0	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/C	N/C	N/C	N/A	N/A
			EU	≤ 1.0	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/A	N/C	N/C	N/A	N/A
			USDA	≤ 1.0	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/C	N/C	N/C	N/A	N/A
			AUS	≤ 1.0	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/C	N/C	N/A	≤ 2.5	> 0.0
			CAF	≤ 0.8	≤ 15.0	N/A	≤ 0.90	≤ /0.15/	N/C	N/C	N/A	≤ 2.5	> 0.0
Olive-pomace oils	OPO		IOC	≤ 1.0	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/A	N/C	N/C	N/A	N/A
			Codex	≤ 1.0	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/C	N/C	N/C	N/A	N/A
			EU	≤ 1.0	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/A	N/C	N/C	N/A	N/A
			USDA	≤ 1.0	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/C	N/C	N/C	N/A	N/A
			AUS	≤ 1.0	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/C	N/C	N/A	≤ 2.5	> 0.0
			CAF	≤ 0.8	≤ 15.0	N/A	≤ 1.70	≤ /0.18/	N/C	N/C	N/A	≤ 2.5	N/A
	ROPO		IOC	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/A	N/C	N/C	N/A	N/A
			Codex	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/C	N/C	N/C	N/A	N/A
			EU	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/A	N/C	N/C	N/A	N/A
			USDA	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/C	N/C	N/C	N/A	N/A
			AUS	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/C	N/C	N/A	≤ 2.5	N/A
			CAF	≤ 0.3	≤ 5.0	N/A	≤ 2.0	≤ /0.20/	N/C	N/C	N/A	≤ 2.5	N/A

N.B:
- In all the tables presented in this paper, the used abbreviations are listed in the Abbreviations section at the end of the paper.
- COPO has not been included in this table because no limit, for none of the quality parameters, was fixed by the regulatory systems reviewed in this study.

sub-categories, the first one including those oils fitting for direct consumption, which are: EVOO, virgin olive oil (VOO) and ordinary virgin olive oil (OVOO); and the second one constituted by lampante virgin olive oils (LVOO), also called lampante olive oil, that is not fitting for direct consumption but gives rise, after a refining procedure, to refined olive oil (ROO), and olive oil (OO) (consisting of a blend of refined olive oil and virgin olive oils). Furthermore, within the context of these legislations, the category of olive-pomace oils is divided in three grades: crude olive-pomace oil (COPO); refined olive-pomace oil (ROPO) and olive-pomace oil (OPO). Figure 1 illustrates the way to obtain the oils belonging to each one of the mentioned categories. However, despite the similarities among the considered legislations regarding olive oil terminology of nomenclature, some differences can be revealed. In particular, the OVOO category is just considered by the IOC and Codex legislations

(indeed, regarding EU Regulation, the ordinary virgin olive oil category has been deleted since 2001 with the regulation EU 1513/2001); Codex does not consider the LVOO and COPO categories; the LVOO category is denominated "crude olive oil" in the regulation standards adopted by the Department of Food and Agriculture of the State of California, and both this standard legislation and the Australian Standards use the terms crude olive-pomace oil, refined olive-pomace blend, and refined olive-pomace oil for designating the three categories of olive-pomace oils. Therefore, the existence of the described heterogeneous terminology will likely cause certain confusion.

Olive oil quality criteria

The comprehensive official quality control of olive oil requires both diverse analytical determinations and a sensory evaluation; the analytical determination of a considerable number of physico-chemical parameters considered as indicator of hydrolytic modification, oxidation, and freshness status of olive oil has to be carried out, and furthermore, the evaluation of its sensory quality by a panel test recognized by the standardizing body is also needed. Table 1 summarizes the most frequently required physico-chemical and sensory quality indices, as well as their threshold limits according to the regulatory legislations considered in this paper. They mainly include:

– **Content of free fatty acids (FFAs)**: these compounds are the product of TAGs hydrolytic degradation that can occur, during olive oil manufacturing process and storage, due to the action of enzymes (lipase) naturally present in the olive fruit and/or caused by enzymes produced by micro-organisms which grow on the fruit (De Oliveira et al., 2010). Olive oils obtained from healthy fruits, regardless of the cultivar, processed just after harvesting, often show very low FFAs content. Official method for the determination of FFAs content (International Organization of Standardization (ISO) 660 (ISO, 2009a) and American Oil chemists Society (AOCS) Cd 3d-63 (AOCS, 1999)) is based on acid/base titration using potassium hydroxide with phenolphthalein as an indicator, and the results are reported as percentage of oleic acid.

All considered regulatory olive oil legislations establish an upper limit for distinguishing olive oil commercial categories according to FAAs content. However, some differences can be observed considering these limits (Table 1). Indeed, as far as virgin olive oils category is concerned, the Californian regulation indicates lower FAAs content limits for defining the different grades belonging to this category. Thus, while 0.8% and 2.0% are the limits fixed by IOC, EU, Codex, USDA and Australian standards for EVOO and VOO grades, respectively, the Californian regulation establishes 0.5% and 1.0%, respectively, as the upper limits for defining the same categories. Other differences that can be emphasized are that while EU, USDA and Australian standards classify virgin olive oils with FFAs content upper the limit of 2.0% as LVOO, this limit is much lower in Californian regulation which considers the oils with FFAs content upper 1.0% as LVOO. IOC regulation, however, fixed a higher upper limit (>3.3%) to classify a virgin olive oil as LVOO. IOC and Codex

standards are the only examples of regulations which set the limit in 3.3%, so the oils with FFAs values below (or equal) to that value will be considered as OVOO. With regard to the remaining olive oils and olive-pomace oils categories, the reviewed regulation systems require the same FFAs content threshold ($\leq 0.3\%$) for both ROO and ROPO grades, whereas the Californian regulation indicates lower FFAs content limit for defining the OO and OPO ($\leq 0.8\%$), comparatively to the other reviewed regulation systems that establish an upper limit of 1.0%.

– **Peroxide value (PV)**: is an indicator of the primary oxidation status of the olive oil, which can be calculated by measuring the concentration of hydroperoxides, which constitute the first compounds to be formed in the degradation process of the olive oil unsaturated FAs. These compounds are not stable; their value increases, reaches a maximum and then decreases because of their further degradation into secondary oxidation products (such as ketones, aldehydes, and conjugated dienes) (Mariotti, 2014). The official method for the determination of PV (ISO 3960 (ISO, 2007) or AOCS Cd 8b-90 (AOCS, 2003)) is based on the iodometric titration of iodine liberated from potassium iodide after reacting with the peroxides present in the oil samples. Results are expressed as milliequivalent of active oxygen per kilogram of olive oil (meq O_2/kg oil). In general, PV upper limit established for olive oil grading are the same on all the standard legislations considered in the current study, with the exception of the limits established by Californian legislation for the EVOO (being 15 meq O_2/kg the upper limit required by this legislation, whereas the other legislations are a bit more permissive, fixing this limit in 20 meq O_2/kg), and by Australian and Californian legislations for the LVOO (being 20 meq O_2/kg the lower limit set by these legislations, while this parameter is not contemplated for LVOO in the other regulations).

– **Ultraviolet specific extinction coefficients**: conventionally indicated by K_{232} and K_{268} or K_{270} and obtained by the spectrophotometric measurements, in the ultraviolet, of extinctions of the olive oil sample diluted in isooctane or cyclohexane at the wavelengths corresponding to the maximum absorption of the conjugated dienes and trienes, respectively, at about 232 and 268 or 270 nm (ISO 3656 (ISO, 2011) or AOCS Ch 5–91 (AOCS, 1991a)). Besides, the absorption around 270 nm could also be caused by substances formed during earth treatment (olive oil is treated with a decolorizing agent (i.e. an absorbent earth)) during the refining process. In addition to these parameters, ΔK value is often calculated according to the following equation: $\Delta K = K_{max} - [1/2(K_{max}+4 + K_{max}-4)]$ where K_{max} is the specific extinction at the wavelength for maximum absorption at 268 or 270 nm. The maximum allowed values of K_{232}, K_{268} or K_{270} and ΔK for the different grades of olive oils and olive-pomace oils are included in Table 1. Some differences among the considered regulatory systems can be found. Thus, for EVOO, the maximum permitted values of K_{232}, K_{268} or K_{270} (2.5 and 0.22, respectively) and ΔK ($\leq /0.01/$) are the same for practically all the legislative standards. Likewise,

when the VOO category is considered, all the reviewed legislative standards require the same threshold values of K_{232}, K_{268} or K_{270} (2.6 and 0.25, respectively) and ΔK ($\leq$ /0.01/). Moreover, in the case of LVOO, only Californian and Australian legislations fix a limit for K_{232}, K_{268} or K_{270}, and ΔK (being >2.60, >0.25 for K_{232}, K_{268} or K_{270}, respectively, in both legislations, whereas for ΔK the value of /0.01/ is the upper limit in the Californian legislation and the down limit in Australian regulation). In addition, for OO, ROO, and OPO grades, no limit is fixed for K_{232}, being the limits for K_{268} or K_{270} and ΔK the same for all the legislative systems taken into account (Table 1).

– **Content of fatty acid alkyl esters (FAAEs):** this quality criterion has been recently adopted by IOC and EU for the assessment of EVOO quality. However, it is not considered by the other olive oil regulatory standards so far. FAAE compounds result from the esterification of free fatty acids with low molecular weight alcohols (mainly methanol and ethanol) yielding methyl and ethyl esters (Pérez-Camino et al., 2002; Boggia et al., 2014). The olive oil content in terms of these compounds was related to the health conditions of processed olive fruits. Indeed, damaged olive fruits were reported to be susceptible to undergo a hydrolytic process (lipolysis of TAGs with liberation of FFAs) and fermentative degradations (pectin demethylation and sugar fermentation), which create appropriate conditions for the synthesis of FAAEs (Biedermann et al., 2008). Furthermore, olive fruits storage before processing was reported to be a factor that increases the formation of these compounds. Other factors, such as inappropriate practices during oil extraction, catalyze the esterification reaction which increases the amount of these compounds in the obtained oils. In addition to their role as a quality parameter, FAAEs content has been reported as a relevant tool for detecting EVOO adulteration with low quality virgin olive oils, that have undergone a mild deodorization treatment conducted at a moderate temperature ($\leq$100 °C), which remove volatile compounds that are responsible for their undesirable sensory attributes (Pérez-Camino et al., 2008). With regard to the analytical determination of FAAEs, the official method (COI/T.20/Doc. No 28 (IOC, 2010a)) requires a preliminary separation of these compounds from the oil by means of a classical column chromatography, using silica gel as adsorbent, with hexane and ethyl ether as eluents; then, the solvents are evaporated by a rotary evaporator, and finally, the fraction containing the methyl and ethyl esters is diluted with *n*-heptane or *iso*-octane and analyzed by a gas chromatography (GC) system for further identification and quantification purposes. IOC and EU regulate both the content of fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs). A legal limit of 75 mg/kg for the sum of FAME and FAEE, or superior than 75 mg/kg and inferior than or equal to 150 mg/kg for the sum of FAME and FAEE (if the ratio of FAEE/FAME is below 1.5) was fixed for oils produced over the crop season 2012/2013 EU Commission Regulation No 61/(2011). However, from the crop season of 2013/2014, only FAEE content is considered, with a maximum value of 40, 35, and 30 mg/kg for oils produced during 2013/2014, 2014/2015 and 2015/2016, respectively (EU Commission Implementing Regulation No 1348/2013). After the crop season of 2016, the maximum value of FAEEs for the EVOO grade is going to be lower than or equal to 35 mg/kg (EU Commission Delegated Regulation 2016/2095).

– **Content of pyropheophytins (PPPs):** determination of PPPs content is only required by Australian and Californian standards for EVOO freshness evaluation. PPPs are formed during olive oil extraction and storage, due to the degradation of chlorophyll pigments (pheophytinization and a certain degree of allomerization). Chlorophyll breaks down to pheophytin *a*, then converts to pyropheophytins *a* as a result of the loss of the carbomethoxy group at carbon 13 (C13)(Aparicio-Ruiz et al., 2010; Guillaume et al., 2014). The generated amount of pyropheophytins *a* remains small, but their content in olive oil increases during the storage depending on various factors, such as olive fruits variety, ripeness, and seasonal conditions (Gallardo-Guerrero et al., 2005). For this reason, the PPPs *a* content in terms of the ratio of pyropheophytin *a* divided by total pheophytins *a* -which is independent of these factors (Aparicio et al., 2013b)- was considered as a freshness parameter of EVOO. The standard method for the determination of PPPs content in olive oil (ISO 29841 (ISO, 2009b)) involves their separation using a miniaturized column chromatography on silica gel and chromatographic analysis using a reverse phase liquid chromatography with a photometric or fluorescence detector. In both Australian and Californian standards, as can be seen in Table 1, a legal limit of 17% of PPPs *a* is set for classifying a virgin olive oil as EVOO.

– **1,2-diacylglycerols (1,2-DAGs):** this is a quality and freshness parameter just considered by Australian and Californian standards for grading olive oil as EVOO. The estimation of this parameter is made by calculating the mass fraction ratio between 1,2-DAGs and the sum of 1,2-DAGs and 1,3-DAGs. DAGs are present in virgin olive oils in low amounts (between 1% and 3%) as intermediate products of the biosynthesis of TAGs (1,2-DAGs) or as products of enzymatic or chemical hydrolysis of TAGs (1,3-DAGs) (Pérez-Camino et al., 2001). During storage, the 1,2-DAGs undergo isomerization, yielding 1,3-DAGs, that are more stable. Consequently, assessing the amounts of these isomeric forms could be informative about the age and the freshness of virgin olive oils. Currently, the official method for the determination of this quality criterion (ISO 29822 (ISO, 2009c)) includes the separation of these isomeric forms on a silica gel chromatography column, derivatization (sylilation), and GC analysis. To classify a virgin olive oil as an EVOO, both Australian and Californian standards have set as minimum level the value of 35% for the ratio between 1,2-DAGs and the sum of 1,2-DAGs and 1,3-DAGs (Table 1).

– **Sensory quality:** pleasant sensory characteristics of olive oil are one of the main reasons for the acceptability and

preference of consumers of this foodstuff. In addition, the cultivation of various olive tree varieties in different pedo-climatic conditions and the use of diverse agronomical and technological techniques for olive extraction and production are the main factors behind the existence, in the olive oil market, of a myriad of olive oils with very distinctive flavor characteristics. The official method for the sensory evaluation of olive oil (COI/T.20/Doc. No 15 (IOC, 2007)) consists on a panel test method applied by a fully selected and trained taste panel recognized by the regulatory body. The method determines the category of olive oil according to the detection and intensity of sensory positive and negative attributes in the analyzed oil. Fruitiness, bitterness and pungency are sensory positive attributes determined by the panelists; whereas fusty-muddy, mustiness-humidity, winey-vinegary, frostbitten olives and rancid constitute the main defects. The panelists provide an intensity value of each attribute, and then the median values of olive oil fruitiness (MeF) and of the most perceived defect (MeD) are calculated. Finally, each grade of olive oil is defined according to the obtained results (Table 1). Thus, when no negative attributes are detected, and the MeF is superior to zero, all the regulatory standards classify the virgin olive oil as EVOO. However, some differences exist among these regulatory systems regarding the sensory evaluation of the other categories. Indeed, in the case of VOO grade, IOC and EU standards fix a maximum value of MeD of 3.5; whereas the other standards trades demand lower values (lower than or equal to 2.5). USDA, Australian and Californian standards classify all olive oils with a MeD superior to 2.5 as LVOO (also, in the case of USDA regulation, an olive oil is classified as LVOO when MeD is less than or equal to 2.5 and the MeF is equal to 0). EU standards, however, consider a higher value (3.5) (or when MeD is less than or equal to 3.5 and the MeF is equal to 0), and the IOC standards threshold is much higher for the LVOO category (value of MeD superior to 6 is established). Both IOC and Codex are the only ones defining olive oils with MeD value between 3.5 and 6 (or when MeD is less than or equal to 3.5 and the MeF is equal to 0), and between 2.5 and 6 (or when MeD is less than or equal to 2.5 and the MeF is equal to 0), respectively, as OVOO. For the other categories, mainly ROO, OO, OPO, and ROPO, the Australian and Californian standards are the only ones which set a limit value of MeD (2.5) for defining these grades. However, for the COPO category, neither regulatory trade limits of MeD nor MeF have been established.

Olive oil purity criteria

In accordance with the regulations concerning olive oil authentication, the olive oil genuineness is defined by values with the lowest and/or highest limits for the content of the selected purity criteria specified by these legislations. Such criteria are related to the amount of diverse groups of chemical compounds in olive oil. In contrast to quality criteria for which some parameters are not considered by all the reviewed olive oil standards trades, the contemplated purity criteria are the same

for every legislation (even if the fixed thresholds show some differences). Nine purity criteria are considered; the limits for each parameter in different grades of olive oils and olive-pomace oils are given in Tables 2 and 3, respectively.

– **Fatty acid composition (%)**: FAs are the main constituents of olive oil forming part of TAGs molecules. Olive oil is characterized by the predominance of monounsaturated (in particular, oleic acid), the low percentage of saturated and a very low percentage of polyunsaturated FAs. According to the official methods, these compounds are evaluated by means of the analysis of methyl esters of FAs using GC with flame ionization detector (FID) (preparation of methyl esters in accordance with AOCS Ce 2–66 (AOCS, 2009) or ISO 5509 (ISO, 2000) or COI/T.20/Doc.24 (IOC, 2001a), and analysis of these compounds by GC-FID according to ISO 5508 (ISO, 1990) or AOCS Ch 2–91 (AOCS, 1991b)).

The limits of variability of the content of olive FAs of olive oils and olive-pomace oils, expressed as percentage of total FAs, as set by the different reviewed regulations are reported in Tables 2 and 3. From these Tables, it can be seen that such limits are consistent for each category of olive oils and olive-pomace oils with the exception of behenic acid for which the upper limit is a bit higher for olive-pomace oils category (0.3%) in comparison with olive oils categories (0.2%). The first remarkable observation that can be revealed when analyzing data from Tables 2 and 3 is that in contrast to Australian, IOC, Codex, EU, and USDA regulations that include the content of 13 FAs as purity criteria, in Californian legislation only the determination of the content of 6 FAs (myristic, heptadecenoic, stearic, arachidic, behenic, and lignoceric) is mandatory. Besides, as shown in these Tables, the percentages of some FAs (palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and stearic (C18:0)) are expected to vary within a quite large range, whilst the other FAs (myristic (C14:0), heptadecanoic (C17:0), heptadecenoic (C17:1), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0) and lignoceric (C24:0)) are found at lower levels than 1.5% and only their upper limits are established. In addition, with regard to the value of the limits set by these regulations (Tables 2 and 3), IOC and EU standards establish the same limits for all regulated FAs, whereas some differences can be observed with the other regulation standards. Thus, with the exception of C18:0, C20:0, C22:0, and C24:0 FAs for which the same upper limits are fixed by all the regulations considered by the current study, the thresholds of the other regulated FAs show some differences. The disparity can be illustrated, for instance, with the case of linolenic fatty acid; there no limit according to the Codex and Californian regulations; however, IOC and EU regulations establish a limit of 1%, whereas USA and AUS standards set a higher limit (1.5%).

– **Fatty acids *trans* isomers content (%)**: the normal arrangement of double bonds in unsaturated FAs in olive oil is *cis* configuration. The presence of *trans* isomers of oleic (*trans* C18:1), linoleic and linolenic acids (C18:2T+C18:3T), in percentages exceeding the

established limits (Tables 2 and 3), can indicate adulteration of virgin olive oils with hydrogenated seed oils, ROO and OPO, among others (Aparicio et al., 2013b). These compounds are determined in accordance with COI/T.20/Doc. No 17 (IOC, 2001b), or ISO 15304 (ISO, 2002) or AOCS Ce 1f-96 (AOCS, 1996a). Concerning the upper limits fixed for these parameters, all the reviewed regulations consider the same values.

– **Difference between actual and theoretical content of triacylglycerols (ΔECN42)**: in contrast to many seed oils, the chemical composition of olive oil shows an abundance of TAGs with equivalent carbon number (ECN) 44, 46, 48, and 50, whereas TAGs with ECN40 and ECN42 are absent or found at trace amounts (Angerosa et al., 2006). Therefore, the determination of the difference between the experimental values of TAGs ECN42 obtained by HPLC with refractive index detector and the theoretical value (ECN 42 theoretical) calculated from the fatty acid composition by GC-FID is used to detect blends of virgin olive oils with unsaturated oils. This parameter is determined according to COI/T.20/Doc. No. 20 (IOC, 2010b) or AOCS Ce 5b-89 (AOCS, 1989). All the reviewed regulations establish the same values as upper limit (Tables 2 and 3) for each one of the olive oils and olive-pomace oils grades defined by these regulations.

– **Sterols**: these compounds constitute one of the main chemical classes of the olive oil unsaponifiable fraction. The determination of olive oil sterol total content as well as its individual composition (content of cholesterol, brassicasterol, campesterol, stigmasterol, Δ7-stigmastenol and apparent β-sitosterol (the sum of contents of Δ5,23 and Δ5,24 stigmastadienols, clerosterol, β-sitosterol, sitostanol, and Δ5-avenasterol), is required by some trade standards to detect possible adulteration of olive oil with foreign oils (Youseff et al., 2014). Official methods for the analysis of sterols in olive oil (COI/T.20/Doc. No. 10 (IOC, 2001c), or ISO 12228 (ISO, 1999a) or AOCS Ch 6–91 (AOCS, 1991c)) involve several steps. First, olive oil saponification is required for the separation of saponifiable and unsaponifiable fractions, then separation by thin-layer chromatography on silica gel plates and derivatization of the sterols have to be carried out. The sterols as trimethylsilyl derivatives are identified and quantified, afterwards, by means of a capillary GC-FID platform.

As can be seen in Table 2, concerning the total sterols content in olive oil categories, Californian regulation is the only one that establishes no limit for grading olive oils into EVOO, VOO and LVOO categories, while the other regulations set a minimal value of 1000 mg/1000 g. Regarding the ROO and OO categories, all the considered regulations set a minimal value of 1000 mg/1000 g. In the case of pomace oils, all the reviewed regulations fix the same minimal values (Table 3). Besides, when the individual sterols content are considered, some differences can be found for both olive oils and olive-pomace oils categories. Within this context, as noticed for FAs composition, Californian standards is the only one requiring the determination of a restricted number of sterol compounds (specifically two compounds: brassicasterol and stigmasterol) (Tables 2 and 3). Thus, IOC, EU and Codex regulations consider the same values for all the regulated compounds; however, USDA, Californian and Australian standards show some differences as can be observed in the tables. For example, Codex, EU and IOC regulations fix an upper limit of 4.0% for the campesterol content, and a decision tree is proposed to verify the authenticity of oils having contents between 4 and 4.5%. However, USDA regulations fix the maximum content for the campesterol on 4.5% (even though it requires the authentication of the oils showing content between 4 and 4.5%); Australian regulation allows higher content for this compound, 4.8%; and no limit is established by Californian regulation.

– **Triterpene dialcohols (sum of erythrodiol and uvaol)**: they are also part of the unsaponifiable fraction of olive oil and their determination is usually carried out together with the sterol fraction. These compounds are mainly found in the fruit skin, so that they are detected at higher concentrations in pomace that undergoes solvent extraction (Habib et al., 2015). For this reason, percentage of erythrodiol and uvaol in relation to those of sterols is considered as a suitable authenticity index to detect possible fraudulent admixtures of virgin olive oils with olive-pomace oils. As shown by Tables 2 and 3, all the reviewed regulations fix a value of 4.5% as the maximum content for virgin olive oils, ROO and OO on erythrodiol and uvaol, except for Californian regulation which establish no limit. In the case of olive-pomace oils, both Codex and Californian regulations establish no limit for grading tested oils, whereas the remaining standards legislations fix the same value (>4.5%).

– **Wax esters**: they are a group of esters of FAs and long-chain aliphatic alcohols accumulated in the skin of olive fruits and, therefore, they are found in considerably higher amounts in olive-pomace oils than virgin olive oils (Tena et al., 2015). Hence, wax content is used to detect virgin olive oils adulteration with olive-pomace oils. Furthermore, this determination can be used as a quality parameter, considering total aliphatic alcohols content and/or the sum of erythrodiol and uvaol. In the unsaponifiable fraction of olive oils, three classes of waxy compounds can be detected: waxes with chain lengths lower than 40 (C36 and C38), others as C40 and C42, and waxes with 44 or more carbon atoms (C44 and C46). The official methods for the determination of wax content (COI/T.20/Doc. No. 18 (IOC, 2003a) or AOCS Ch 8–02 (AOCS, 2002)) are based on their separation from the olive oil unsaponifiable fraction by silica gel chromatography and analysis by capillary GC-FID. The waxes content is expressed as the sum of C40, C42, C44 and C46 waxes; however, in the case of EVOO and VOO, IOC and EU just consider the C42, C44 and C46 waxes and establish a maximum value of 150 mg/kg (Table 2). For the other categories, similar values are established by all the reviewed regulations.

– **Total aliphatic alcohols content**: these compounds are found at significantly higher concentration levels in olive-pomace oils than in virgin olive oils

(Gandul-Rojas and Mínguez-Mosquera, 2006). The main aliphatic alcohols components detected in the unsaponifiable fraction of virgin olive oils are docosanol (C22), tetracosanol (C24), hexacosanol (C26), and octacosanol (C28). Other aliphatic alcohols, such as tricosanol (C23), pentacosanol (C25), and heptacosanol (C27) are present in low amounts. The standard methodology for the determination of aliphatic alcohols (COI/T.20/Doc. No. 26 (IOC, 2003b)) includes their separation from the oil unsaponifiable fraction by chromatography on a basic silica gel plate and their analysis and quantification by using GC-FID with a capillary column. The total content of these compounds (expressed by the sum of the concentrations of individual aliphatic alcohols), in combination with other purity parameters (erythrodiol and uvaol, and wax content) is used to distinguish the presence of LVOO and olive-pomace oils in virgin olive oils. All the reviewed standards regulations consider an olive oil as LVOO when the wax content is between 300 and 350 mg/kg, if the total aliphatic alcohol content is <350 mg/kg or the erythrodiol + uvaol content is <3.5%. In contrast, if the total aliphatic alcohol content is >350 mg/kg, the erythrodiol+uvaol content is >3.5% and the oil shows a wax content between 300 mg/kg and 350 mg/kg, the sample will be considered as COPO.

– **Stigmastadienes**: these compounds are formed in olive oils during the refining process as a consequence of the acid catalyzed sterol dehydration reaction in the course of bleaching process, or during the deodorization process, promoted by high temperatures (Crews et al., 2014). Among these compounds, stigmasta-3,5-diene originated from the dehydration of β-sitosterol is the most abundant. Therefore, its determination in olive oils (COI/T.20/Doc. No. 11 (IOC, 2001d), or ISO 15778–1(ISO, 1999b) or AOCS Cd 26–96 (AOCS, 1996b)) by means of preparative chromatography and the subsequent analysis by GC-FID is an important indicator of the presence of refined oils in virgin olive oils, even at very low concentrations (Cert et al., 1994; Angerosa et al., 2006). Some differences can be found regarding the limits set by the reviewed standards regulations in the case of EVOO and VOO categories (Table 2). Indeed, while IOC and EU regulation establish an upper limit of 0.05 ppm, the other regulations allow a higher content (0.15 for Codex and USA regulations and 0.10 for Australian and Californian standards). For the LVOO category, an upper limit of 0.50 ppm of stigmasta-3,5-diene has been fixed by all the reviewed regulations. For the remaining olive oils and olive-pomace oils, the determination of this parameter is not required by any of the reviewed regulations (Tables 2 and 3).

– **2-glyceryl monopalmitate (2P)**: this parameter characterizes the percentage of palmitic acid at the 2-position of TAGs by means of 2P evaluation. In virgin olive oils only about 2% of the amount of the palmitic acid present is bonded on position 2 of TAGs compounds, whilst in oils artificially esterified the bonding with glycerol occurs in a random manner and significantly increases that

percentage. Therefore, the determination of virgin olive oil content on 2P is used for the detection of admixtures with esterified oils (Boskou, 2015). The concentration of 2P is determined in accordance with COI/T.20/Doc. No. 23 (IOC, 2006) or ISO 12872 (ISO, 2010), after hydrolysis of TAGs by enzymatic digestion with pancreatic lipase which only hydrolyzes the ester bonds in positions 1 and 3, leaving intact the bond in position 2 of glycerol. The method also implies the separation by silica gel chromatography, silanization, and the analysis with capillary GC-FID. Limits adopted by IOC, EU, and USDA regulations are the same for the olive oils and olive-pomace oils categories, with the exception of ROO and OO categories, for which no limits are established by USDA regulation. In these standards, the upper limit (%) of 2P is assigned according the oil content on palmitic acid. In contrast, in the other reviewed regulations (Codex, Australian and Californian), the content on palmitic acid is not considered, and higher content of 2P is allowed (Tables 2 and 3). As can be also seen in these tables, in the case of Californian standards, the content of 2P is only regulated for oils from the categories ROO and OO.

Recent progress and trends in olive oil authentication

Given the drawbacks and limitations that some of the official analytical methods used for the authentication of virgin olive oils show regarding different aspects, a number of alternative analytical methods and techniques have been suggested over the past decade.

Advances in analytical methods to determine olive oil quality indices

Considering the physicochemical olive oil quality criteria (Table 4), for the determination of FFAs, several spectroscopic methods, including Near-infrared (NIR) (Marquez et al., 2005; Cayuela et al., 2009), Visible/Near Infrared (Vis/NIR) (Cayuela Sánchez et al., 2013; García Martín 2015), Fourier transform infrared (FT-IR) (Bendini et al., 2007) and Fourier transform-Raman (FT-Raman) (Muik et al., 2003) have been proposed for the determination of olive oil acidity reaching significantly good results. Furthermore, others analytical techniques based on flow injection analysis (FIA) in automated systems (Bonastre et al., 2004), electrochemical methods using electrical impedance spectroscopy detector (Grossi et al., 2014), enzymatic methods (Ben Rejeb and Gargouri, 2011) and capillary electrophoresis (CE) (Balesteros et al., 2007) have been proposed for the determination of virgin olive oil FFAs. Further details about the recent analytical methods proposed for the determination of olive oil FFAs content can be found in De Oliveira et al. (2010).

Besides, some papers have focused on the development of analytical methods for the PV evaluation in virgin olive oils, based on either direct or indirect measurement of hydroperoxides. They include the development and application of a direct parallel flow injection multichannel spectrophotometric method (Thomaidis et al., 2000), the use of electrochemical sensors (Adhoum and Monser, 2008), the application of

Table 2. Comparative analysis of the threshold values of purity criteria established for olive oils grades by the different reviewed olive oil regulatory systems.

Olive oils grades

EVOO+ VOO

	IOC	EU	Codex	USDA	AUS	CAF
Fatty acids composition (%)						
Myristic	≤ 0.03	≤ 0.03		≤ 0.05	≤ 0.05	
Palmitic	7.50 - 20.0	7.50 - 20.0			7.0 - 20.0	N/C
Palmitoleic	0.30 - 3.50	0.30 - 3.50		0.30		
Heptadecanoic	≤ 0.40	≤ 0.40				
Heptadecenoic	≤ 0.60	≤ 0.60		≤ 0.30	≤ 0.40	N/C
Stearic	0.50 - 5.0	0.50 - 5.0				
Oleic	55.0 - 83.0	55.0 - 83.0		53.0 - 85.0	53.0 - 85.0	
Linoleic	2.5 - 21.0	2.5 - 21.0		3.5 - 21.0	2.50 - 22.0	N/C
Linolenic	≤ 1.00	≤ 1.00	N/C	≤ 1.50	≤ 1.50	
Arachidic				≤ 0.60		
Eicosenoic			≤ 0.40		≤ 0.50	N/C
Behenic				≤ 0.20		
Lignoceric				≤ 0.20		
C18:1 T (%)				≤ 0.05		
C18:2 T+C18:3 T(%)				≤ 0.05		
ΔECN42				≤ 0.2/		
Sterols composition (%)						
Cholesterol			≤ 0.5	≤ 0.5		N/C
Brassicasterol						
Campesterol (camp)	≤ 4.0	≤ 4.0		≤ 4.5	≤ 4.8	N/C
Stigmasterol	< camp	< camp			≤ 1.9	
Delta-7-stigmastenol*	≥93.0	≥93.0	≤ 0.5			N/C
Apparent β-sitosterol					≥92.5	
Total sterol content (mg/1000g)	≥1000	≥1000				N/A
Erythrodiol+Uvaol (%)	≤ 4.5	≤ 4.5				N/A
Wax (mg/kg)	≤ 150	≤ 150		≤ 250	≤ 250	
Aliphatic alcohols (mg/kg)						
Stigmastadienes (%)	≤ 0.05	≤ 0.05	≤ 0.15		≤ 0.10	N/A
ΣP (%)						
If C:16:0 ≤ 14.0%	≤ 0.9	≤ 0.9	≤ 1.5	≤ 0.9	≤ 1.5	
If C:16:0 > 14.0%	≤ 1.0	≤ 1.0		≤ 1.0		

OVOO

	IOC	Codex	EU	USDA	AUS	CAF
Myristic	≤ 0.03	≤ 0.05	≤ 0.03		≤ 0.05	
Palmitic	7.50 - 20.00	7.50 - 20.0			7.0 - 20.0	N/C
Palmitoleic	0.30 - 3.50					
Heptadecanoic	≤ 0.40	≤ 0.30	≤ 0.40		≤ 0.30	
Heptadecenoic	≤ 0.60	≤ 0.30	≤ 0.60		≤ 0.40	N/C
Stearic	0.50 - 5.00					
Oleic	55.00 - 83.00	55.0 - 83.0			53.0 - 85.0	
Linoleic	2.5 - 21.0	3.5 - 21.0	2.5 - 21.0	3.5 - 21.0	2.50 - 22.0	N/C
Linolenic	≤ 1.00	N/C	≤ 1.00		≤ 1.50	
Arachidic	≤ 0.60					
Eicosenoic	≤ 0.40		≤ 0.40		≤ 0.50	N/C
Behenic	≤ 0.20			≤ 0.20		
Lignoceric	≤ 0.20			≤ 0.20		
C18:1 T (%)	≤ 0.05			≤ 0.1		
C18:2 T+C18:3 T(%)	≤ 0.05			≤ 0.1		
ΔECN42	≤ 0.2/	≤ 0.2/		≤ 0.3/		
Cholesterol	≤ 0.5	≤ 0.5	≤ 0.1	≤ 0.5		N/C
Brassicasterol						
Campesterol (camp)	≤ 4.0	≤ 4.0	≤ 4.0	≤ 4.5	≤ 4.8	N/C
Stigmasterol	< camp	< camp				
Delta-7-stigmastenol*	≤ 0.5	≤ 0.5		≤ 0.5		N/C
Apparent β-sitosterol	≥93.0	≥93.0		≥93.0	≥92.5	
Total sterol content (mg/1000g)	≥1000	≥1000		≥1000		N/A
Erythrodiol+Uvaol (%)	≤ 4.5	≤ 4.5		≤ 4.5		N/A
Wax (mg/kg)	≤ 250	≤ 250		≤ 300		
Stigmastadienes (%)	≤ 0.1	≤ 0.15			≤ 0.50	
If C:16:0 ≤ 14.0%	≤ 0.9	≤ 1.5	≤ 0.9	≤ 1.5	≤ 1.5	N/A
If C:16:0 > 14.0%	≤ 1.0		≤ 1.1			

LVOO

	IOC	EU	USDA	AUS	CAF
Myristic	≤ 0.03	≤ 0.03		≤ 0.05	
Palmitic	7.50 - 20.0	7.50 - 20.0		7.0 - 20.0	N/C
Palmitoleic			0.30 - 3.50		
Heptadecanoic	≤ 0.40	≤ 0.40		≤ 0.30	
Heptadecenoic	≤ 0.60	≤ 0.60	≤ 0.30	≤ 0.40	N/C
Stearic			0.50 - 5.0		
Oleic	55.0 - 83.0	55.0 - 83.0		53.0 - 85.0	
Linoleic	2.5 - 21.0	2.5 - 21.0	3.5 - 21.0	2.50 - 22.0	N/C
Linolenic	≤ 1.00	≤ 1.00		≤ 1.50	
Arachidic			≤ 0.60		
Eicosenoic		≤ 0.40		≤ 0.50	N/C
Behenic			≤ 0.20		
Lignoceric			≤ 0.20		
C18:1 T (%)			≤ 0.1		
C18:2 T+C18:3 T(%)			≤ 0.1		
ΔECN42			≤ 0.3/		
Cholesterol		≤ 0.1	≤ 0.5		N/C
Campesterol (camp)	≤ 4.0	≤ 4.0	≤ 4.5	≤ 4.8	N/C
Stigmasterol			N/A		
Delta-7-stigmastenol*			≤ 0.5		N/C
Apparent β-sitosterol	≥93.0		≥93.0		
Total sterol content (mg/1000g)	≥1000		≥1000		N/A
Erythrodiol+Uvaol (%)	≤ 4.5		≤ 4.5		N/A
Wax (mg/kg)			≤ 300		
Stigmastadienes (%)			≤ 0.50		
If C:16:0 ≤ 14.0%	≤ 0.9	≤ 0.9		≤ 1.5	N/A
If C:16:0 > 14.0%	≤ 1.0**	≤ 1.1		≤ 1.8	

An olive oil is classified as LVOO when the wax content is between 300 and 350 mg/kg, if the total aliphatic alcohol content is between 300 and 350 mg/kg.

ROO+OO

	IOC	EU	Codex	USDA	AUS	CAF
Myristic	≤ 0.03	≤ 0.03		≤ 0.05		
Palmitic	7.50 - 20.0	7.50 - 20.0	0.30 - 3.50	7.0 - 20.0	7.0 - 20.0	N/C
Heptadecanoic	≤ 0.40	≤ 0.40		≤ 0.30		
Heptadecenoic	≤ 0.60	≤ 0.60	≤ 0.3	≤ 0.3	≤ 0.40	N/C
Stearic			0.50 - 5.0		
Oleic	55.0 - 83.0	55.0 - 83.0		53.0 - 85.0	53.0 - 85.0	
Linoleic	2.5 - 21.0	2.5 - 21.0	3.5 - 21.0	3.5 - 21.0	2.50 - 22.0	N/C
Linolenic	≤ 1.00	≤ 1.00	N/C	≤ 1.50	≤ 1.50	
Arachidic				≤ 0.60		
Eicosenoic			≤ 0.40		≤ 0.50	N/C
Behenic				≤ 0.20		
Lignoceric				≤ 0.20		
C18:1 T (%)				≤ 0.20		
C18:2 T+C18:3 T(%)				≤ 0.30		
ΔECN42				≤ 0.3/		
Cholesterol			≤ 0.5	≤ 0.5		N/C
Campesterol (camp)	≤ 4.0	≤ 4.0		≤ 4.5	≤ 4.8	N/C
Stigmasterol			< camp		≤ 1.9	
Delta-7-stigmastenol*			≤ 0.5			N/C
Apparent β-sitosterol	≥93.0		≥93.0	≥1000	≥92.5	
Total sterol content (mg/1000g)	≥1000					N/A
Erythrodiol+Uvaol (%)	≤ 4.5			≤ 4.5		N/A
Wax (mg/kg)	≤350			≤350		
Stigmastadienes (%)			N/A			
If C:16:0 ≤ 14.0%	≤ 0.9	≤ 0.9	≤ 1.8	N/A	≤ 1.8	
If C:16:0 > 14.0%	≤ 1.0**					

Aliphatic alcohols (mg/kg): An olive oil is classified as LVOO when the wax content is between 300 and 350 mg/kg, if the total aliphatic alcohol content is < 350 mg/kg or the erythrodiol + uvaol content is < 3.5%.

* When Δ-7-stigmastenol levels are within the range 0.5-0.8 (for extra virgin and virgin olive oils) a decision tree is proposed to verify the authenticity of the oils by IOC and EU regulations.
** ≤ 1.1 for RVOO grade in EU regulation.

Table 3. Comparative analysis of the threshold values of purity criteria established for olive-pomace oils grades by the different reviewed olive oil regulatory systems.

		OPO						ROPO						COPO				
		IOC	EU	Codex	USDA	AUS	CAF	IOC	EU	Codex	USDA	AUS	CAF	IOC	EU	USDA	AUS	CAF
Fatty acids composition (%)	Myristic	≤0.03			≤0.05	≤0.05		≤0.03			≤0.05	≤0.05		≤0.03		≤0.05	≤0.05	
	Palmitic	7.50–20.0			0.30–3.50	7.0–20.0	N/C	7.50–20.0			0.30–3.50	7.0–20.0	N/C	7.50–20.0		0.30–3.50	7.0–20.0	N/C
	Palmitoleic																	
	Heptadecanoic	≤0.40			≤0.30	≤0.30	N/C	≤0.40			≤0.30	≤0.30	N/C	≤0.40		≤0.30	≤0.30	N/C
	Heptadecenoic	≤0.60			≤0.30	≤0.40	N/C	≤0.60			≤0.30	≤0.40	N/C	≤0.60		≤0.30	≤0.40	N/C
	Stearic				0.50–5.0						0.50–5.0					0.50–5.0		
	Oleic	55.0–83.0				53.0–85.0		55.0–83.0				53.0–85.0		55.0–83.0			53.0–85.0	
	Linoleic	2.5–21.0			3.5–21.0	2.5–22.0	N/C	2.5–21.0			3.5–21.0	2.5–22.0	N/C	2.5–21.0		3.5–21.0	2.5–22.0	N/C
	Linolenic	≤1.00		N/C		≤1.50		≤1.00		N/A		≤1.50		≤1.00		≤1.50		
	Arachidic				≤0.60						≤0.60					≤0.60		
	Eicosenoic	≤0.40				≤0.50	N/C	≤0.40				≤0.50	N/C	≤0.40			≤0.50	N/C
	Behenic				≤0.30						≤0.30					≤0.30		
	Lignoceric				≤0.20						≤0.20					≤0.20		
	C18:1 T (%)				≤0.40						≤0.40					≤0.20		
	C18:2 T+C18:3 T (%)				≤0.35						≤0.35					≤0.10		
	ΔECN42 (%)				≤0.5/						≤0.5/					≤0.6/		
Sterols composition (%)	Cholesterol				≤0.5		N/C				≤0.5		N/C			≤0.5		N/C
	Brassicasterol	≤0.2				≤0.1		≤0.2				≤0.1		≤0.2			≤0.1	
	Campesterol (camp)	≤4.0			≤4.5	≤4.8	N/C	≤4.0			≤4.5	≤4.8	N/C	≤4.0		≤4.5	≤4.8	N/C
	Stigmasterol			<camp		≤1.9				<camp		≤1.9			N/A		≤1.9	
	Delta-7-stigmastenol*				≤0.5						≤0.5					≤0.5		N/C
	Apparent β-sitosterol	≥93.0				≥92.5	N/C	≥93.0				≥92.5	N/C	≥93.0			≥92.5	
	Total sterol content (mg/1000g)				≥1600		N/A				≥1800		N/A			≥2500		N/A
	Erythrodiol+Uvaol (%)	> 4.5		N/A		> 4.5	N/A	> 4.5		N/A		> 4.5	N/A	≥93.0		> 4.5	≥92.5	N/A
	Wax (mg/kg)				>350						>350					>350		
	Aliphatic alcohols (mg/kg)	\<If the total aliphatic alcohol content is > 350 mg/kg (for olive pomace oils (crude and refined)) a decision tree is proposed to verify the authenticity of the oils by IOC and EU regulations.\>						\<If the total aliphatic alcohol content is > 350 mg/kg, the erythrodiol+uvaol content is > 3.5% and the oil shows a wax content between 300 mg/kg and 350 mg/kg, olive oil is considered as COPO\>								N/A		
	Stigmastadienes (%)				N/A						N/A					N/A		
	2P (%)	≤1.2		≤2.2	≤1.2	≤2.2	N/A	≤1.4		≤1.4	≤1.4	≤2.2	N/A	≤1.4			≤2.2	N/A

* When Δ-7-stigmastenol levels are within the range 0.5–0.7 (for olive pomace oils (crude and refined)) a decision tree is proposed to verify the authenticity of the oils by IOC and EU regulations.

Table 4. Representative examples of recent analytical methodologies proposed for olive oil quality parameters determination.

Analytical techniques	Instrumental measurements	Data treatment	Main findings	References
Vibrational spectroscopic techniques	NIR	PLS	Real-time evaluation of olive oil FFAs with a standard error of prediction of 0.999	Marquez et al., 2005
		PLS	Development of chemometric models with good predictive potential for olive oil FFAs determination by directly measuring the fruit	Cayuela et al., 2009
		PLS	Predictive models with good performance for the determination of PV, sensory attributes and others olive oil analytical parameters and compounds	Inarejos-García et al., 2013
	Vis/NIR	PLS	Development, with good performances, of predictive models for the determination of FFAs, PV, and conjugated dienes	Cayuela et al., 2013
		PLS, MCUVE and SPA	Improving accuracy of Vis/NIR chemometric models for olive oil FFAs determination by using longer path lengths and long wavelengths of the visible	García Martín 2015
	ATR-FTIR	PLS	Development of predictive models with very good performances for rapid and accurate measurements of FFAs and PV	Bendini et al., 2007a
	FT-Raman	PLS	Development of chemometric models with very good predictive ability for rapid and accurate measurement of olive and olive oil FFAs	Muik et al.,2003
	FT-NIR	PLS	Calibration model for free acidity of virgin olive oils with satisfactory predictive ability was developed using partial least squares regression	Bendini et al., 2007b
	FT-MIR	PLS, OLS	Rapid determination of olive oil PV. Results were in agreement with results obtained with the official standard method	Pizarro et al., 2013a
	FT-IR	PLS	Developed chemometric models allowed the determination FAAES with an accuracy similar to the reference official standard method	Valli et al.,2013
Chromatographic techniques	GC-FID	ANOVA	Solid-phase microextraction is an efficient alternative method for FAAEs preparation	Perez-Camino et al.,2008
	GC-EI MS	PCA and PBr	Discrimination between EVOO and low quality olive oils based on FAAEs direct determination and multivariate data analysis	Boggia et al., 2014
Electrophoretic techniques	CE	ANOVA	Ethanolic extraction of the long-chain FFAs followed by quantitative determination of oil FFAs by electrokinetic chromatography	Balesteros et al.,2007
Electro-chemical methods	Electrical impedance spectroscopy	NLR	FFAs determination with a good accuracy applying electrical conductance to olive oil emulsion with hydro-alcoholic solution	Grossi et al.,2014
	Electrochemical sensor	ANOVA	Method successfully applied to the measurement of olive oil PV with an excellent agreement with results obtained with the official standard procedure	Adhoum and Monser, 2008
	Determination of changes in the electrical conductivity value	ANOVA and LR	Determination of PV value with accuracy similar to official standard methods. Evaluation of changes in the electrical conductivity values of the aqueous phase during the reaction of potassium iodide with the hydroperoxides presented in oil samples	Yang et al.,2014
	Opto-electronic system	PLS	Real time Determination of PV value with good accuracy	Grossi et al.,2015
	TDR	PLS	Predictive models for rapid determination of FAAES in olive oil samples with good accuracy with respect to official method	Berardinelli et al., 2013
Flow injection analysis	FIA in combination with a distributed expert system	ANOVA	On-line rapid and accurate chemical quality control of olive oil (FFAs, PV, K_{232} and K_{272})	Bonastre et al., 2004
Enzymatic methods	Enzymatic reaction. UV spectrophotometry determination	ANOVA	Determination of FFAs with good accuracy by enzymatic oxidation of free polyunsaturated fatty acids present in oil samples with lipoxygenase and spectrophotometric detection of the hydroperoxy-FAs produced at 234 nm	Ben Rejeb and Gargouri, 2011
Chemiluminescence Spectroscopy	Chemiluminescent reaction of alkaline luminol and the hydroperoxides of oil	ANOVA and LR	Determination of PV value with excellent agreement with the results obtained with the official standard procedure	Tsiaka et al.,2013
Hyperspectral imaging techniques	Hyperspectral image sensorsr	GA, LASSO, SPA and MLR	Real time determination of PV value and FFAs with good accuracy	Martínez Gila et al.,2015
Biosensors	Electronic tongue	LDA	The electronic tongue showed satisfactory correct prediction capability of the overall intensity sensory perception of EVOO	Veloso et al., 2015
	Electronic Nose sensors equipped with 10 Metal Oxide Semiconductor (MOS) sensors	PCA and LR	Good reliability and discriminating power among olive oils of different quality (EVOO and LVOO) and among different intensities of fruity and rancid attributes. Results were in good agreement with those obtained by the official panel test method	Apetrei and Apetrei, 2014

electrical conductivity methods (Yang et al., 2014), the use of chemiluminescent methods (Tsiaka et al., 2013), the application of stepwise orthogonalization of predictors to mid-infrared (MIR) spectra (Pizarro et al., 2013a), the use of NIR spectroscopy (Inarejos-García et al., 2012), the utilization of an on-line system based on hyperspectral information (Martínez Gila et al., 2015) and an opto-electronic system (Grossi et al., 2015).

As far as the olive oil content on FAAEs is concerned, to date, several analytical methods have been developed and applied for the determination of FAMEs and FAEEs in virgin olive oils. A solid phase extraction (SPE) using silica cartridges was first proposed by Perez-Camino et al. (2008), trying to achieve an efficient extraction protocol. Another analytical approach combining the FT-IR screening of olive oil and partial least-squares (PLS) analysis has been also applied to determine these compounds (Valli et al., 2013). Furthermore, a rapid procedure based on the screening of FAAEs in virgin olive oils using time-domain reflectometry (TDR) and PLS analysis was developed and applied with noticeable success (Berardinelli et al., 2013). An approach based on direct thermo-desorbed and cryo-focalized in the cooled injector of a gas chromatography coupled to electron impact mass spectrometry (GC-EI MS) can be also mentioned; the authors used principal component analysis (PCA) data treatment (Boggia et al., 2014).

Even though the illustrated examples of methods for the determination of the physicochemical olive oil quality criteria (Table 4) offer some advantages when compared to the conventional analytical methods used by official regulations (they represent, in general, simple, efficient and nondestructive methodologies), they also exhibit certain limitations, such as, requiring expensive instrumentation, the need of frequent calibration, the fact that most of the proposed methods have been validated only on small sample-sets, and the circumstance that the different procedures must be separately calibrated for different types of virgin olive oils. For these reasons, each one of these alternative methodologies should be adapted taking into account necessity, cost, accessibility, analysis time (number of samples analyzed per hour), sample preparation requirements (with or without previous treatment) and sensitivity, among other features.

As far as the olive sensory quality evaluation is concerned, various instrumental techniques have been proposed (Table 4), mainly based on establishing the link or association between virgin olive oil's volatile compounds composition and its sensory attributes (positive and/or negative). It is nowadays well-known that numerous volatile compounds (with diverse molecular weight, chemical nature, odor thresholds, and probably present in olive oil at very low amounts) are distinctive to the aroma, and hence, to the sensory quality of olive oil (Kalua et al., 2007). However, owing to the complex chemical composition of the volatile fraction of virgin olive oils and the fact that most of the volatile components are present in this matrix at very low amounts, there is a need for highly sensitive analytical methods for the characterization and quantification of these compounds. Therefore, a great number of analytical strategies including chemical pre-concentration, separation and detection techniques have been developed and applied to the olive oil aroma characterization (Gomes da Silva et al., 2012). The combination of these analytical methods with multivariate data analysis techniques has proved to be useful for the sensory classification of virgin olive oils. However, it is also necessary to explain that in many papers that try to correlate the information about the volatile fraction composition with negative and positive attributes to classify virgin olive oils, the description of this complex relationship is tentative, because no information is often given about the odor threshold and activity of the identified compounds. Furthermore, in some instances, the aroma attributes should not be associated to a single compound, since they can result from the interaction of very similar odorants (in terms of aroma and structural terms) present in olive oil at low concentrations (even below their sensory threshold), but, in certain cases, exerting a concerted action (Angerosa et al., 2004). This challenging situation has opened up the way to the application of new olfaction instrumentation, in particular GC-olfactory (GC-O), and chemical sensor technologies (electronic tongue and noses), combined with multivariate data processing methods, which have been used with considerable success to classify olive oil according to their sensory quality (Sinelli et al., 2010; Escuderos et al., 2011; Savarese et al., 2013; Veloso et al., 2015).

Evolution of analytical methods to detect olive oil adulteration

The rapid and reliable detection of adulteration (with a proper degree of sensitivity and selectivity) is a very challenging issue in the field of virgin olive oil authentication. Indeed, the tedious and, sometimes, time-consuming procedures of the conventional analytical methods approaches need to be improved or replaced by faster and precise techniques. In this sense, during the last decade, numerous analytical procedures (including sample preparation, analysis, data acquisition and processing) have been developed and proposed to control the adulteration of virgin olive oil (Table 5). They have garnered general acceptance as powerful methods, offering some advantages such as high separation efficiency and resolution, rapid analysis and minimal consumption of reagents and samples, which make them attractive alternatives to the conventional analytical methods used, so far, for virgin olive oil adulteration control. In this section, we have considered different method categories, being the most relevant the following ones:

– **Vibrational spectroscopic techniques**: vibrational spectroscopic techniques based on both infrared and mid-infrared absorptions (FT-IR, FT-MIR, NIR, and MIR) and Raman scattering, have demonstrated their great potential as promising tools to uncover olive oil adulteration over the last years; they offer important advantages over the conventional analytical methods used in this area, in particular, regarding the needed volume of reagents, rapid measurements and fast data acquisition, relatively low cost, samples handling and their non-destructive nature (analysis is performed directly on intact samples or with only minimal sample preparation), etc. Table 5 shows a selected number of applications of vibrational-spectroscopy-based methods for virgin olive oils adulteration control. As can be observed, these applications can be roughly divided into two broad categories.

The first is mainly related to alternative applications to conventional methods for the determination of some purity criteria. The second category deals with the rapid adulteration detection, identification of the type of adulterant and the quantification of this adulteration. In both cases, given the nature of the data sets obtained (generation of typical spectra of analyzed samples), chemometric techniques are usually required to develop predicting models that correlate the complex spectra to the level of a compound, class, or parameter to be predicted.

Belonging to the first category, vibrational spectroscopy such as NIR (Galtier et al., 2007), MIR (Dupuy et al., 2010), FT-NIR (Azizian et al., 2015), Attenuated Total Reflection Fourier Transfer Infrared (ATR-FTIR) (Maggio et al., 2009), and Raman scattering (Korifi et al., 2011) have been favorably applied to monitor the content of FAs, *trans* FAs and TAGs in virgin olive oil samples (Table 5). In these studies, the small estimation errors achieved through the application of chemometrics to the spectral data, demonstrated the quality of the developed models and the suitability of these techniques to the determination of these purity criteria of olive oil.

Furthermore, with regard to the second category of applications of vibrational spectroscopy to virgin olive oil adulteration control, a large number of studies have been published over the last years about NIR (Christy et al., 2004; Kasemsumran et al., 2005), Vis/NIR (Mignani et al., 2011), MIR (Gurdeniz and Ozen, 2009), FT-IR (Lerma-García et al., 2010; Rohman et al., 2015), ATR-FTIR (De la Mata et al., 2012; Aftab et al., 2014), Raman (Lopez-Diez et al., 2003; Zou et al., 2009; Zhang et al., 2011), Vis-Raman (El-Abassy et al., 2009), and FT-Raman (Heise et al., 2005) spectroscopy. These techniques have been employed to develop rapid and simple methods to detect adulterations and to determine the nature and quantity of the adulterant/s in the olive oil samples under study. The application of statistical data evaluation allowed establishing approaches with high capability and great potential to detect EVOO's adulteration and identify the adulterants´ nature (Table 5).

- **Nuclear magnetic resonance (NMR) spectroscopy**: NMR spectroscopy techniques (^{1}H, ^{13}C, ^{31}P), have been extensively utilized in virgin olive oil adulteration control, both for quantitative analysis of some purity criteria and for targeted or untargeted fingerprinting approaches. These analytical approaches (considering the methodology development, advances and applications in the field of olive oil adulteration) have been comprehensively described in various interesting critical review papers (Mannina and Sobolev, 2011; Dais and Hatzakis, 2013). The authors basically showed that NMR spectroscopy techniques and the subsequent use of suitable chemometric techniques seem to be a simple, fast and powerful approach for the quantitative analysis of olive oils TAGs, *trans* and *cis* FAs and sterols. In the mentioned very interesting papers, the authors also highlight that NMR can apply fingerprinting approaches allowing the detection of adulterants (low price olive oils or vegetable oils) in

EVOOs. The detection limit and the high discriminative capability of the models developed using NMR and chemometric treatments suggest their use as plausible alternative to the official methods.

- **Mass spectrometry**: various MS methodologies have been established (not requiring prior separation) to be applied for virgin olive oils adulteration control. When no chromatographic or electrophoretic previous separation is coupled to MS, an overall mass spectrum of all the sample's compounds may be obtained in a short analysis time. In the studies employing direct infusion MS in this field, electrospray ionization (ESI), atmospheric pressure photoionization ion sources (APPI), and matrix-assisted laser desorption/ionization (MALDI) have been used for the detection and identification of the most common EVOO's adulterant vegetable oils, in particular hazelnut oil. Within this context, Goodacre et al. (2002) used a direct infusion ESI-MS approach combined with chemometric treatments (PCA and cluster analysis (CA)); the results were very promising and showed that the obtained spectra generated very interesting information and allowed a good discrimination between EVOO and adulterated oils without the need of any chromatographic separation. Using a similar approach, more recently, Alves et al. (2013) demonstrated the suitability of combining the spectral information achieved by ESI-MS with a chemometric data analysis using partial least squares discriminant analysis (PLS-DA) for discriminating EVOO from others vegetable oils commonly used as adulterants, particularly OVOO, corn, sunflower, soybean and canola oils. Most lately, besides applying PLS-DA to ESI-MS data, PLS treatment was used to build predictive models for the detection of EVOO adulteration with four adulterant oils (soybean, corn, sunflower and canola) (Alves et al., 2014). Furthermore, Gómez-Ariza et al., (2006) described, in a comparative study, the potential of ESI-MS and APPI-MS for the control of EVOO adulteration with hazelnut oil; both methods seemed to be optimum to virgin olive oil adulteration detection in short time (approximately 1 min per sample). Alternatively, approaches coupling FIA to time-of-flight mass spectrometry (TOF MS) equipped with a MALDI source have been described, and their capability to detect EVOO adulteration with hazelnut oil has been evaluated (Calvano et al., 2010; Calvano et al., 2012). Chapagain and Wiesman (2009) also proposed a worthy example, where a fingerprinting method based on MALDI-TOF MS was applied as reliable and fast strategy for the effective determination of FAs and TAGs composition in virgin olive oil samples without any required derivatization. The application of chemometric treatments to the obtained data, allowed the authors to achieve the correct discrimination of the studied virgin olive oils from others common adulterant vegetable oils. An analytical methodology based on direct analysis in real time coupled to high-resolution time-of-flight (DART-TOF MS) and linear discriminant analysis (LDA) as chemometric approach for the data treatment that was also developed and successfully applied to differentiate non-adulterated EVOO from

those adulterated with OPO, OO, and hazelnut oil (Vacla-vik et al., 2009). Some other examples concerning the use of MS as an appropriate tool for the EVOO adulteration control can be illustrated, for instance, the potential of combining headspace-mass spectrometry (HS-MS) and chemometric analysis to detect adulteration of olive oil samples with different levels of hazelnut oil (Peña et al., 2005), sunflower and OPO (Lorenzo et al., 2002) has been tested.

Even though these fingerprinting MS approaches represent attractive analytical alternatives to olive oil adulteration control, especially for their minimal requirements of sample preparation, no need of chemical derivatization or chromatographic separation, short analysis time, and their environmentally friendly nature, they obviously show some drawbacks too. The major disadvantage of MS based-techniques is that they are one of the most expensive analytical techniques to be used (both in terms of the initial investment and the subsequent maintenance costs).

− **Chromatographic techniques**: outstanding advances have been made to fulfill the goal of improving the current official analytical methods (based on chromatographic techniques (both HPLC and GC)) in terms of sample preparation, instrumental analysis, data processing and interpretation for the efficient control of olive oil adulteration. Some examples illustrating these improvements are given in Table 5. Work has been mainly made regarding sample preparation and selecting powerful detection systems. Indeed, methods using SPE prior separation of free and esterified sterols (Mathison and Holstege, 2013), and wax (Pérez-Camino et al., 2012) have been proposed. The main advantages of using SPE are: relatively short preparation time, reduced solvent and sample consumption, and the possibility of handling several samples simultaneously. As mentioned above, the use of potent detection systems such as MS, coupled to chromatographic separation techniques for the structural and quantitative analysis of some VOO purity criteria has represent one of the growing areas. In this sense, Cañabate-Cañabate-Díaz et al. (2007) proposed, for the first time, the analysis of sterols and triterpenic dialcohols from the unsaponifiable fraction of virgin olive oils, ROO, OPO and COPO using a HPLC-APCI MS analytical platform, obtaining a proper separation of cholesterol, stigmasterol, β-sitosterol, sitostanol, campesterol, erythrodiol, and uvaol. The same analytical platform was used to develop a rapid and effective method for the characterization of sterols and triterpenic dialcohols from the unsaponifiable fraction of virgin olive oils (Segura-Carretero et al., 2008; Zarrouk et al., 2010), allowing the structural characterization and the quantification of the main sterolic and triterpenic dialcohols compounds occurring in virgin olive oil shortening substantially the sample preparation procedure. By using similar approaches, some other authors chose HPLC-APCI MS for the detailed characterization of TAGs profiles of virgin olive oil and others vegetable oils (Holčapek and Lísa, 2009); moreover, when PCA was applied for the treatment of the obtained data, adulterated

virgin olive oil with sunflower oil could be detected even at a very low levels (1%). In like manner, a direct injection HPLC-APCI MS/MS method was proposed for the characterization of EVOO's TAGs profiles, showing the potential of the developed methodology when it is combined to PCA, for the detection of EVOO's adulteration with soybean oil (Fasciotti and Pereira Netto, 2010). Furthermore, over the last years, various fast and reliable approaches based on fingerprinting methods (in either targeted or untargeted mode using chromatographic techniques, lonely or in conjunction with MS detectors, and combined to chemometrics) were found to be valuable to provide a solution to EVOO adulteration control. Among these approaches, it is possible to mention as example the use of sterols profile determined by liquid chromatography with ultraviolet absorption detection and chemometrics (PCA, hierarchical cluster analysis (HCA), and PLS-DA) to build discriminative models which exhibited good predictive capability allowing the correct classification of virgin olive oils and other vegetable edible oils (Bagur-González et al., 2015); the use of TAGs profile, as determined by GC-MS, combined with chemometrics (genetic algorithm partial least squares (GA-PLS), PLS and Soft independent modeling of class analogies (SIMCA)) for the identification and quantification of EVOO adulteration with others vegetable oils (sunflower, corn, seeds, sesame and soya) (Ruiz-Samblás et al., 2012); or the quantification of virgin olive oil in blends with other vegetable oils using a targeted fingerprinting approach combining the TAGs profile determined by HPLC coupled to a Charged Aerosol Detector (CAD) and chemometrics (interval PLS (iPLS) and PLS) (De La Mata-Espinosa et al., 2011). Some other examples regarding this topic and the control of VOO adulteration using chemometrics and chromatographic methods of TAGs profile can be found in an interesting review authored by Bosque-Sendra et al., 2012.

Although the afore-mentioned studies provide evidences that advances in chromatographic techniques appear to solve some drawbacks of the conventional methods used to control olive oils adulteration, the exhaustive overview of these studies also reveals some weak points that make difficult their adoption as alternative methods to officially guarantee VOO authenticity. Some of them are: the fact that most of the studies used a limited number of samples; the analyzed oils are usually coming from restricted geographical areas and belong to few varieties; and the use of MS detectors, which increases the overall method costs.

− **Other analytical approaches**: a number of important analytical methods have been developed and suggested for virgin olive oils adulteration control purposes using other emerging analytical techniques (Table 5). The following are some pertinent examples:

− Various genetic and deoxyribonucleic acid (DNA) based techniques have been proposed as useful procedures for the qualitative and quantitative determination of adulterant vegetable oils and other lower-price virgin olive oils and olive-pomace oils in EVOO (Rabiei and Enferadi

2012; Ben-Ayed et al., 2013; Vietina et al., 2013). The application of these techniques seem to provide some advantages, such as increased specificity and sensitivity, high durability of DNA molecules, as well as the fact that they are independent from environmental conditions (compared to other authenticity compounds) and show a reliable performance with highly steady processed samples. Diverse molecular markers have been typically used for VOO adulteration control, mainly simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR) and single nucleotide polymorphisms (SNP) (Ben-Ayed et al., 2013; Ou et al., 2015). Various authors showed, however, that the reliability and reproducibility of these methods are widely conditioned by the quality of the DNA extracted from studied oils. For this reason, tremendous effort has been done to develop reliable and effective DNA extraction methods (Raieta et al., 2015) and to extend the use of polymerase chain reaction (PCR) to amplify the extracted microsatellite markers (Wu et al., 2011; Vietina et al., 2013).

– Electronic nose and electronic tongue technologies, in combination with chemometrics, have been successfully applied for the detection of adulteration of EVOO with different kinds of olive oils, pomace oils and/or vegetable oils (Mildner-Szkudlarz and Jeleń 2010; Apetrei and Apetrei 2014).

– Thermal techniques: thermal properties (measured both in cooling and heating regimes) of EVOO have been reported to widely correlate with its chemical composition (Chiavaro et al., 2007). In this context, olive oil FAs composition was successfully determined using an approach that combine differential scanning calorimetry (DSC) and PLS regression (Cerretani et al., 2011). Furthermore, thermo-analytical techniques, in particular DSC, have been suggested as a valuable tool to fight against olive oil adulteration. Thus, Ferrari et al. (2007), and Van Wetten et al. (2015) described DSC methods to authenticate olive oils and other edible oils; Chiavaro et al. (2008a) developed a technique based on DSC to differentiate olive oils of five distinct commercial categories, and to detect adulteration of EVOO with refined hazelnut oil (Chiavaro et al., 2008b) and/or high oleic sunflower oil (Chiavaro et al., 2009).

– Isotopic techniques: although the reported applications in this category are, to date, limited, these techniques have shown great potential for virgin olive oil adulteration control. Thus, methods such as: stable isotope ratio analysis and $^{13}C/^{12}C$ measured using elemental analyzer-isotopic ratio coupled to MS, or determined by a gas chromatography-combustion-isotopic ratio MS (GC/C-IRMS) have demonstrated to be useful for detecting the adulteration of olive oil with olive-pomace oils or with other vegetable oils (Angerosa et al., 1997; Spangenberg, 1998)

– Electrophoretic techniques: analytical methods based on capillary electrophoresis coupled to MS or ultraviolet have demonstrated to be valuable and helpful tools to guarantee the authenticity of olive oil and/or detect adulteration with other oils (Sánchez-Hernández et al., 2011; Monasterio et al., 2013).

Trends and advances in analytical approaches to trace the geographical and varietal origin of virgin olive oils

As stated in the first section, the geographical and varietal origin authentication of virgin olive oil remain not legally regulated by official analytical methods. However, over the last years, and due to the increasing interest of consumers for the labeled geographical origin and monovarietal virgin olive oils, tremendous efforts have been devoted to the development of robust and reliable analytical strategies to verify their declared varietal and geographical provenance. In this sense, differentiation of virgin olive oil according to geographic origin and/or variety has been performed by using mainly three strategies: targeted analyses, the use of profiling approaches and the utilization of more untargeted ones. The first one is based on identifying various olive oil's compounds, determining their content, and correlating the obtained data with the geographical and/or varietal origins. The second one includes the qualitative and/or quantitative determination of a larger set of olive oil compounds that are related, considering their chemical nature and/or biosynthesis pathway. The third strategy implies the use of fingerprinting approaches using a chemometric screening of the whole sample fingerprint in order to identify key markers that differentiate the area of production and/or cultivars of interest. Regardless of the approach applied, chemometric models built by using different multivariate data analysis have been used to get a correct classification of the samples' varietal and/or geographical origin. Relevant literature examples have been summarized in Table 5. Indeed, several studies have been undertaken to develop chemometric models for classifying olive oils according to their area of production and/or varietal origins, based on a range of chemical compounds such as TAGs (Gökçebağ et al., 2013), FAs (Diraman et al., 2010; Diraman et al., 2011; Martínez et al., 2014), phenolic compounds (Alkan et al., 2012; Bajoub et al., 2014), pigments (Cichelli and Pertesana, 2004), sterols (Lukić et al., 2013; Giacalone et al., 2015), and volatile compounds (Pouliarekou et al., 2011). Besides, in some studies dealing with the olive oil geographical and varietal origin verification, data from several major and minor compounds have been combined into one model, in order to exploit the different information provided by each type of compounds (Yorulmaz et al., 2014; Bajoub et al., 2015). As far as fingerprinting approaches are concerned, we find stimulating examples based on separative and MS techniques (Lerma-García et al., 2011; Riccio et al., 2011), vibrational spectrometric techniques (Hennessy et al., 2009; Lin et al., 2012), NMR (Petrakis et al., 2008; Longobardi et al., 2012), DNA-based techniques (Martins-Lopes et al., 2008; Melucci, et al., 2016), electronic nose and electronic tongues techniques (Dias et al., 2014; Melucci, et al., 2016), etc. In all the mentioned instances, the fingerprints were treated by different chemometric techniques and allowed the authentication of geographical and/or varietal provenance of the studied oils. Another tactic which is becoming quite popular is the simultaneous use of data from different analytical techniques (the so-called data-fusion), since the information provided by each of them might

Table 5. Representative examples of recent analytical methodologies proposed for olive oil adulteration control (alternative to conventional method to determine purity criteria (category 1); rapid adulteration detection/quantification and identification of the adulterant oils (category 2)) and botanical and geographical origins assessment.

Analytical techniques	Instrumental measurement	Adulteration control		Origin authentication		Data treatment	Main findings	Reference
		Category 1	Category 2	Geographical	Varietal			
Vibrational spectroscopic techniques	NIR		***		***	PLS-DA	Rapid and efficient quantification of the main FAs and TAGs on French PDO olive oils and their discrimination according to geographic origin	Galtier et al., 2007
	NIR	***		***		PCA and PLS	Successful discrimination and quantification of olive oil adulteration with vegetable oils	Christy et al., 2004
	NIR	***		***		PLS and PLS-DA	Discrimination, detection and quantification of olive oil adulteration with vegetable oils	Kasemsumran et al., 2005
	Vis/NIR	***		***		PCA and PLS-DA	Quantification and identification of EVOO adulteration with lower-grade olive and olive pomace oils	Mignani et al., 2011
	Vis/NIR	***	***		***	GA-PLS	Rapid and efficient classification of studied virgin olive oils according to their geographic origin.	Lin et al., 2012
	FT-NIR		***	***		PLS	Prediction of EVOO FA composition with satisfactory accuracy and identification of the kind and amount of adulterant vegetable oils in EVOO	Azizian et al., 2015
	MIR	***		***		PCA, PLS and PLS-DA	Detection and quantification of olive oil adulteration with different edible oils	Gurdeniz and Ozen, 2009
	NIR and MIR		***		***	PLS, PLS-DA and H-PLS	Quantitative analysis of FAs and TAGs compounds, and geographical classification of the studied oils	Dupuy et al., 2010
	NIR and MIR			***		PLS	Quantification of the main olive oil FAs. Results were in agreement of those obtained using official method	Maggio et al., 2009
	ATR-FTIR	***			***	HCA, PCA, PLS and PLS-DA	Fast and accurate discrimination between olive oils from different grades and varieties and blended olive oils with different edible oils	De la Mata et al., 2012
	ATR-FTIR	***	***	***		PCA, LDA, PLS-DA	Successful discrimination between Ligurian (Italy) and non-Ligurian olive oils	Hennessy et al., 2009
	SB-ATR-FTIR	***		***		PLS and PCS	EVOO adulteration with ROO detection and quantification	Aftab et al., 2014
	FT-IR	***		***		LDA and MLR	Detection and quantification of EVOO adulteration with different edible oils at concentration lower than 5%	Lerma-García et al., 2010
	FT-IR	***		***		LDA, PCr and PLS	Quantification of pumpkin seed oil blended into EVOO	Rohman et al., 2015
	FT-Raman	***		***		PLS	Efficient chemometric models for the detection of olive oil adulteration by sunflower oil	Heise et al., 2005
	FT-Raman	***		***		PCA and GA-PLS	Rapid and accurate detection and quantitative assessment of the olive oil adulteration with hazelnut oils	Lopez-Diez et al., 2003
	Raman	***		***		***	Rapid discrimination between various grades of olive oil and seed oils and detection of olive oil adulteration with above 5% of edible oils	Zou et al., 2009
	Raman	***		***		SVM	Rapid detection and quantitative evaluation of adulterated olive oil with vegetable oils	Zhang et al., 2011
	Vis/Raman	***		***		PCA and PLS	Fast monitoring and detection of EVOO adulteration with sunflower oil	El-Abassy et al., 2009

Table 5. Representative examples of recent analytical methodologies proposed for olive oil adulteration control (alternative to conventional method to determine purity criteria (category 1); rapid adulteration detection/quantification and identification of the adulterant oils (category 2)) and botanical and geographical origins assessment. (*Continued*)

	Technique	Description	Statistical method	Reference
Mass spectrometry	ESI MS	Accurate and rapid discrimination between EVOO and OVO grades and detection of adulteration of EVOO with vegetable oils	PLS-DA	Alves et al., 2013
	ESI MS and APPI MS	Both methods seem to be rapid procedures with high discrimination power allowing detection of olive oil adulteration, especially by hazelnut oils	PCA, LDA	Gómez-Ariza et al., 2006
		Characterization of the polar components in hazelnut and EVOO. Identification of some of them as EVOO adulteration markers	***	Calvano et al., 2010
	MALDI-TOF MS	Identification of some phospholipids compounds as markers of EVOO adulteration with hazelnut oil	***	Calvano et al., 2012
		Fast and reliable characterization of TAGs and FAs profiles in virgin olive oils without any derivatization	ANOVA	Chapagain and Wiesman, 2009
	DART-TOF MS	Rapid and easy differentiation of EVOO, OPO and OO grades and detection of EVOO adulteration with hazelnut oil based on TAGs profiles	LDA	Vaclavik et al., 2009
	HS-MS	Reliable analytical procedure to detect olive oil adulteration with hazelnut oil	CA, PLS, PCA, PCr and MLR	Peña et al., 2005
	HS-MS	Rapid and reliable method to differentiate the adulterated olive oil (with sunflower oil and OPO) from the non-adulterated olive oils	LDA	Lorenzo et al., 2002
Chromatography		Improved method simplifying the preparation step for rapid and reliable determination of sterols, uvaol, and erythrodiol using SPE	LR	Mathison and Holstege, 2013
		Improved method simplifying the preparation step for rapid and accurate evaluation of olive oil waxy fraction using SPE	ANOVA	Pérez-Camino et al., 2012
	GC-FID	Geographical classification of Extremadura Spanish olive oils according to their origin based on FAs composition	ANOVA and LDA	Martínez et al., 2014
		Successful assessment of varietal origin of monovarietal Turkish virgin olive oils by sterols and triterpenoids profiling	PCA and LDA	Lukić et al., 2013
		Reliable and efficient discrimination between Italian ad non Italian virgin olive oil based on sterolic fraction profiling and chemometrics	PCA and SIMCA	Giacalone et al., 2015
		Quali-quantitative evaluation of the main sterols and triterpenic dialcohols from the unsaponifiable fraction of VOO, ROO, OPO and COPO	***	Cañabate-Díaz et al., 2007
	HPLC-APCI MS	Separation and identification of 15 sterols and 2 triterpenic dialcohols from the unsaponifiable fraction without using thin-layer chromatography	***	Segura-Carretero et al., 2008
		Characterization of TAGs and FAs profiles of olive oil and others vegetable oils and detection of olive oil adulteration with sunflower oil	PCA	Holčapek and Lísa, 2009
	HPLC-APCI MS/MS	Characterization and quantification of the main sterols and triterpenic dialcohols compounds occurring in virgin olive oil without previous derivatization	PCA, CA	Zarrouk et al., 2010
		Characterization of EVOO TAGs profiles and detection of possible adulterations with soybean oil	PCA	Fasciotti and Pereira Netto, 2010
	HPLC-UV	Fingerprinting of the sterolic fraction of virgin and pomace olive oils and some vegetable oils	PCA, HCA, PLS-DA	Bagur-González et al., 2015
		Using TAGs profiles to trace the geographical and varietal origin of Turkish olive oils	PCA and HCA	Gökçebağ et al., 2013
	HPLC-UV & HPLC-FLD	Classification of monovarietal virgin olive oils by pigments compounds profiling	PCA and HCA	Cichelli and Pertesana, 2004
	HPLC-CAD	TAGs fingerprinting for the detection and quantification of EVOO adulteration with others edible vegetable oils	iPLS and PLS	De La Mata-Espinosa et al., 2011

Category	Technique	Reference	Application	Chemometric methods
	GC-MS	Pouliarekou et al., 2011	Correct geographical and varietal classification of Western Greek monovarietal virgin olive oil based on volatile compounds profiling	ANOVA and LDA
	GC-MS	Ruiz-Samblás et al., 2012	Reliable identification and quantification of EVOO adulteration with others vegetable oils	GA-PLS, PLS and SIMCA
	HPLC-ESI MS	Bajoub et al., 2014	Geographical classification of North Moroccan monovarietal virgin olive oils by phenolic compounds profiling	ANOVA, PCA and LDA
	GC-FID and HPLC with different detectors	Bajoub et al., 2015	Geographical classification of North Moroccan monovarietal virgin olive oils combining their composition (major and minor compounds) and chemometrics	ANOVA, PCA and LDA
	UHPLC-APCI MS	Lerma-García et al., 2011	Rapid fingerprinting of sterols and use of chemometrics allowing the correct varietal classification of the studied oils	LDA
NMR	1H NMR	Longobardi et al., 2012	Correct classification of studied virgin olive oils according to their geographic origin	ANOVA, PCA, CA and NCM
Other techniques	DNA-based techniques	Vietina et al., 2013	Efficient and rapid identification of olive oil adulteration with vegetable oils	***
Other techniques	DNA-based techniques	Martins-Lopes et al., 2008	The efficiency of RAPD, ISSR, and SSR molecular markers combined with chemometrics was tested and validated as geographical and varietal origin tracing approach	PCA
Other techniques	Electronic nose	Mildner-Szkudlarz and Jeleń, 2010	Very good results were obtained en terms of the detection of EVOO adulteration by rapeseed and sunflower oils, as well as predicting the percentage of adulteration	PCA and PLS
Other techniques	Electronic nose	Dias et al., 2014	Rapid and efficient fingerprinting approach for the classification of virgin olive oils according to their varietal origin	LDA
Other techniques	Voltammetric E-tongue	Apetrei and Apetrei, 2014	Detection and identification of EVOO adulteration with sunflower, soybean and corn oils at concentrations lower than 10%	ANOVA, PCA, PLS and PLS-DA
Other techniques	Thermal techniques: DSC	Cerretani et al., 2011	Rapid and efficient characterization and quantification of the main FAs occurring in different olive oils grades, OPO and three seed oils (canola, sunflower and hazelnut oils)	PLS
Other techniques		Wetten et al., 2015	Rapid and efficient detection of EVOO adulteration with sunflower oil	***
Other techniques	Isotopic techniques	Angerosa et al., 1997	$^{13}C/^{12}C$ isotope ratio measurements were successfully used for the detection of olive oil adulteration with pomace oil	***
Other techniques	Electrophoretic techniques: CE-MS/MS	Sánchez-Hernández et al., 2011	Sensitive method for determining six non-protein amino acids in vegetable oils (soybean, sunflower and corn oils) and olive oil samples and their use as novel markers for the detection of adulterations	***

– Colored cells in light green in this table indicate that the quoted application belongs to that category. /

*** means Not belonging to.

be different and complementary (Karabagias et al., 2013; Pizarro et al., 2013b). Undoubtedly, it can be concluded that important work has been done over the last years in the field of virgin olive oil geographic and varietal origin assessment. However, in spite of these advances, this issue is still far from being completely resolved. Certainly, universal analytical methods for the determination of the geographical/varietal origin of virgin olive oil do not really exist mainly due to the restricted character of most of the studies carried out in this field (in terms of analyzed samples, studied varieties and considered geographical areas). The latter mentioned items represent a kind of barrier or obstacle which complicates the acceptance of the discriminative models proposed by these studies and impede that those methodologies become more widespread. To face this situation there is a need for further comprehensive and long-term (including multiple seasons) studies with higher number of samples collected from the main olive-growing areas over the world, representing the main varieties cultivated worldwide. Such studies could lead to build a large database that would make possible the geographical and varietal traceability of the most representative olive oils around the world.

Conclusions and future trends

The worldwide proliferation of olive oil quality and authenticity standards regulations, driven predominantly by the trade globalization of this product and the emergence of new producing and consuming countries outside the Mediterranean region, has stimulated the discussion and debate about the harmonization of olive oil standards and trade regulations, which should take into account the natural variation of olive oil composition due to environmental conditions and agro-technological practices.

However, even if the assessment of the quality, authenticity, and origin (geographical and/or varietal) traceability of this product are fields of paramount importance, several challenges need to be faced. Indeed, although conventional methods are still being used, the recent findings herein reviewed, highlight that new approaches based on the use of advanced analytical techniques and subsequent data mining and analysis by applying chemometrics, open up very interesting perspectives to overcome the limitations of the conventional analytical methods. In this respect, sophisticated technologies such as vibrational spectroscopic techniques, NMR spectroscopy, MS, biosensors, and DNA-based approaches represent promising alternatives for the authentication and traceability of olive oil, because of their sensitivity, high-throughput, reproducibility and robustness in comparison with conventional methods used till now. Thus, as discussed throughout this paper, these strategies, applied on nontargeted and/or targeted studies, have played (and will play) a key role in overcoming huge challenges in the authentication and traceability of olive oil.

Unfortunately, in spite of the wide number of published reports in which olive oil quality control, authentication, and traceability have been successfully carried out taking advantage of these advanced analytical methodologies, two major deficiencies have been identified from the studies reviewed herewith: (a) most of the proposed methods were developed using a

limited number of olive oil samples coming from restricted varietal origins and geographical areas, fact which appears to limit their use on a wider scale; and (b) some of the discussed methods, even if their potential was proved, are very costly, and, therefore, cannot be used for routine analysis. Certainly, the main drawbacks of some of the reviewed techniques (i.e. MS, NMR and DNA-based methodologies) are related to the cost of the instruments, which often are not accessible for many olive oil laboratories. For these reasons, it is assumed that they will not become an alternative to conventional methods in a short-term scenario.

Hence, it seems necessary to conceive further developments which should aim at improving the representativeness of the studied samples to the main olive-growing areas and cultivated varieties together with the employment of cost-effective analytical techniques.

Abbreviations

1,2-DAGs	1,2-diacylglycerols
2P	2-glyceryl monopalmitate
AOCS	American Oil Chemists' Society
AFLP	amplified fragment length polymorphism
APPI	atmospheric pressure photoionization ion sources
ATR-FTIR	attenuated total reflection Fourier transfer infrared
AUS	Australian Standards
CAF	Californian Standards
CAD	charged aerosol detector
CA	cluster analysis
CE	capillary electrophoresis
Codex	Codex Alimentarius
COPO	crude olive-pomace oil
DNA	deoxyribonucleic acid
DAGs	diacylglycerols
ΔECN42	difference between actual and theoretical content of triacylglycerols
DSC	differential scanning calorimetry
DART	direct analysis in real time
ESI	electrospray ionization
ECN	equivalent carbon number
EC	European Commission
EU	European Union
EVOO	extra virgin olive oil
FAAEs	fatty acid alkyl esters
FAEEs	fatty acids ethyl esters
FAMEs	fatty acids methyl esters
FAs	fatty acids
FID	flame ionization detector
FIA	Flow injection analysis
FLD	Fluorescence detector
FT-IR	Fourier transform infrared
FT-MIR	Fourier transform-mid-infrared
FT-Raman	Fourier transform-Raman
FFAs	free fatty acids
GC-EI MS	gas chromatography coupled to electron impact mass spectrometry
GC-O	GC-olfactory

GA-PLS	genetic algorithm partial least squares
HS-MS	headspace-mass spectrometry
HCA	hierarchical cluster analysis
H-PLS	hierarchical partial least-squares
HPLC	high pressure liquid chromatography
IOC	International Olive Council
ISO	International Organization for Standardization
ISSR	inter-simple sequence repeats
iPLS	interval partial least-squares
LVOO	lampante virgin olive oil
LASSO	least absolute shrinkage and selection operator
LDA	linear discriminant analysis
LR	linear regression
MS	mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
MeD	median of olive oil defects
MeF	median of olive oil fruitiness
MIR	mid-infrared
MCUVE	Monte Carlo uninformative variable elimination
MLR	multiple linear regression
NCM	nearest class mean
N/A	not applicable
N/C	not considered
NIR	near-infrared
NMR	nuclear magnetic resonance
OO	olive oil
OPO	olive-pomace oil
ANOVA	one way analysis of variance
OVOO	ordinary virgin olive oil
PLS-DA	partial least squares discriminant analysis
PLS	partial least-squares
PBr	passing-bablok regression
PV	peroxide values
PCR	polymerase chain reaction
PCA	principal component analysis
PCS	principal component spectra diagnostic
PCr	principle component regression
PPPs	pyropheophytins
RAPD	random amplified polymorphic DNA
ROO	refined olive oil
ROPO	refined olive-pomace oil
OLS	ordinary least squares
SSR	simple sequence repeats
SNP	single nucleotide polymorphisms
SIMCA	soft independent modeling of class analogies
SPE	solid phase extraction
SPA	successive projections algorithm
SVM	support vector machine
TDR	time-domain reflectometry
TOF MS	time-of-flight mass spectrometry
TAGs	triacylglycerols
K_{232} and K_{268} or K_{270}	ultraviolet specific extinction coefficients
UV	Ultraviolet
USDA	United States Department of Agriculture
VOO	virgin olive oil
Vis/NIR	Visible/near infrared
Vis/Raman	Visible/Raman

References

Adhoum, N. and Monser, L. (2008). Electrochemical sensor for hydroperoxides determination based on Prussian blue film modified electrode. *Sensors and Actuators B.* **133**:588–592.

Aftab, A.K., Rubina, S., Abdul, H.L., Razia, S., Mahroz, A.K. and Dildar, H. M. (2014). Quantification of pure refined olive oil adulterant in extra virgin olive oil using diamond cell ATR-FTIR spectroscopy. *J. Chem. Soc. Pakistan* **36**:699–706.

Alkan, D., Tokatli, F. and Ozen, B. (2012). Phenolic characterization and geographical classification of commercial extra virgin olive oils produced in Turkey. *J. Am. Oil. Chem. Soc.* **89**:261–268.

Alves, J.O., Botelho, B.G., Sena, M.M. and Augusti, R. (2013). Electrospray ionization mass spectrometry and partial least squares discriminant analysis applied to the quality control of olive oil. *J. Mass. Spectrom.* **48**:1109–1115.

Alves, J.O., Sena, M.M. and Augusti, R. (2014). Multivariate calibration applied to ESI mass spectrometry data: a tool to quantify adulteration in extra virgin olive oil with inexpensive edible oils. *Anal. Methods* **6**:7502–7509.

Angerosa, F., Camera, L., Cumitini, S., Gleixner, G. and Reniero, F. (1997). Carbon stable isotopes and olive oil adulteration with pomace oil. *J. Agric. Food Chem.* **45**:3044–3048.

Angerosa, F., Campestre, C. and Giansante, L. (2006). Analysis and authentication. **In**: Olive Oil Chemistry and Technology, pp. 114–172. Boskou, D., Ed., Champaign, Illinois, USA.

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

AOCS Official method Ce 5b-89. (1989). Triglycerides in Vegetable Oils by HPLC.AOCS Press, Champaign, IL, USA.

AOCS Official Method Ch 5–91. (1991a). Determination of Specific Extinction of Oils and Fats, Ultraviolet Absorption. AOCS Press, Champaign, IL, USA.

AOCS Official Method Ch 2–91. (1991b). Determination of Fatty Acids in Olive Oils by Capillary GLC. AOCS Press, Champaign, IL, USA.

AOCS Official Method Ch 6–91. (1991c). Determination of the Composition of the Sterol Fraction of Animal and Vegetable Oils and Fats by TLC and Capillary GLC. AOCS Press, Champaign, IL, USA.

AOCS Official Method Ce 1f-96. (1996a). Determination of Cis- and Trans-Fatty Acids in Hydrogenated and Refined Oils and Fats by Capillary GLC. AOCS Press, Champaign, IL, USA.

AOCS Official Method Cd 26–96. (1996b). Stigmastadienes in Vegetable Oils. AOCS Press, Champaign, IL, USA.

AOCS Official Method Cd 3d 63. (1999). Acid Value. AOCS Press, Champaign, IL, USA.

AOCS Official Method Ch 8–02. (2002). Determination of Wax Content by Capillary Column Gas-Liquid Chromatography. AOCS Press, Champaign, IL, USA.

AOCS Official Method Cd 8b-90. (2003). Peroxide value acetic acid-isooctane Method. AOCS Press, Champaign, IL, USA.

AOCS Official Method Ce 2–66. (2009). Preparation of Methyl Esters of Fatty Acids. AOCS Press, Champaign, IL, USA.

Aparicio, R., Conte, L.S. and -Jochen, F.H. (2013a). Olive oil authentication. **In**: Handbook of Olive Oil Analysis and Properties, pp. 589–653. Aparicio, R., Harwood, J., Eds, Springer, New York, USA.

Aparicio, R., Morales, M.T., Aparicio-Ruiz, R., Tena, N. and García-González, D.L. (2013b). Authenticity of olive oil: Mapping and comparing official methods and promising alternatives. *Food Res. Int.* **54**:2025–2038.

Aparicio-Ruiz, R., Mínguez-Mosquera, M.I. and Gandul-Rojas, B. (2010). Thermal degradation kinetics of chlorophyll pigments in virgin olive oils. *J. Agric. Food Chem.* **58**:6200–6208.

Apetrei, I.M. and Apetrei, C. (2014). Detection of virgin olive oil adulteration using a voltammetric e-tongue. *Comput. Electron. Agric.* **108**:148–154.

Azizian, H., Mossoba, M.M., Fardin-Kia, A.R., Delmonte, P., Karunathilaka, S.R. and Kramer, J.K.G. (2015). Novel, rapid identification, and quantification of adulterants in extra virgin olive oil using near-infrared spectroscopy and chemometrics. *Lipids* **50**:705–718.

Bagur-González, M.G., Pérez-Castaño, E., Sánchez-Viñas, M. and Gázquez-Evangelista, D. (2015). Using the liquid-chromatographic-fingerprint of sterols fraction to discriminate virgin olive from other edible oils. *J. Chromatogr. A* **1380**:64–70.

Bajoub, A., Carrasco-Pancorbo, A., Ajal, E.A., Ouazzani, N. and Fernández-Gutiérrez, A. (2014). Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of North Moroccan virgin olive oils. *Food Chem.* **166**:292–300.

Bajoub, A., Hurtado-Fernández, E., Ajal, E.A., Fernández-Gutiérrez, A., Carrasco-Pancorbo, A. and Ouazzani, N. (2015). Quality and chemical profiles of monovarietal north Moroccan olive oils from "Picholine Marocaine" cultivar: Registration database development and geographical discrimination. *Food Chem.* **179**:127–136.

Balesteros, M.R., Tavares, M.F., Ribeiro, S.J., Polachini, F.C., Messaddeq, Y. and Oliveira, M.A. (2007). Determination of olive oil acidity by CE. *Electrophoresis* **28**:3731–3736.

Ben-Ayed, R., Kamoun-Grati, N. and Rebai, A. (2013). An overview of the authentication of olive tree and oil. *Compr. Rev. Food Sci. Food. Saf.* **12**:218–227.

Bendini, A., Cerretani, L., Di Virgilio, F., Belloni, P., Bonoli-Carbognin, M. and Lercker, G. (2007a). Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. *J. Food Qual.* **30**:424–437.

Bendini, A., Cerretani, L., Di Virgilio, F., Belloni, P., Lercker, G., and Toschi, T.G. (2007b). In-process monitoring in industrial olive mill by means of FT-NIR. *Eur. J. Lipid Sci. Technol.* **109**:498–504.

Ben Rejeb, I. and Gargouri, M. (2011). Determination of olive oil acidity using an enzymatic method. *Anal.Lett.* **44**:1454–1462.

Berardinelli, A., Ragni, L., Bendini, A., Valli, E., Conte, L., Guarnieri, A. and Toschi, T.G. (2013). Rapid screening of fatty acid alkyl esters in olive oils by time domain reflectometry. *J. Agric. Food. Chem.* **61**:10919–10924.

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

Boggia, R., Borgogni, C., Hysenaj, V., Leardi, R. and Zunin, P. (2014). Direct GC-(EI) MS determination of fatty acid alkyl esters in olive oils. *Talanta* **119**:60–67.

Bonastre, A., Ors, R. and Peris, M. (2004). Advanced automation of a flow injection analysis system for quality control of olive oil through the use of a distributed expert system. *Anal. Chim. Acta.* **506**:189–195.

Boskou, D. (2015). Olive oil. In: Specialty Oils and Fats in Food and Nutrition Properties, Processing and Applications, pp. 3–38. Geoff, T., Ed., Woodhead Publishing Elsevier Science, Oxford, UK.

Boskou, D. (2011). Olive oil. **In**: Vegetable Oils in Food Technology Composition, Properties and Uses, pp. 243–271. Gunstone, F.D., Ed., Wiley-Blackwell, Oxford, UK.

Bosque-Sendra, J.M., Cuadros-Rodríguez, L., Ruiz-Samblás, C. and De la Mata, A.P. (2012). Combining chromatography and chemometrics for the characterization and authentication of fats and oils from triacylglycerol compositional data-A review. *Anal.Chim.Acta.* **724**:1–11.

California Department of Food and Agriculture. (2014). Grade and labeling standards for olive oil, refined-olive oil and olive-pomace oil. Available from: http://www.cdfa.ca.gov/mkt/mkt/pdf/CA_Olive_Oil_Standards.pdf. Accessed 2015 November 15.

Calvano, C.D., Aresta, A. and Zambonin, C.G. (2010). Detection of hazelnut oil in extra-virgin olive oil by analysis of polar components bymicro-solid phase extraction based on hydrophilic liquid chromatography and MALDI-TOF mass spectrometry. *J. Mass Spectrom.* **45**:981–988.

Calvano, C.D., De Ceglie, C., D'Accolti, L. and Zambonin, C.G. (2012). MALDI-TOF mass spectrometry detection of extra-virgin olive oil adulteration with hazelnut oil by analysis of phospholipids using an ionic liquid as matrix and extraction solvent. *Food Chem.* **134**:1192–1198.

Cañabate-Díaz, B., Segura Carretero, A., Fernández-Gutiérrez, A., Belmonte Vega, A., Garrido Frenich, A., Martínez Vidal, J.L. and Duran Martos, J. (2007). Separation and determination of sterols in olive oil by HPLC-MS. *Food Chem.* **102**:593–598.

Cayuela, J.A., García, J.M. and Caliani, N. (2009). NIR prediction of fruit moisture, free acidity and oil content in intact olives. *Grasas y Aceites* **60**:194–202.

Cayuela, S.J.A., Moreda, W. and García, J.M. (2013). Rapid determination of olive oil oxidative stability and its major quality parameters using vis/NIR transmittance spectroscopy. *J. Agric. Food Chem.* **61**:8056–8062.

Ceci, L.N. and Carelli, A.A. (2007). Characterization of monovarietal argentinian olive oils from new productive zones. *J. Am. Oil. Chem. Soc.* **84**:1125–1136.

Cerretani, L., Maggio, R.M., Barnaba, C., Toschi, T.G. and Chiavaro, E. (2011). Application of partial least square regression to differential scanning calorimetry data for fatty acid quantitation in olive oil. *Food Chem.* **127**:1899–1904.

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

Chapagain, B.P. and Wiesman, Z. (2009). MALDI-TOF/MS fingerprinting of triacylglycerols (TAGs) in olive oils produced in the Israeli Negev desert. *J. Agric. Food Chem.* **57**:1135–1142.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L., Bonoli, M., Bendini, A. and Lercker, G. (2007). Monovarietal extra virgin olive oils: Correlation between thermal properties and chemical composition. *J. Agric. Food Chem.* **55**:10779–10786.

Chiavaro, E., Rodriguez-Estrada, M.T., Barnaba, C., Vittadini, E., Cerretani, L. and Bendini, A. (2008a). Differential scanning calorimetry: A potential tool for discrimination of olive oil commercial categories. *Anal. Chim. Acta* **625**:215–226.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L. and Bendini, A. (2008b). Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. *Food Chem.* **110**:248–256.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L., Capelli, L. and Bendini, A. (2009). Differential scanning calorimetry detection of high oleic sunflower oil as an adulterant in extra-virgin olive oil. *J. Food Lipids* **16**:227–244.

Christy, A.A., Kasemsumran, S., Du, Y. and Ozaki, Y. (2004). The detection and quantification of adulteration in olive oil by near-infrared spectroscopy and chemometrics. *Anal. Sci.* **20**:935–940.

Cichelli, A. and Pertesana, G.P. (2004). High-performance liquid chromatographic analysis of chlorophylls, pheophytins and carotenoids in virgin olive oils: Chemometric approach to variety classification. *J. Chromatogr. A* **1046**:141–146.

Codex Alimentarius. (1991). Codex standards for olive oils and olive pomace oils. Available from: http://www.codexalimentarius.org/input/download/standards/88/CXS_033e.pdf. Accessed 2014 November 15.

Goodacre, R., Vaidyanathan, S., Bianchi, G. and Kell, D.B. (2002). Metabolic profiling using direct infusion electrospray ionisation mass spectrometry for the characterisation of olive oils. *The Analyst* **127**:1457–1462.

Crews, C., Pye, C. and Macarthur, R. (2014). An improved rapid stigmastadiene test to detect addition of refined oil to extra virgin olive oil. *Food Res. Int.* **60**:117–122.

Dabbou, S., Sifi, S., Rjiba, I., Esposto, S., Taticchi, A., Servili, M., Montedoro, G.F. and Hammami, M. (2010). Effect of pedoclimatic conditions on the chemical composition of the Sigoise olive cultivar. *Chem. Biodivers.* **7**:898–908.

Dais, P. and Hatzakis, E. (2013). Quality assessment and authentication of virgin olive oil by NMR spectroscopy: A critical review. *Anal. Chim. Acta* **765**:1–27.

De La Mata-Espinosa, P., Bosque-Sendra, J.M., Bro, R. and Cuadros-Rodríguez, L. (2011). Olive oil quantification of edible vegetable oil blends using triacylglycerols chromatographic fingerprints and chemometric tools. *Talanta* **85**:177–182.

De la Mata, P., Dominguez-Vidal, A., Bosque-Sendra, J.M., Ruiz-Medina, A., Cuadros-Rodríguez, L. and Ayora-Cañada, M.J. (2012). Olive oil assessment in edible oil blends by means of ATR-FTIR and chemometrics. *Food Control* **23**:449–455.

De Oliveira, D.N. and Catharino, R.R. (2015). Direct metabolic fingerprinting of olive oils using STELDI-MS. *J. Food Compos. Anal.* **38**:131–134.

De Oliveira, M.A.L., Balesteros, M.R., Faria, A.F. and Vaz, F.A.S. (2010). Determination of olive oil acidity. **In**: Olives and Olive Oil in Health and Disease Prevention, pp. 545–552. Preedy, V.R. and Watson, R.R., Eds, Academic Press, San Diego, CA, USA.

Dias, L.G., Fernandes, A., Veloso, A.C.A., Machado, A.A.S.C., Pereira, J.A. and Peres, A.M. (2014). Single-cultivar extra virgin olive oil classification using a potentiometric electronic tongue. *Food Chem.* **160**:321–329.

Diraman, H., Saygi, H. and Hişil, Y. (2010). Relationship between geographical origin and fatty acid composition of Turkish virgin olive oils for two harvest years. *J. Am. Oil Chem. Soc.* **87**:781–789.

Diraman, H., Saygi, H. and Hişil, Y. (2011). Geographical classification of Turkish virgin olive oils from the Aegean region for two harvest years based on their fatty acid profiles. *J. Am. Oil. Chem. Soc.* **88**:1905–1915.

Di Vita, G., D'Amico, M., La Via, G. and Caniglia, E. (2013). Quality perception of PDO extra-virgin olive oil: Which attributes most influence Italian consumers?. *Agric. Econ. Rev.* **14**:46–58.

Dupuy, N., Galtier, O., Ollivier, D., Vanloot, P. and Artaud, J. (2010). Comparison between NIR, MIR, concatenated NIR and MIR analysis and hierarchical PLS model. Application to virgin olive oil analysis. *Anal. Chim. Acta* **666**:23–31.

EEC. (1991). Official Journal of the European Communities (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

El-Abassy, R.M., Donfack, P. and Materny, A. (2009). Visible Raman spectroscopy for the discrimination of olive oils from different vegetable oils and the detection of adulteration. *J. Raman Spectrosc.* **40**:1284–1289.

Escuderos, M.E., Sánchez, S. and Jiménez, A. (2011). Quartz crystal microbalance (QCM) sensor arrays selection for olive oil sensory evaluation. *Food Chem.* **124**:857–862.

EU. (2011). Commission Regulation No 61/2011 of 24 January 2011 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Comm.* **L23**:1–14.

EU. (2013). Commission Implementing Regulation No 1348/2013 of 16 December 2013 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Comm.* **L338**:31–67.

EU. (2016). Commission Delegated Regulation 2016/2095 of 26 September 2016 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Comm.* **L326**:1–6.

Fasciotti, M. and Pereira Netto, A.D. (2010). Optimization and application of methods of triacylglycerol evaluation for characterization of olive oil adulteration by soybean oil with HPLC-APCI-MS-MS. *Talanta* **81**:1116–1125.

Ferrari, C., Angiuli, M., Tombari, E., Righetti, M.C., Matteoli, E. and Salvetti, G. (2007). Promoting calorimetry for olive oil authentication. *Thermochim. Acta* **459**:58–63.

Gallardo-Guerrero, L., Gandul-Rojas, B., Roca, M. and Mínguez-Mosquera, M.I. (2005). Effect of storage on the original pigment profile of Spanish virgin olive oil. *J. Am. Oil. Chem. Soc.* **82**:33–39.

Galtier, O., Dupuy, N., Le Dréau, Y., Ollivier, D., Pinatel, C., Kister, J. and Artaud, J. (2007). Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. *Anal. Chim. Acta.* **595**:136–144.

Gandul-Rojas, B. and Mínguez-Mosquera, M.I. (2006). Olive processing. **In**: Handbook of Fruits and Fruit Processing, pp. 491–517. Hui, Y.H., Barta, J., Cano, M.P., Gusek, T.W., Sidhu, J.S. and Sinha, N.K., Eds, Wiley-Blackwell Publishing, Ames, Iowa, USA.

García Martín, J.F. (2015). Optical path length and wavelength selection using Vis/NIR spectroscopy for olive oil's free acidity determination. *Int. J. Food Sci. Technol.* **50**:1461–1467.

García, R., Martins, N. and Cabrita, M.J. (2013). Putative markers of adulteration of extra virgin olive oil with refined olive oil: Prospects and limitations. *Food Res. Int.* **54**:2039–2044.

Giacalone, R., Giuliano, S., Gulotta, E., Monfreda, M. and Presti, G. (2015). Origin assessment of EV olive oils by esterified sterols analysis. *Food Chem.* **188**:279–285.

Gökçebağ, M., Dıraman, H. and Özdemir, D. (2013). Classification of Turkish monocultivar (*Ayvalık and Memecik* cv.) virgin olive oils from north and south zones of Aegean region based on their triacyglycerol profiles. *J. Am. Oil. Chem. Soc.* **90**:1661–1671.

Gomes da Silva, M.D.R., Costa Freitas, A.M., Cabrita, M.J.B. and Garcia, R. (2012). Olive oil composition: Volatile compounds. **In**: Olive Oil Constituents, Quality, Health Properties and Bioconversions, pp. 18–46. Boskou, D., Ed., InTech-Oppen Access Company, Rijeka, Croitia.

Gómez-Ariza, J.L., Arias-Borrego, A., García-Barrera,T. and 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**:859–869.

Grossi, M., Di Lecce, G., Toschi, T.G. and Riccò, B. (2014). Fast and accurate determination of olive oil acidity by electrochemical impedance spectroscopy. *IEEE Sens. J.* **14**:2947–2954.

Grossi, M., Di Lecce, G., Arru, M., Toschi, T.G. and Riccò, B. (2015). An opto-electronic system for in-situ determination of peroxide value and total phenol content in olive oil. *J. Food Eng.* **146**:1–7.

Guillaume, C., Gertz, C. and Ravetti, L. (2014). Pyropheophytin a and 1,2-Diacyl-glycerols over time under different storage conditions in natural olive oils. *J. Am. Oil Chem. Soc.* **91**:697–709.

Gurdeniz, G. and Ozen, B. (2009). Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. *Food Chem.* **116**:519–525.

Habib, L., Jraij, A., Khreich, N., Charcosset, C. and Greige-Gerges, H. (2015). Effect of erythrodiol, a natural pentacyclic triterpene from olive oil, on the lipid membrane properties. *J. Membr. Biol.* **248**:1079–1087.

Heise, H.M., Damm, U., Lampen, P.H., Davies, A.N. and Mcintyre, P.S. (2005). Spectral variable selection for partial least squares calibration applied to authentication and quantification of extra virgin olive oils using Fourier transform Raman spectroscopy. *Appl. Spectrosc.* **59**:1286–1294.

Hennessy, S., Downey, G. and O'Donnell, C.P. (2009). Confirmation of food origin claims by fourier transform infrared spectroscopy and chemometrics: extra virgin olive oil from Liguria. *J. Agric. Food. Chem.* **57**:1735–1741.

Holčapek, M. and Lísa, M. (2009). Statistical evaluation of triacylglycerol composition by HPLC/APCI-MS. *Lipid Technol.* **21**:261–265.

Inarejos-García, A.M., Gómez-Alonso, S., Fregapane, G. and Salvador, M.D. (2012). Evaluation of minor components, sensory characteristics and quality of virgin olive oil by Near Infrared (NIR) spectroscopy. *Food Res. Int.* **50**:250–258.

IOC. (2001a). COI/T.20/Doc. 24. Regulation on the Preparation of the Fatty Acids Methyl Esters from Olive Oil and Olive-Pomace Oil. International Olive Council, Madrid, Spain.

IOC. (2001b). COI/T.20/DOC. No 17. Regulation on the Determination of Trans Unsaturated Fatty Acids by Capillary Column Gas Chromatography. International Olive Council, Madrid, Spain.

IOC. (2001c). COI/T.20/Doc. No. 10. Regulation on the Determination of the Composition and Content of Sterols by Capillary- Column Gas Chromatography. International Olive Council, Madrid, Spain.

IOC. (2001d). COI/T.20/Doc. No. 11. Regulation on the Determination of Stigmastadienes in Vegetable Oils. International Olive Council, Madrid, Spain.

IOC. (2003a). COI/T.20/Doc. No. 18. Regulation on the Determination of Wax Content by Capillary Column. International Olive Council, Madrid, Spain.

IOC. (2003b). COI/T.20/Doc. No. 26. Regulation on the Determination of Aliphatic Alcohols Content by Capillary Gas Chromatography. International Olive Council, Madrid, Spain.

IOC. (2006). COI/T.20/Doc. No. 23. Regulation on the Determination of the Percentage of 2-Glyceryl Monopalmitate. International Olive Council, Madrid, Spain.

IOC. (2007). COI/T.20/Doc. No 15. Regulation on the Sensory Analysis of Olive Oil Method for the Organoleptic Assessment of Virgin Olive Oil. International Olive Council, Madrid, Spain.

IOC. (2010a). COI/T.20/Doc. No 28. Regulation on the Determination of the Content of Waxes, Fatty Acid Methyl Esters and Fatty Acid Ethyl Esters by Capillary Gas Chromatography. International Olive Council, Madrid, Spain.

IOC. (2010b). COI/T.20/Doc. No. 20. Regulation on the Determination of the Difference Between Actual and Theoretical Content of Triacyglycerols with ECN 42. International Olive Council, Madrid, Spain.

IOC. (2013). International trade standard applying to olive oils and olive-pomace oils. Available from: http://www.internationaloliveoil.org/documents/viewfile/3615-normaen1. Accessed 2014 November 15.

IOC. (2014). Data on olive oil production and consumption. Available from: http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures. Accessed 2015 July 20.

IOC. (2015). Data on olive oil production and consumption. Available from: http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures. Accessed 2015 December 10.

IOC. (2016). Trade standard applying to olive oils and olivepomace oils. COI/T.15/NC No 3/Rev. 11 July 2016. Available from: http://www.internationaloliveoil.org/estaticos/view/222-standards. Accessed 2017 April 3.

ISO 5508. (1990). Animal and Vegetable Fats And Oils – Analysis by Gas Chromatography of Methyl Esters of Fatty Acids. International Standardization Organization, Geneva.

ISO 12228. (1999a). Animal and Vegetable Fats And Oils – Determination of Individual and Total Sterols Contents—Gas Chromatographic Method. International Standardization Organization, Geneva.

ISO 15788. (1999b). Animal and Vegetable Fats And Oils – Determination of Stigmastadienes in Vegetable Oils—Part 1: Method Using Capillary-Column Gas Chromatography. International Standardization Organization, Geneva.

ISO 5509. (2000). Animal and Vegetable Fats And Oils—Preparation of Methyl Esters of Fatty Acids. International Standardization Organization, Geneva.

ISO 15304. (2002). Animal and Vegetable Fats And Oils—Determination of the Content of Trans Fatty Acid Isomers of Vegetable Fats and Oils-Gas Chromatographic Method. International Standardization Organization, Geneva.

ISO 3960. (2007). Animal and Vegetable Fats And Oils – Determination of Peroxide Value- Iodometric (Visual) Endpoint Determination. International Standardization Organization, Geneva.

ISO 660. (2009a). Animal and vegetable fats and oils – determination of acid value and acidity.

ISO 29841. (2009b). Vegetable Fats and Oils – Determination of the Degradation Products of Chlorophylls a and a' (pheophytins a, a' and pyropheophytins). International Standardization Organization, Geneva.

ISO 29822. (2009c). Vegetable Fats and Oils—Isomeric Diacylglycerols—Determination of Relative Amounts of 1,2- and 1,3-Diacylglycerols. International Standardization Organization, Geneva.

ISO 12872. (2010). Olive Oils and Olive-Pomace Oils – Determination of the 2-Glyceryl Monopalmitate Content. International Standardization Organization, Geneva.

ISO 3656. (2011). Animal and Vegetable Fats and Oils – Determination of Ultraviolet Absorbance Expressed as Specific UV Extinction. International Standardization Organization, Geneva.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D. and Robards, K. (2007). Olive oil volatile compounds, flavour development and quality: A critical review. Food Chem. 100:273–286.

Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I. and Kontominas, M.G. (2013). Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses. Food Res. Int. 54:1950–1958.

Kasemsumran, S., Kang, N., Christy, A. and Ozaki, Y. (2005). Partial least squares processing of near–infrared spectra for discrimination and quantification of adulterated olive oils. Spectrosc. Lett. 38:839–851.

Korifi, R., Le Dréau, Y., Molinet, J., Artaud, J. and Dupuy, N. (2011). Composition and authentication of virgin olive oil from French PDO

regions by chemometric treatment of Raman spectra. J. Raman Spectrosc. 42:1540–1547.

Lerma-García, M.J., Ramis-Ramos, G., Herrero-Martínez, J.M. and Simó-Alfonso, E.F. (2010). Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. Food Chem. 118:78–83.

Lerma-García, M.J., Simó-Alfonso, E.F., Méndez, A., Lliberia, J.L. and Herrero-Martínez, J.M. (2011). Classification of extra virgin olive oils according to their genetic variety using linear discriminant analysis of sterol profiles established by ultra-performance liquid chromatography with mass spectrometry detection. Food Res. Int. 44:103–108.

Lin, P., Chen, Y. and He, Y. (2012). Identification of geographical origin of olive oil using visible and near-infrared spectroscopy technique combined with chemometrics. Food Bioprocess Technol. 5:235–242.

Longobardi, F., Ventrella, A., Napoli, C., Humpfer, E., Schütz, B., Schäfer, H., Kontominas, M.G. and Sacco, A. (2012). Classification of olive oils according to geographical origin by using 1H NMR fingerprinting combined with multivariate analysis. Food Chem. 130:177–183.

Lopez-Diez, E.C., Bianchi, G. and Goodacre, R. (2003). Rapid quantitative qssessment of the adulteration of virgin olive oils with hazelnut oils using raman spectroscopy and chemometrics. J. Agric. Food Chem. 51:6145–6150.

Lorenzo, I.M., Pavón, J.L.P., Laespada, M.E.F., Pinto, C.G. and Cordero, B. M. (2002). Detection of adulterants in olive oil by headspace–mass spectrometry. J. Chromatogr. A 945:221–230.

Lukić, M., Lukić, I., Krapac, M., Sladonja, B. and Piližota, V. (2013). Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening. Food Chem. 136:251–258.

Maggio, R.M., Kaufman, T.S., Del Carlo, M., Cerretani, L., Bendini, A., Cichelli, A. and Compagnone, D. (2009). Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. Food Chem. 114:1549–1554.

Mannina, L. and Sobolev, A.P. (2011). High resolution NMR characterization of olive oils in terms of quality, authenticity and geographical origin. Magn.Reson. Chem. 49:3–11.

Mariotti, M. (2014). Virgin olive oil: definition and standards. In: The Extra-Virgin Olive Oil Handbook, pp. 11–19. Peri, C., Ed., John Wiley & Sons, Oxford, UK.

Marquez, A.J., Díaz, A.M. and Reguera, M.I.P. (2005). Using optical NIR sensor for on-line virgin olive oils characterization. Sensors Actuat. B-Chem. 107:64–68.

Martínez, M., Fuentes, M., Franco, N., Sánchez, J. and De Miguel, C. (2014). Fatty acid profiles of virgin olive oils from the five olive-growing zones of Extremadura (Spain). J. Am. Oil. Chem. Soc. 91:1921–1929.

Martínez Gila, D.M., Cano Marchal, P., Gámez García, J. and Gómez Ortega, J. (2015). On-line system based on hyperspectral information to estimate acidity, moisture and peroxides in olive oil samples. Comput. Electron. Agric. 116:1–7.

Martins-Lopes, P., Gomes, S., Santos, E. and Guedes-Pinto, H. (2008). DNA markers for Portuguese olive oil fingerprinting. J. Agric. Food Chem. 56:11786–11791.

Mathison, B. and Holstege, D. (2013). A rapid method to determine sterol, erythrodiol, and uvaol concentrations in olive oil. J. Agric. Food Chem. 61:4506–4513.

Melucci, D., Bendini, A., Tesini, F., Barbieri, S., Zappi, A., Vichi, S., Conte, L. and Toschi, T.G. (2016). Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics. Food Chem. 204:263–273.

Mignani, A.G., Ciaccheri, L., Ottevaere, H., Thienpont, H., Conte, L., Marega, M., Cichelli, A., Attilio, C. and Cimato, A. (2011). Visible and near-infrared absorption spectroscopy by an integrating sphere and optical fibers for quantifying and discriminating the adulteration of extra virgin olive oil from Tuscany. Anal. Bioanal. Chem. 399:1315–1324.

Mildner-Szkudlarz, S. and Jeleń, H.H. (2010). Detection of olive oil adulteration with rapeseed and sunflower oils using MOS electronic nose and SMPE-MS. J. Food. Qual. 33:21–41.

Monasterio, R., Fernandez, M.A. and Silva, M.F. (2013). Olive oil by capillary electrophoresis: Characterization and genuineness. *J. Agric. Food Chem.* **61**:4477–4496.

Muik, B., Lendl, B., Molina-Díaz, A. and Ayora-Cañada, M.J. (2003). Direct, reagent-free determination of free fatty acid content in olive oil and olives by Fourier transform Raman spectrometry. *Anal. Chim. Acta* **487**:211–220.

Ou, G., Hu, R., Zhang, L., Li, P., Luo, X. and Zhang, Z. (2015). Advanced detection methods for traceability of origin and authenticity of olive oils. *Anal. Methods* **7**:5731–5739.

Peña, F., Cárdenas, S., Gallego, M. and Valcárcel, M. (2005). Direct olive oil authentication: Detection of adulteration of olive oil with hazelnut oil by direct coupling of headspace and mass spectrometry, and multivariate regression techniques. *J. Chromatogr. A* **1074**:215–221.

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

Pérez-Camino, M.C., Gómez-Coca, R.B. and Moreda, W. (2012). Waxy fraction containing long-chain aliphatic aldehydes in virgin olive oils. *Food Chem.* **132**:1451–1456.

Pérez-Camino, M.C., Moreda, W. and Cert, A. (2001). Effect of olive fruit quality and oil storage practices on the diacylglycerol content of virgin olive oils. *J. Agric. Food Chem.* **49**:699–704.

Pérez-Camino, M.C., Moreda, W., Mateos, R. and Cert, A. (2002). Determination of esters of fatty acids with low molecular weight alcohols in olive oils. *J. Agric. Food Chem.* **50**:4721–4725.

Petrakis, P.V., Agiomyrgianaki, A., Christophoridou, S., Spyros, A. and Dais, P. (2008). Geographical characterization of Greek virgin olive oils (Cv. Koroneiki) using ^{1}H and ^{31}P NMR fingerprinting with canonical discriminant analysis and classification binary trees. *J. Agric. Food Chem.* **56**:3200–3207.

Pizarro, C., Esteban-Díez, I., Rodríguez-Tecedor, S. and González-Sáiz, J. M. (2013a). Determination of the peroxide value in extra virgin olive oils through the application of the stepwise orthogonalisation of predictors to mid-infrared spectra. *Food Control* **34**:158–167.

Pizarro, C., Rodríguez-Tecedor, S., Pérez-Del-Notario, N., Esteban-Díez, I. and González-Sáiz, J.M. (2013b). Classification of Spanish extra virgin olive oils by data fusion of visible spectroscopic fingerprints and chemical descriptors. *Food Chem.* **138**:915–922.

Pouliarekou, E., Badeka, A., Tasioula-Margari, M., Kontakos, S., Longobardi, F. and Kontominas, M.G. (2011). Characterization and classification of Western Greek olive oils according to cultivar and geographical origin based on volatile compounds. *J. Chromatogr. A* **1218**:7534–7542.

Rabiei, Z. and Enferadi, S.T. (2012). Traceability of origin and authenticity of olive oil. **In**: Olive Oil Constituents, Quality, Health Properties and Bioconversions, pp. 163–184. Boskou, D., Ed., InTech-Oppen Access Company, Rijeka, Croitia.

Raieta, K., Muccillo, L. and Colantuoni, V. (2015). A novel reliable method of DNA extraction from olive oil suitable for molecular traceability. *Food Chem.* **172**:596–602.

Riccio, M.F., Sawaya, A.C.H.F., Abdelnur, P.V., Saraiva, S.A., Haddad, R., Eberlin, M.N. and Catharino, R.R. (2011). Easy ambient sonic-spray ionization mass spectrometric of olive oils: Quality control and certification of geographical origin. *Anal. Lett.* **44**:1489–1497.

Rohman, A., Che Man, Y.B. and Nurrulhidayah, A.F. (2015). Fourier-transform infrared spectra combined with chemometrics and fatty acid composition for analysis of pumpkin seed oil blended into olive oil. *Int. J. Food Prop.* **18**:1086–1096.

Ruiz-Samblás, C., Marini, F., Cuadros-Rodríguez, L. and González-Casado, A. (2012). Quantification of blending of olive oils and edible vegetable oils by triacylglycerol fingerprint gas chromatography and chemometric tools. *J. Chromatogr. B* **910**:71–77.

Sánchez-Hernández, L., Marina, M.L. and Crego, A.L. (2011). A capillary electrophoresis-tandem mass spectrometry methodology for the determination of non-protein amino acids in vegetable oils as novel markers for the detection of adulterations in olive oils. *J. Chromatogr. A* **1218**:4944–4951.

Savarese, M., Caporaso, N. and Parisini, C. (2013). Application of an electronic nose for the evaluation of rancidity and shelf life in virgin olive oil. *Electron. Int. Interdiscip. Conf.* **12**:361–366.

Segura-Carretero, A., Carrasco-Pancorbo, A., Cortacero, S., Gori, A., Cerretani, L. and Fernández-Gutiérrez, A. (2008). A simplified method for HPLC-MS analysis of sterols in vegetable oil. *Eur. J. Lipid. Sci. Technol.* **110**:1142–1149.

Sinelli, N., Cerretani, L., Di Egidio, V., Bendini, A. and 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 Res. Int.* **43**:369–375.

Spangenberg, J.E. (1998). Characterization of olive oil by carbon isotope analysis of individual fatty acids: Implications for authentication and palaeoclimatological changes in the Mediterranean Basin. *Mineral. Mag.* **62**:1438–1438.

Standards Australia. (2011). Australian standard olive oils and olive-pomace oils. Available from: http://www.aph.gov.au/DocumentStore. ashx?id=ca0c9dd8-b5ee-45fa-b7e3-3078219d7be9. Accessed 2015 November 15.

Tena, N., Wang, S.C., Aparicio-Ruiz, R., García-González, D.L. and Aparicio, R. (2015). In-Depth assessment of analytical methods for olive oil purity, safety, and quality characterization. *J. Agric. Food Chem.* **63**:4509–4526.

Thomaidis, N.S., Georgiou, C.A. and Polissiou, M.G. (2000). Direct parallel flow injection multichannel spectrophotometric determination of olive oil iodine value. *Anal. Chim. Acta* **405**:239–245.

Toschi, T. G., Bendini, A., Lozano-Sánchez, J., Segura-Carretero, A. and Conte, L. (2013). Misdescription of edible oils: Flowcharts of analytical choices in a forensic view. *Eur. J. Lipid Sci. Technol.* **115**:1205–1223.

Tsiaka, T., Christodouleas, D.C. and Calokerinos, A.C. (2013). Development of a chemiluminescent method for the evaluation of total hydroperoxide content of edible oils. *Food Res. Int.* **54**:2069–2074.

USDA. (2010). United States standards for grades of olive oil and olive-pomace oil. Available from: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3011889. Accessed 2015 November 15.

Vaclavik, L., Cajka, T., Hrbek, V. and Hajslova, J. (2009). Ambient mass spectrometry employing direct analysis in real time (DART) ion source for olive oil quality and authenticity assessment. *Anal. Chim. Acta* **645**:56–63.

Valli, E., Bendini, A., Maggio, R.M., Cerretani, L., Toschi, T.G., Casiraghi, E. and Lercker, G. (2013). Detection of low-quality extra virgin olive oils by fatty acid alkyl esters evaluation: A preliminary and fast mid-infrared spectroscopy discrimination by a chemometric approach. *Int. J. Food Sci. Technol.* **48**:548–555.

Valli E., Bendini, A., Berardinelli, A., Ragni, L., Riccò, B., Grossi, M. and Gallina Toschi T. (2016). Rapid and innovative instrumental approaches for quality and authenticity of olive oils. *Eur. J. Lipid Sci. Technol.* **118**:1601–1619.

Van Wetten, I.A., Van Herwaarden, A.W., Splinter, R., Boerrigter-Eenling, R. and Van Ruth, S.M. (2015). Detection of sunflower oil in extra virgin olive oil by fast differential scanning calorimetry. *Thermochim. Acta* **603**:237–243.

Veloso, A.C.A., Dias, L.G., Rodrigues, N., Pereira, J.A. and Peres, A.M. (2015). Sensory intensity assessment of olive oils using an electronic tongue. *Talanta* **146**:585–593.

Vietina, M., Agrimonti, C. and Marmiroli, N. (2013). Detection of plant oil DNA using high resolution melting (HRM) post PCR analysis: A tool for disclosure of olive oil adulteration. *Food Chem.* **141**:3820–3826.

Wu, Y., Zhang, H., Han, J., Wang, B., Wang, W., Ju, X. and Chen, Y. (2011). PCR-CE-SSCP applied to detect cheap oil blended in olive oil. *Eur. Food. Res. Technol.* **233**:313–324.

Yang, Y., Li, Q., Yu, X., Chen, X. and Wang, Y. (2014). A novel method for determining peroxide value of edible oils using electrical conductivity. *Food Control* **39**:198–203.

Youseff, R., Soubh, L. and Alassaf, Z. (2014). Detection of vegetable oils adulteration using desmethylsterols composition. *Int. J. Pharm. Sci. Rev. Res.* **28**:229–233.

Yorulmaz, A., Yavuz, H. and Tekin, A. (2014). Characterization of Turkish olive oils by triacylglycerol structures and sterol profiles. *J. Am. Oil. Chem. Soc.* **91**:2077–2090.

Zabaras, D. (2010). Olive oil adulteration with hazelnut oil and analytical approaches for its detection. **In**: Olives and Olive Oil in Health and

Disease Prevention, pp. 441–450. Preedy, V.R. and Watson, R.R., Eds., Academic Press, San Diego, CA, USA.

Zarrouk, W., Carrasco-Pancorbo, A., Segura-Carretero, A., Fernández-Gutiérrez, A. and Zarrouk, M. (2010). Exploratory characterization of the unsaponifiable fraction of Tunisian virgin olive oils by a global approach with HPLC-APCI-IT MS/MS analysis. *J. Agric. Food Chem.* **58**:6418–6426.

Zhang, X.F., Zou, M.Q., Qi, X.H., Liu, F., Zhang, C. and Yin, F. (2011). Quantitative detection of adulterated olive oil by Raman spectroscopy and chemometrics. *J. Raman Spectrosc.* **42**:1784–1788.

Zou, M.Q., Zhang, X.F., Xiao-Hua, Q.I., Han-Lu, M., Dong, Y., Chun-Wei, L.I.U., Guo, X.U. N. and Wang, H. (2009). Rapid authentication of olive oil adulteration by raman spectrometry. *J. Agric. Food. Chem.* **57**:6001–6006.