



# Importance of some minor compounds in olive oil authenticity and quality

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## ABSTRACT

**Background:** Consumption and production of olive oils have been increasing steadily worldwide mainly due to proven health benefits and sensorial characteristics of olive oil. At the same time, rising demand makes it harder to protect olive oil genuineness; therefore, inauthentic products have been always a serious problem in olive oil industry.

**Scope and approach:** Some minor compounds such as pigments (chlorophylls and carotenoids) including their derivatives pyropheophytins (PPPs), diacylglycerols (DAGs) and fatty acid ethyl esters (FAEEs) are all prominent compounds with their discriminatory and descriptive properties. Among several different approaches, use of these components to differentiate genuine and adulterated olive oils could be a promising choice since it is harder to mimic these compounds in fake mixtures. Recent studies focus on these compounds as authentication and quality tools for olive oil and potential of these compounds are aimed to be reviewed.

**Key findings and conclusions:** Results from literature indicated that these parameters could be used in both authenticity and quality determination of olive oils with some limitations. Pigments were found to be more promising in geographical and/or varietal classification. All of the discussed components have successful applications in determination of olive oil quality with respect to storage history and oil grades. However, in detection of certain types of adulteration techniques such as soft deodorization, reviewed parameters did not work effectively alone. Regulations could be updated with these findings and use of combined parameters including discussed compounds could be further investigated for unsolved authentication problems.

## 1. Introduction

Well-established health effects and desirable sensory properties of olive oil are the major driving forces for the high economical value of this product. Major components of olive oil are triacylglycerols and this oil also contains various minor components such as chlorophylls, carotenoids, phenolic compounds and squalene (Yan, Oey, van Leeuwen, & van Ruth, 2018).

Minor components of virgin olive oil which does not need to go through refining steps are highly preserved during mechanical extraction (Olmo-García et al., 2019). Minor compounds are not only significant for physicochemical characteristics of the product, but they are also correlated with taste and nutritional value (Olmo-García et al., 2019). In addition, they are important markers for olive oil quality, purity and authenticity (Olmo-García et al., 2018; Tena, Wang, Aparicio-Ruiz, García-González, & Aparicio, 2015). Therefore, the concentrations and types of minor compounds are of great importance for both the consumers and the manufacturers (Olmo-García et al., 2018). The quality and quantity of these metabolites are affected by olive variety, growth conditions of olives, extraction and refining

procedures of oil as well as storage conditions (Dais & Hatzakis, 2013).

Besides their health-promoting effects, minor components (volatiles, phenolic compounds, terpenoids, sterols, etc.) are also found to be more successful descriptors of olive oil compared to major metabolites due to the fact that it is hard to mimic minor compounds during preparation of illegal formulations (Dais & Hatzakis, 2013). Importance of minor compound composition has become even more significant since olive fruits have been started to be cultivated outside the Mediterranean zones. Even for the same olive type, differences in olive growth locations are also leading to compositional differences between oils obtained from relatively new areas and the products from traditional olive producer countries (Aparicio, Morales, Aparicio-Ruiz, Tena, & García-González, 2013). As a result, olive oils from new cultivation areas could be out of the limits set by official regulatory agencies mainly based on Mediterranean countries (Uncu, Ozen, & Tokatli, 2019). In addition, some traditional but minor cultivars, even grown in the Mediterranean region could still have chemical compounds out of the described limits (García González, Aparicio, & Aparicio-Ruiz, 2018). Thus, the data of the minor compounds of olive oils have become more valuable for statistical evaluation as a significant part of authentication studies (Dais

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& Hatzakis, 2013).

As a solution to these emerging problems, new chemical parameters mainly exploiting minor compounds of olive oil have been put into action as quality and/or authenticity indicators (Dais & Hatzakis, 2013). If the official and recently proposed methods are examined, it could be seen that methods that determine quality and adulteration in general are intertwined with each other. Therefore, effect of the various constituents of olive oil on the quality and authenticity are examined together in this review. Fatty acid alkyl esters (FAAEs), diacylglycerols (DAGs), natural color pigments, particularly pyropheophytins (PPPs) as the degradation product of chlorophylls and phenolic compounds are regarded as some of the potential quality and authenticity indicators of olive oil (European Commission, 2013).

Several recent reviews and studies in the literature provide information regarding the current regulations about olive oil as well as their methods of analysis (Bajoub, Bendini, Fernández-Gutiérrez, & Carrasco-Pancorbo, 2018; Conte et al., 2019; Tena et al., 2015). Some novel techniques (Surface-enhanced Raman spectroscopy (SERS), biosensors, microfluidic devices) have also been applied in olive oil authentication (Bremer, Smits, & Haasnoot, 2009; Deng et al., 2018; Du et al., 2019; McIntosh et al., 2016) and in determination of some bioactive compounds of olive oils especially polyphenolic content (Al Mughairy, Al-Lawati, & Suliman, 2019; Camerlingo, Portaccio, Delfino, & Lepore, 2019; Hammami, Kuliček, & Raouafi, 2016; Ramos, Contreras, & Macías, 2020). Some well-known minor compounds such as sterols, stigmastadienes, aliphatic hydrocarbons and phenolic compounds along with major compounds (triacylglycerols, fatty acid contents) which have official limits in regulations were evaluated in detail in the previous reviews (Aparicio, Conte, & Fiebig, 2013; Arvanitoyannis & Vlachos, 2007; Ben-Ayed, Kamoun-Grati, & Rebai, 2013; Boskou, 2008; García González et al., 2018; Montealegre, Alegre, & García-Ruiz, 2010). Olive oil is very rich in terms of phenolic compounds such as hydroxytyrosol, tyrosol and oleuropein. Because of the well-established health effects of these compounds they have been extensively studied and there are several reviews about these compounds (Alu'datt et al., 2017; Boskou, 2015) and literature reviews are even available on certain individual phenolic compounds (Parkinson & Keast, 2014; Wani et al., 2018). However, there is not any comprehensive and critical review in the literature focusing on emerging minor compounds, FAAEs, DAGs and pigments, and emphasizing their importance in olive oil studies although many studies in the literature indicated their potential on different quality issues such as detection of different types of adulteration and determining olive grade or storage history. Therefore, it was aimed to review the several minor compounds (FAAEs, color compounds with their derivatives (e.g. PPPs), DAGs with derivatives (e.g. monochloropropanediol esters (MCPDEs) and glycidyl esters (GEs)) that have been studied in recent years in terms of the authenticity and quality of olive oil.

## 2. Recent problems regarding authentication of olive oils

Olive oil industry must deal with various authentication and quality problems. Mixing of various edible oils such as sunflower, canola and soybean oils with olive oil is one of the most common type of

adulteration problems for olive oil. However, detection of these mixtures is becoming easier and faster. Therefore, fraudsters are constantly introducing new mixtures and causing new problems to oil industry. Despite the progresses in analytical methods, developments may still not be enough to find absolute solutions to some of the major problems (European Commission, 2013). One of these cases is addition of soft-deodorized virgin olive oil to extra virgin olive oil and this type of mixing could not be detected by standard methods (Kulling et al., 2019). Some proposed solutions for this problem include the determination of PPPs and alkyl esters (Aparicio-Ruiz, Romero, García-González, Oliver-Pozo, & Aparicio, 2017a).

Another issue is related with Protected Designation of Origin (PDO) status which is granted to products coming from certain regions. A label showing PDO status could be placed on the product and these products are generally sold at higher prices in the markets. Olive oils which do not have this status could be intentionally mislabeled to obtain extra profit. Detection of this type of adulteration becomes a more difficult task especially after cultivation areas are spreading to non-Mediterranean countries (Australia, Argentina, Chile, South Africa, USA, etc.). Therefore, it has been proposed to build larger chemical data bases by using minor and major compounds of olive oil for geographical authentication (García González et al., 2018).

Another problem is related with the freshness of olive oils. To obtain extra earnings, fraudsters add old olive oil samples from previous harvest year into the fresh olive oil. This is an emerging adulteration case and there is an update in European Union regulation (EU, 2012) about olive oil labelling requirements indicating the freshness of olive oil. According to the regulation, harvest year could be placed on the label only if 100% of the olive oil is from the olives harvested in the same year. However, there is not any official method in the literature to determine this type of adulteration. It has been proposed that new quality parameters such as FAAEs, pigments (PPPs, carotenoids, etc.) and DAGs have potential for olive oil quality and authenticity (European Commission, 2013).

Production of fake extra virgin olive oil mixtures is another type of fraud. A recent report on deliberately mislabeling the mixture of olive oil made with refined olive oil as extra virgin olive oil was the case occurred in 2018 which was detected by compulsory controls (Kulling et al., 2019). Another case was also reported in 2019 by Europol in which chlorophyll,  $\beta$ -carotene and soya oil were added to sunflower oil to prepare a fake olive oil. The last two adulteration examples were detected easily by existing regulations based on methods using chromatographic techniques (Kulling et al., 2019). In order to solve emerging issues in olive oil, official methods have been updated regularly as a result of new scientific findings about the quality and authenticity of olive oils. Examples of several relatively new regulations about minor components of olive oil mentioned in the paragraphs above are provided in Table 1.

## 3. Application of minor components in quality and authentication studies

Minor components of olive oils have been quite useful in investigation of both quality issues such as monitoring of oxidation as

**Table 1**  
Official regulations about reviewed parameters of olive oil quality and authenticity.

Parameters	Legislations
Fatty acid ethyl esters (FAEEs)	Quality criteria defined in International Olive Council (IOC) (2019) and EU (2016) regulations which state that olive oil could be graded as extra virgin only if it contains ethyl esters less than or equal to 35 mg/kg
Diacylglycerols (DAGs)	Quality and freshness indicator only found in Australian (Standards Australia, 2011) and Californian (California Department of Food and Agriculture, 2014) standards to grade olive oil as extra virgin under certain conditions. Both standards define threshold value for 1,2-DAGs as 35% as the ratio between 1,2- to total 1,2- and 1,3- DAGs
Pyropheophytins (PPPs)	Used in freshness evaluation by both Australian (Standards Australia, 2011) and Californian (California Department of Food and Agriculture, 2014) standards. According to both standards olive oils are graded as extra virgin when they contain less than or equal to 17% of PPPs

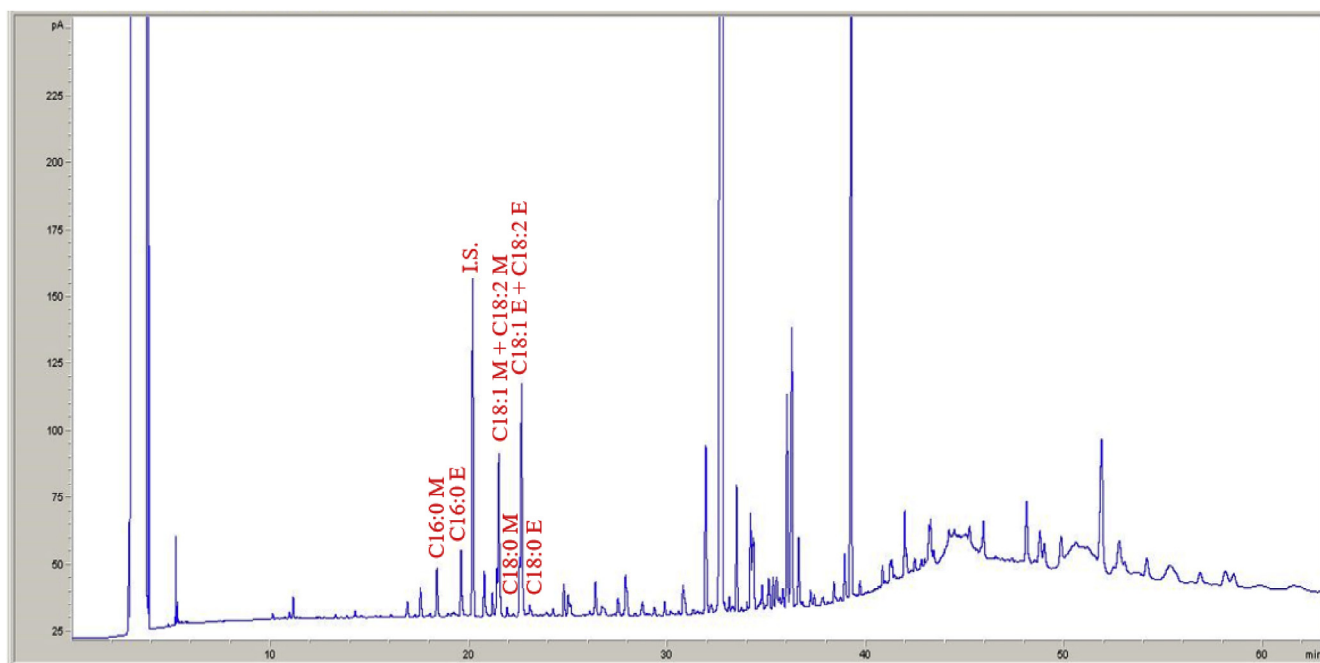


Fig. 1. Sample GC chromatogram of alkyl esters of an olive oil according to International Olive Council method (IOC, 2010).

well as the stability, storage history, and also different types of adulteration of olive oil (Dais & Hatzakis, 2013; Uncu & Ozen, 2019). Some of these issues were successfully addressed and included in official regulations as mentioned in the previous parts. Determination of some relatively new parameters (MCPDs, GEs, FAEs, DAGs, and PPPs) are the emerging approaches in the field for the unsolved issues. Below, these parameters are explained in detail including their strong and weak sides with up to date applications.

### 3.1. Fatty acid alkyl esters (FAEs)

Fatty acid alkyl esters (FAEs) are produced by enzymatic reaction of free fatty acids with low molecular weight alcohols, mainly methanol and ethanol, under acidic conditions yielding methyl (FAME) and ethyl esters (FAEE), respectively (Bajoub et al., 2018; Pérez-Camino, Moreda, Mateos, & Cert, 2002; Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo, & Moreda, 2008). Critical levels of FAEs (sum of FAME and FAEE) for olive oil have been defined first by a Commission Regulation (EU) No 61/2011 (EU, 2011) as a quality parameter since the formation of these compounds indicates fermentation (mainly ethanol formation) as well as degradation processes (mainly methanol formation) occurred during storage (Purcaro, Barp, & Conte, 2015). In addition, it is not possible to remove FAEs without leaving by-products such as stigmastadiene in high temperature treatments (Purcaro et al., 2015). All of these make FAEs as suitable markers for olive oil quality as well as sensorial assessment (Biedermann, Bongartz, Mariani, & Grob, 2008). Moreover, storage and processing conditions of olive fruit are also other factors for FAEs formation (Caponio et al., 2018; Jabeur, Zribi, Abdelhedi, & Bouaziz, 2015; Squeo et al., 2017). It was observed that oil that was produced from olives stored in closed plastic bags rather than in perforated plastic containers have higher concentrations of FAEs due to fermentation activity in the closed plastic bags (Jabeur et al., 2015).

Former regulation has been amended by substituting FAE (sum of FAME and FAEE) with only FAEE by EU Commission Implementing Regulation 1348/2013 (EU, 2013). Reason for this substitution is that FAEE presence depends on level of its substrate, ethanol, which is produced chemically as a result of fermentative processes. On the other hand, amount of FAMES depends on methanol content, and unlike

ethanol, methanol is physiologically formed during pectin degradation of cell wall as olive fruit ripens (García-Vico et al., 2018). The concentrations of FAEs depend first on the availability of substrates (ethanol and free fatty acids), and then storage time and temperature, agricultural practices (health status of olive fruits) as well as manufacturing conditions (Bajoub et al., 2018; Conte et al., 2019; García-Vico et al., 2018). In two separate studies, ethanol content of olives being precursor of ethyl ester formation in olive oil was investigated with respect to two different parameters as maturation stage (Beltran, Bejaoui, Jimenez, & Sanchez-Ortiz, 2015) and harvesting method (Beltran et al., 2016). It was observed that ethanol content of olive fruit increased during the ripening process (Beltran et al., 2015). Furthermore, ground-picked olives were more susceptible to sensory defects with increasing level of ethanol content compared to tree-picked fruits (Beltran et al., 2016). In another study, FAE levels of olive oils were investigated during storage (Conte et al., 2014). The results indicated that high quality olive oils with initially low content of free ethanol and FAEs did not show any increment of ethyl esters during storage in contrast to lower quality ones. Since these findings confirmed the necessity of an update based on omission of FAME from the regulation and lowering the limit for FAEs, modifications in regulation were done (Conte et al., 2014). As a result, only the amount of FAEs have been used as a threshold value for virgin olive oil in determination of the quality in terms of category after this change. According to the latest EU (2016) and International Olive Oil Council (2019) regulations, olive oil could be graded as extra virgin only if it contains FAEs  $\leq 35$  mg/kg. The target compounds were fractionated by gas chromatography (GC) and determined as sum of ethyl of C16:0, C18:0, C18:1 and C18:2 in official method (Fig. 1). As an alternative method, GC Electron Ionization Mass Spectroscopy (GC-EI-MS) method has been also used in determination of FAEs of olive oils as a fast technique without sample preparation. It was observed that this method was at least as successful as official EU method in discrimination of extra virgin and lower quality olive oils (Boggia, Borgogni, Hysenaj, Leardi, & Zunin, 2014). Moreover, very recently GC-Ion Mobility Spectrometry (GC-IMS) has been used with promising results in quantification of ethanol content in olive oils without sample pretreatment and found as being faster than the method based on GC-FID/MS (del Mar Contreras, Aparicio, & Arce, 2020). In addition, spectroscopic methods have been applied to the

prediction of FAAE content due to their environmentally friendly and easy to use characteristics compared to wet chemical methods. Fourier transform infrared (FTIR) spectroscopy was used in quantification of FAAEs and ratio of ethyl and methyl esters value successfully (Valli et al., 2013). The same type of application was also performed with near infrared (NIR) spectroscopy (Cayuela, 2017; Garrido-Varo, Sánchez, De la Haba, Torres, & Pérez-Marín, 2017). In addition, FTIR and UV–visible spectroscopy separately and in combined form were applied to predict FAAE and FAEE content of olive oils (Uncu et al., 2019). FTIR spectroscopy also achieved discrimination of extra virgin from non-extra virgin olive oils based on FAEEs content (Squeo, Grassi, Paradiso, Alamprese, & Caponio, 2019). Dielectric spectroscopy as time domain reflectometry (TDR) was another method used in screening of FAMES, FAEEs, and FAAEs in olive oils (Berardinelli et al., 2013). In a review paper, determination of various quality parameters of olive oils including FAAEs by different rapid and innovative instrumental approaches were discussed (Valli et al., 2016).

In addition to their quality determining characteristics, these parameters have been also used in detection of mildly refined olive oil which is one of the most recent and common way of adulteration of extra-virgin olive oil. It has been very hard to detect this type of mixing with any other chemical test (Jabeur et al., 2015). FAAE has been firstly proposed as a useful marker to detect soft deodorized olive oils (Pérez-Camino et al., 2008) since this compound is not affected by mild refining conditions significantly. Recent studies are focusing on FAEE contents of olive oils rather than FAAE due to the update in the legislations mentioned in the previous paragraph. Later on, the weak side of this approach as an authentication tool was also discussed in different studies (Aparicio-Ruiz et al., 2017a; García-Vico et al., 2018; Gómez-Coca, Fernandes, Pérez-Camino, & Moreda, 2016). In one of these investigations, it was proven that FAEE content of olive oil could be related with factors other than the quality and health of olives used in olive extraction as opposed to prior knowledge and this could be explained by two main factors (Gómez-Coca et al., 2016). One of these factors is ethanol (precursor of FAEE) formation which had been previously thought to be produced only by fermentation. However, it was found out that healthy fruits could also be the sources of ethanol during maturation which contribute to aroma development (Beltran et al., 2015). Other factor is related to technological aspects such as addition of water during the extraction process and this could change ethanol concentration as well as FAEE formation (Gómez-Coca et al., 2016). As a result, extra virgin olive oil could be out of the limits in a few months' time if FAEE content would be measured (Gómez-Coca et al., 2016). Therefore, in a recent study, it was proposed that strict regulations should take into account of the presence of ethanol basal levels in the oils which were found quite high in many cultivars. As a result, it

becomes an important point to differentiate physiologically formed and fermentative ethanol contents in the olive fruits (García-Vico et al., 2018). In the light of these findings, the latest EU regulation about FAEE might need an update for including the initial ethanol content. In some cases, deodorized low quality (especially rancid) oils might not have very high FAEE content and if this oil is used as an adulterant current critical levels in legislation might not be enough to detect the adulteration. Hence, it could be concluded that FAEEs are suitable adulteration markers for the oils possessing significantly high content of FAEEs compared to virgin olive oils (Conte et al., 2019). Another important factor making FAEEs insufficient in detection of adulteration is masking effect of the certain processing conditions of the soft deodorization on the oils. It was observed that deodorization at 100 °C for 60 min is the optimum condition to remove volatiles responsible for sensory defects without significant losses of quality parameters such as total phenols, PPPs and FAEEs and the critical limits of regulations are still met using these parameters (Aparicio-Ruiz et al., 2017a). Therefore, monitoring FAEEs could only be useful in detecting highly degraded oils with initial concentration already higher than the threshold values of the regulations prior to deodorization process. Otherwise, mixture of soft deodorized olive oil and extra virgin olive oil could not be determined up to 50% with current standard methods (Aparicio-Ruiz et al., 2017a).

Another attention-grabbing point is the relationship between FAEEs content and sensory defects. First comprehensive effort to reveal a relationship between the FAAEs concentration of olive oils and their sensory classification was conducted by Gómez-Coca, Moreda, and Pérez-Camino (2012) and a connection between the FAAEs and fermentative sensory defects was determined (Gómez-Coca et al., 2012). In another study, FAEEs are also correlated with the fermentation processes responsible for sensory defects and it was concluded that their relations could be used to determine olive oils that have undergone mild refining processes (Di Serio et al., 2017). In a recent study, correlation between sensory characteristics and various chemical parameters of Brazilian olive oils were investigated (Zago, Squeo, Bertocini, Difonzo, & Caponio, 2019). A positive correlation was obtained between concentration of FAEE and vinegary defect. Therefore, FAEE amount could be useful not only for authentication but also for quality control of olive oils in terms of sensory characteristics. Other examples of recent applications of alkyl esters in olive oil authentication are listed in Table 2.

### 3.2. Diacylglycerols (DAGs) and derivatives

Diacylglycerols (DAGs) have been considered as another quality parameter especially by some relatively new olive growing areas, USA

**Table 2**

Examples of studies from the literature for the determination of olive oil authenticity and quality using fatty acid alkyl esters (FAAEs).

Aim	Main Findings	Reference
Checking authenticity	FAAEs could be used to detect adulteration of olive oil with mild deodorized low-quality olive oil up to 30%.	Jabeur et al. (2015)
Shelf life prediction	FAEEs could be used to predict shelf-life of olive oil along with main chemical, physicochemical, and sensory characteristics under standard shelf life conditions.	Di Serio et al. (2018)
Detection of adulteration	FAEEs were found successful in the detection of extra virgin olive oil fraud with 2% refined pomace olive oils.	Jabeur, Drira, Rebai, and Bouaziz (2017)
Effect of processing parameters	FAAEs increase from the decanter to the vertical centrifuge during production. Use of water decreases the formation of FAEE and FAME.	Alcalá et al. (2017)
Shelf life determination	After 6 months of storage, FAEEs content of extra virgin olive oil could be off limit although the other quality related parameters (peroxide index, K 232, K 270 and ΔK) were not.	Grompone et al. (2016)
Evaluation of quality	Good correlation was established between FAAEs and free acidity. Moreover, FAAEs as well as many other parameters as free acidity, waxes, stigmastadienes, extinction coefficients, and peroxide values were all negatively correlated to the sensorial characteristics.	Di Loreto et al., 2014
Characterization in terms of PDO	Olive oils from Sicilian region were below the critical limit of FAAEs except some aged ones.	Costa et al. (2017)
Characterization in terms of variety and growing area	Both variety and growing environment of olives have significant effect on qualitative indexes such as free fatty acid, peroxide value, specific extinction coefficient values, waxes, fatty acids, FAAEs content and sterols of olive oils.	Piscopo, De Bruno, Zappia, Ventre, and Poiana (2016)



(particularly California state) and Australia. DAGs are found in virgin olive oil in minor amounts ranging from 1% to 3% and they are generally produced before or during olive oil extraction process. 1,2-DAGs are the intermediate products that form as a result of the incomplete biosynthesis of triacylglycerols (TAGs) while 1,3-DAGs are the products of enzymatic or chemical hydrolysis of TAGs (Pérez-Camino, Moreda, & Cert, 2001). Health status of the olive fruits is one of the major factors determining the amount, type and ratio (1,2- to 1,3-) of DAGs. Olive oils extracted from poor quality olive fruits showed a significant raise of 1,3-DAGs while the product obtained from healthy olive fruits contains almost exclusively 1,2-DAGs (Garcia, Martins, & Cabrita, 2013). In addition, storage conditions and time as well as extraction process (high temperature and water dilution during extraction), presence of macromolecules, and metals had also major effects on DAG ratio of olive oils (Circi et al., 2018; Vlahov, Giuliani, & Del Re, 2010). During storage, the concentration of 1,2-DAGs gradually decreases by isomerization resulting in the formation of more stable 1,3-DAGs. Thus, ratio of these isomeric forms was found to be reliable markers for the freshness (age) and the quality of virgin olive oils (Bajoub et al., 2018). According to both Californian and Australian standards, olive oils are graded as extra virgin if it contains 1,2 DAGs  $\geq 35\%$  in terms of C32 + C34 + C36 and this value actually is the ratio between 1,2-DAGs and total DAGs content known as *D* value. The methods used in the determination of DAGs are based on GC, high performance size exclusion chromatography and high-performance liquid chromatography (HPLC) all of which requires tedious derivatization steps before injection of the sample (Vlahov et al., 2010). GC-FID has been used most commonly to determine fractionated isomeric DAGs in olive oil (Gertz & Fiebig, 2006a). A typical DAG profile for an olive oil sample obtained with GC-FID analysis are shown in Fig. 2. GC-EI-MS is another technique applied to characterize and quantify DAGs without any requirement for a standard which was reported as a problem for the previous method (Zhu, Clegg, Shoemaker, & Wang, 2013). Thin layer chromatography (TLC) coupled with visible (Vis) spectrophotometry was also used as a simple method to quantify DAGs in edible oils (Li, Yu, Yang, & Liu, 2018). As a relatively new approach some spectroscopic methods were also used in DAGs determination. Recently, DAG content of olive oils were predicted from Fourier transform near infrared (FT-NIR) spectroscopic data (Azizian, Wang, Li, & Kramer, 2018;

Willenberg, Matthäus, & Gertz, 2019). In addition, a very recent study investigated the use of FTIR and UV-vis spectroscopic methods jointly and separately to estimate DAGs composition of olive oils (Uncu et al., 2019). Furthermore, NMR spectroscopy in the forms of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$  NMR has been preferred in determination of acylglycerols of olive oil because of its ease of sample handling and rich data generation (several metabolites in single spectrum) as an alternative to wet chemical methods (Dais & Spyros, 2007; Hatzakis, Agiomyriganaki, Kostidis, & Dais, 2011; Vlahov et al., 2010).

Three isomeric classes of DAGs (1,2-, 2,3-, and 1,3-) of extra virgin olive oils stored in different temperatures of 15 °C and 30 °C and time up to 12 months were evaluated in order to observe the effects of these parameters on DAGs content in a study (Cossignani et al., 2007). The results indicated that significant differences existed in the amounts of different DAG classes as well as the ratios between the classes. The samples inspected just after extraction possessed the highest contents in terms of percentage for 1,2-DAGs and the lowest for 1,3- and 2,3-DAGs. On the other hand, the samples kept at 30 °C had the highest content of 1,3 DAGs due to isomerization reaction favored mainly by temperature. Therefore, it was concluded that storage temperature was the most important factor on the DAGs content, and their isomerization provided information regarding the storage conditions as well as the preservation status of olive oils. In addition to the aforementioned parameters, other possible storage factors for the isomerization of DAGs in fresh olive oils were examined for 24 months (Caponio et al., 2013). The results showed that storage time was the significant factor in increasing amounts of 1,3-DAGs due to isomerization causing higher 1,3/1,2 ratio for oils. Besides, it was found that degree of isomerization was also affected by the initial hydrolysis level of the olive oil. However, storage conditions such as the bottle glass color, the light and the air had no effect on isomerization of DAGs except the speed of the reaction. Therefore, it was confirmed that the DAGs ratio could be used as a freshness index for extra virgin olive oil since concentrations of these compounds were not affected by either oil variety or storage conditions (glass color, light, and air) (Caponio et al., 2013). In a similar study (Ayyad et al., 2015), effects of different conditions of storage at 20 °C in darkness and in light, at 4–6 °C in light and at 20 °C in light with argon in the headspace were observed for 14 months. The results confirmed that not only the length of the storage time but also temperature had

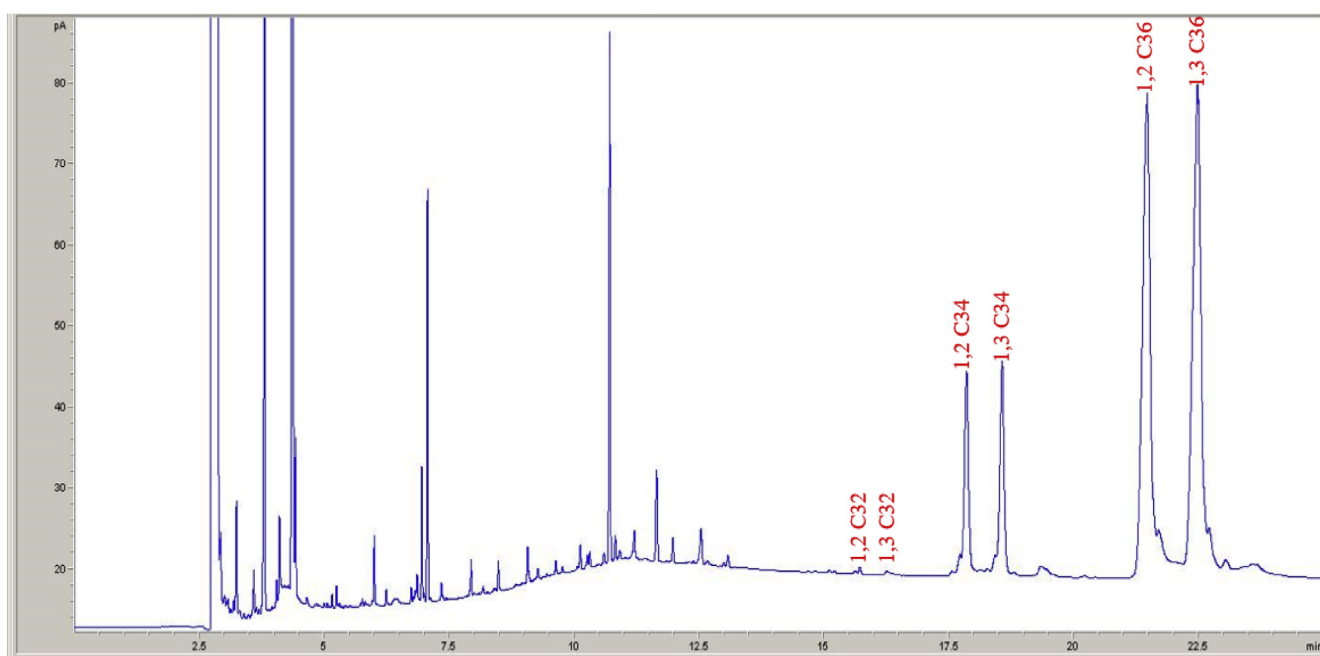


Fig. 2. Typical GC chromatogram of an olive oil showing individual DAGs peaks obtained by analysis according to ISO method (ISO, 2009a).

**Table 3**  
Examples of studies about the use of diacylglycerols in determination of authenticity and quality of olive oils.

Aim	Main Findings	Reference
Quality determination	The <i>D</i> value and total DAGs have potential to determine quality of virgin olive oils, commercial olive oils, refined olive oils, and pomace oils by using $^{31}\text{P}$ NMR	Fronimaki, Spyros, Christophoridou, and Dais (2002)
Quality determination	All types of DAGs content of olive oil could be detected with $^{19}\text{F}$ NMR and then could be used to monitor the quality of olive oil as well as ordinary edible oils	Zhou, Li, Weng, Fang, and Gu (2015)
Adulteration detection of olive oils with hazelnut oil	$^{31}\text{P}$ NMR was used for the quantification of minor compounds as phenolic compounds, DAGs, sterols, and free fatty acids. Detection limit was found at 5% for refined hazelnut oils in refined olive oils	Agiomyriganaki, Petrakis, and Dais (2010)
Adulteration detection of olive oils with seed oils	The detection of olive oil adulteration with various refined seed oils was accomplished by using the combination of $^1\text{H}$ NMR and $^{31}\text{P}$ NMR. Adulteration could be determined as low as 5% by using <i>D</i> value of fresh olive oils.	Vigli, Philippidis, Spyros, and Dais (2003)
Adulteration detection of olive oil with lampante and refined olive oil	High field $^{31}\text{P}$ NMR was used to detect the targeted adulterations at varying levels by determining 1,2-DAGs, 1,3-DAGs, total DAGs, <i>D</i> value, sterols and acidity. 5% was the limit of detection for both adulterant type (refined and lampante)	Fragaki, Spyros, Siragakis, Salivaras, and Dais (2005)
Adulteration detection of olive oil with refined olive oil and seed oil	$^{19}\text{F}$ NMR and $^1\text{H}$ NMR were compared to detect lower grade oil in olive oil with respect to DAGs content and $^{19}\text{F}$ NMR was suitable for detection of refined olive oil while $^1\text{H}$ NMR was suitable for seed oil for the same type of application	Jiang, Li, Chen, and Weng (2018)
Cultivar characterization	Geographical classification of Turkish and Slovenian extra virgin olive oils by $^1\text{H}$ NMR spectra was performed in terms of aldehydes, phenolic compounds, terpenes and DAGs as major discriminants	Özdemir et al. (2018)
Geographical characterization	Fatty acids, phenolics, DAGs, total free sterols, free acidity, and iodine number were used to determine geographical identity of olive oil samples up with 87% success rate by means of $^1\text{H}$ and $^{31}\text{P}$ NMR spectroscopy	Petrakis, Agiomyriganaki, Christophoridou, Spyros, and Dais (2008)
Effect of storage conditions	1,2-DAGs were found very promising to control overall olive oil quality and freshness as well as easily indicate any problems during the storage of the olive oil	Guillaume et al. (2014)

effects on isomerization of DAGs. Inert gas was not that effective in the protection of olive oils from isomerization under storage in the light. In another study (Salvo, Rotondo, La Torre, Cicero, & Dugo, 2017),  $^1\text{H}$  NMR spectroscopy was also used in monitoring of olive oil aging with respect to DAG content. The olive oils were stored in the dark and at room temperature for one year. It was already known that the isomerization rate was affected by the free fatty acidity, additionally it was proven that the presence of specific macromolecules (lipases) had effect on DAG content as well (Salvo et al., 2017).

The studies mentioned so far focused on the investigation of the changes in olive oil DAGs content with different parameters during storage. However, kinetic studies were also performed to correlate the age of olive oil with DAGs concentration (Dais & Spyros, 2007). Kinetics of DAG formation and isomerization in virgin olive oil were formulated in terms of *D* and free fatty acid values by using  $^{31}\text{P}$  NMR spectroscopy (Spyros, Philippidis, & Dais, 2004). Robust prediction models were obtained between actual and theoretical storage times up to 10–12 months (Spyros et al., 2004). In another study, a more comprehensive mathematical expression was established for the determination of shelf life of olive oils with respect to many parameters such as alkyl esters, volatiles and 1,2-DAGs etc. (Di Serio, Giansante, Di Loreto, & Di Giacinto, 2018). In a recent study, artificial intelligence derived system as adaptive neuro-fuzzy inference predicted the oxidative stability of virgin olive oil during storage as a function of time, temperature, DAGs as well as other well studied parameters (Arabameri et al., 2019). According to this study, minor constituents including DAGs were found as the most important factors influencing the preservation status and freshness of olive oils during storage. Furthermore, it was concluded that the changes in DAGs content could be a good indicator for olive oil oxidative stability. While the direct effect of DAGs concentration on olive oil sensory characteristics during storage was not observed, they are still essential in determination of aging. As a result of aging, degradation of various health promoting components of olive oil such as tocopherol and phenolic compounds were also observed which further decreased the nutritional and sensory characteristics by increasing rancidity (Dais & Spyros, 2007). Therefore, it becomes an important point to know the storage history of olive oil to be sure about its actual quality. Relation between DAG concentration and storage time could also mean that these compounds can be used in detection of adulteration of fresh olive oils with old oils.

In addition to their applicability in quality determination, DAGs are used as a tool in authenticity determination of olive oils. It is known that fresh extra virgin olive oil samples do not contain high amounts of total DAGs (1–3% mainly 1,2-DAGs) compared to lower quality olive oils such as refined olive oils (4–5% mainly 1,2-DAGs) and pomace olive oils (15–20% mainly 1,2-DAGs). Moreover, the isomerization from 1,2-DAGs to 1,3-DAGs results an immediate equilibrium state in refined olive oils (Dais & Spyros, 2007). In this respect, adulteration of virgin olive oil with deodorized oils was inspected with a study in which 1,2- and 1,3-DAG isomers in olive oil were determined with solid phase extraction followed by GC analysis (Pérez-Camino et al., 2001). The results indicated that the relationship between acidity and total DAGs were not an efficient indicator for the genuineness of olive oils. While the 1,3-/1,2-DAGs ratio was found useful in authentication of virgin olive oils as well as in determining the oil aging and evaluating the storage conditions (Pérez-Camino et al., 2001). Therefore, the studies on olive oil authenticity have been focused on the ratio of DAGs as *D* value rather than total content of these compounds. However, the increase of 1,3-DAG could be also due to the long storage of olive oil. Therefore, any change in *D* value may not necessarily be a sign of adulteration (Dais & Hatzakis, 2013). Aforementioned studies deal with only DAGs and their derivatives. However, NMR metabolic profiling which quantifies DAGs as well as many other parameters simultaneously and NMR fingerprinting were also proposed as efficient tools in adulteration detection of olive oil. In the literature, there are various studies which used NMR spectroscopy to identify DAGs content as well as other important authenticity parameters for the determination of olive oil adulteration as shown in detail in Table 3. In general, DAGs were regarded as quality parameters to grade olive oil. However, the methodological approach based on investigation of many physico-chemical parameters together as in the previous examples was also valid for the classification studies of olive oil with respect PDO and variety in terms of their DAGs contents. There are several examples of the use of DAGs content in classification and/or differentiation as well as adulteration and quality determination of olive oils (Table 3).

More recently, authentication studies have been investigating monochloropropanediol esters (MCPDE) as (2- and 3-MCPD) and glycidyl esters (GEs) presence in olive oils and in other vegetable oils (Kamikata et al., 2019; Yan et al., 2018). MCPDEs and GEs are the minor compounds derived from DAGs and MAGs, respectively through

refining processes (Yan et al., 2018). These compounds are formed during the deodorization step of the refining process and they are also known as heat-induced contaminants. MCPDEs and GEs could be used as indicators of extra virgin olive oil adulteration with refined oils since these compounds were not expected to be present in the extra virgin olive oil produced without any chemical treatment from healthy olive fruits (Kamikata et al., 2019). Besides temperature, pressure, water activity and other processing parameters also speed up the formation of 3-MCPD esters (Weiβhaar, 2008; Yan et al., 2018). In a recent study, it was found that these processing derived contaminants could be used to detect lower grade oils in olive oil in varying limits of detection as 2% when using 3-MCPD esters, 5% for 2-MCPD esters, and 13–14% for GEs (Yan et al., 2018). Especially, quantification of MCPDEs were found to be promising with lower limit of detection compared to GEs. In another study, potential of these compounds as an adulteration detection tool was also emphasized (Kamikata et al., 2019). Determination of these compounds are important not only for adulteration studies but also for the health concerns. It was reported that after consumption of highly contaminated foods with these derivatives gastrointestinal tract can easily convert these compounds to their free forms which are known to have toxicological effects on human (Nguyen & Fromberg, 2020).

### 3.3. Color pigments and derivatives

The color of a virgin olive oil is attributed to the lipophilic chlorophyll and carotenoid pigments present in the olive fruit (Montealegre et al., 2010). Green olives having high chlorophyll content give greenish color to the oils whereas mature olives yield yellowish oils due to their higher carotenoid content. As a result, combination and the proportions of these pigments determine the ultimate color of the olive oils (Lazzerini, Cifelli, & Domenici, 2016). Olive oils contain comparably rich variety of carotenoids ( $\beta$ -carotene, lutein, violaxanthin, neoxanthin and other xanthophylls) and chlorophyll derivatives (chlorophyll *a* and *b*, pheophytin *a* and *b*, and other minor derivatives) (Lazzerini & Domenici, 2017). The level of these pigments in olive oil could go up to almost 100 ppm. The major pigments were reported as pheophytin *a* (up to 25 ppm), followed by  $\beta$ -carotene (up to 15 ppm) and lutein (up to 10 ppm) (Lazzerini et al., 2016); however, amounts may differ depending on various factors. The main factors affecting the concentration of each pigment found in olive oils are highly correlated with the physiochemical characteristics of olive fruits and they rely on botanical as well as geographical origin, environmental conditions (climate and/or irrigation) and also extraction process (mainly malaxation). In addition, the storage conditions of olive oil are also important factors in pigment type and concentration (Gandul-Rojas, Roca, & Gallardo-Guerrero, 2016; Lazzerini, Cifelli, & Domenici, 2017; Lazzerini & Domenici, 2017; Lazzerini et al., 2016).

In the literature, the pigments have been identified mostly by chromatographic techniques and most successfully by HPLC coupled with diode array (DAD), UV–Vis as well as other types of detectors (Lazzerini et al., 2016; Mínguez-Mosquera, Gandul-Rojas, & Gallardo-Guerrero, 1992; Seppanen, Rahmani, & Csallany, 2003). A sample HPLC chromatogram of olive oil pigments is shown in Fig. 3. In addition, total pigment contents of olive oils have been evaluated in terms of chlorophylls at 470 nm and carotenoids at 670 nm after dilution with proper solvent by UV–vis spectrophotometer (Cerretani, Motilva, Romero, Bendini, & Lercker, 2008; Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sánchez, Gómez, & Garrido-Fernández, 1991; Reboredo-Rodríguez et al., 2016).

In the recent years, other spectroscopic techniques are also becoming alternatives to the HPLC and UV–vis spectroscopic methods used in quantification of individual (Domenici et al., 2014) and total pigments of olive oil (Cayuela, Yousfi, Martínez, & García, 2014), respectively. Direct analysis of olive oils with UV–Vis–NIR spectroscopy was found promising compared to timely and waste producing reference analysis of total chlorophylls and carotenoids (Cayuela et al.,

2014). Absorption spectra in the near UV–vis region were mathematically treated by Ayuso, Haro, and Escolar (2004) to reveal its potential uses in color characterization. Then, suitability of near-UV-vis region for the determination of major pigments of olive oils as two carotenoids (lutein and  $\beta$ -carotene) and two chlorophylls (pheophytin *a* and *b*) was proposed in another study (Domenici et al., 2014). This finding was also confirmed with an investigation in which pigment contents of Mediterranean olive oils obtained from UV–vis spectroscopy and HPLC-DAD measurements were compared with similar success (Lazzerini et al., 2017). Moreover, a very recent study (Borello & Domenici, 2019) compared two different approaches for determining olive oil pigments using the near UV–Vis spectroscopy. First method was the standard method (Mínguez Mosquera et al., 1991) based on absorption spectra at single wavelengths (470 and 670 nm) while mathematical deconvolution of the absorption spectra developed in a previous study (Domenici et al., 2014) was the other approach used in the same type of application. The results indicated that overall approach used in standard method was not as effective as newly proposed method in determination of total carotenoids' and chlorophylls' derivatives in olive oils due to the fact that standard method underestimates the contents of both carotenoids and the chlorophyll derivatives compared to whole spectrum (Borello & Domenici, 2019). In another study, use of UV–vis spectroscopy in the whole range of 200–800 nm was found promising in prediction of detailed pigment profile of olive oils compared to FTIR spectroscopy since pigment profile is highly correlated with UV–vis absorption profile (Uncu et al., 2019). Fluorescence spectroscopy was also used in determination of major pigments (chlorophylls *a* and *b* and pheophytins *a* and *b*) of olive oils (Galeano Díaz, Durán Merás, Correa, Roldán, & Rodríguez Cáceres, 2003). Ultra-fast high-performance liquid chromatography with fluorescence excitation–emission detection was the method of choice for the quantification of these pigments directly without previous sample treatment (Lozano, Muñoz de la Peña, Durán-Merás, Espinosa Mansilla, & Escandar, 2013).

Measurement of some pigment compounds has been proposed as a way of determining the quality and adulteration of olive oils (Tena et al., 2015). They are regarded as quality tools due to their relationship with freshness, nutritional and antioxidant properties of olive oils (Lazzerini et al., 2017). Natural color pigments have also been used in authentication of olive oils (Lazzerini et al., 2016). According to one of the studies using chlorophyll and carotenoid pigments of virgin olive oils as authenticity and quality index, total chlorophylls to total carotenoids ratio should be around 1 and also the ratio of minor carotenoids to lutein should be around 0.5 to indicate the authenticity of olive oils (Gandul-Rojas, Cepero, & Mínguez-Mosquera, 2000). Moreover, it was concluded that these thresholds were valid for olive oils in general regardless of fruit variety. In addition, certain pigments such as the percentages of lutein, violaxanthin, and total pigment contents could be used as discriminatory tools for monovarietal virgin olive oils (Gandul-Rojas et al., 2000). Some pigment fractions such as chlorophylls/carotenoids, minor carotenoids/lutein, and percentages of violaxanthin and lutein along with total pigment content were found stable during one year of storage irrespective of the variety and degree of ripeness of the olive fruit (Roca, Gandul-Rojas, Gallardo-Guerrero, & Mínguez-Mosquera, 2003). It was determined that degradation of chlorophylls as a result of pheophytinization reaction started from malaxation step of oil extraction and increased during storage (Aparicio-Ruiz, Aparicio, & García-González, 2014). The chlorophylls *a* and *b* being naturally present in the olive fruit are irreversibly converted into more stable pigments (pheophytins *a* and *b*, orderly) as the central Mg<sup>+2</sup> ion of the porphyrin ring is replaced by two hydrogen atoms, and further to pyropheophytins (PPPs). PPPs are the ultimate degradation products of chlorophyll by the removal of the carboxymethyl group from the pheophytins (García et al., 2013; Giuliani, Cerretani, & Cichelli, 2011). Formation of chlorophyll *a* derivatives (pheophytin *a* and pyropheophytin *a* (PPP *a*)) in small amounts were identified as an indication of oil storage (Roca et al., 2003). This finding

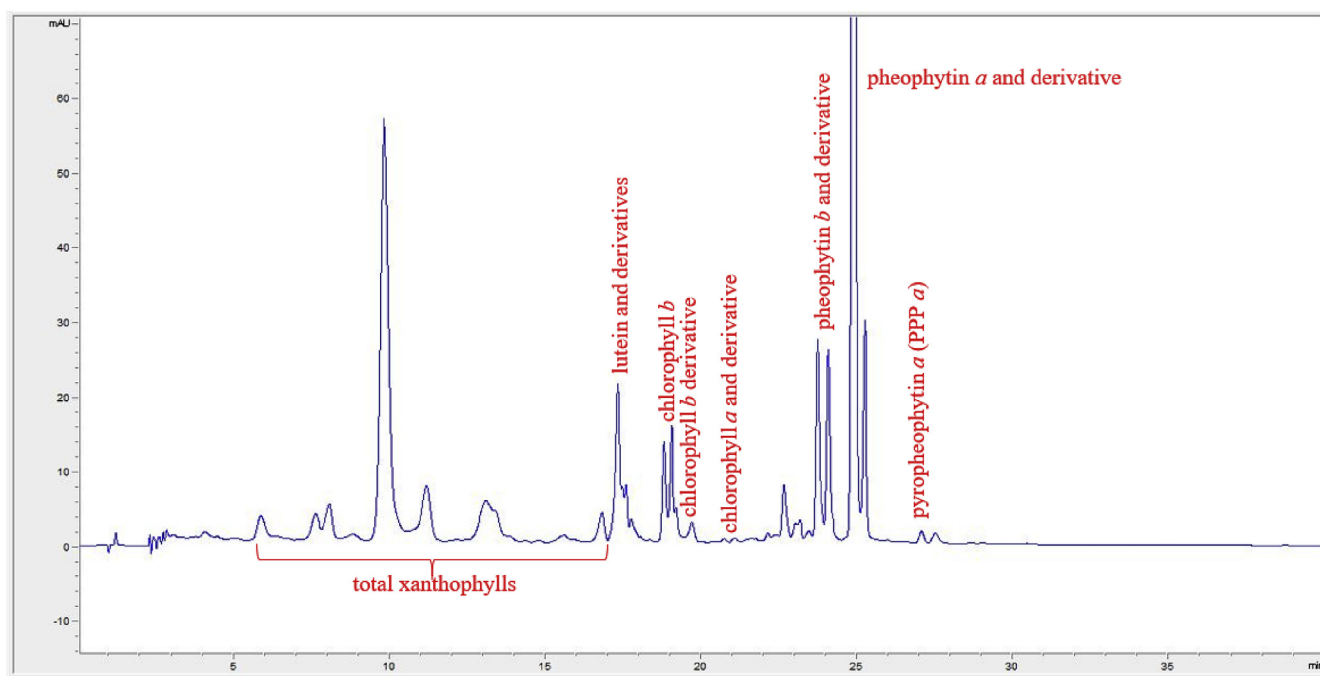


Fig. 3. Pigment chromatogram of an olive oil sample obtained with HPLC analysis described in the literature (Mateos & García-Mesa, 2006).

was also confirmed in another study in which increasing amounts of PPP *an* as a new compound was observed during the storage (Gallardo-Guerrero, Gandul-Rojas, Roca, & Mínguez-Mosquera, 2005) whereas none or trace amounts existed in fresh olive oils (Anniva, Grigoriadou, Psomiadou, & Tsimidou, 2006). It was also indicated that temperature was a significant factor favoring the formation of PPPs. Thus, the content and proportion of PPP *a* in terms of ratio between pheophytin *a* (the precursor pigment) to PPP *a* could indicate the storage conditions of the olive oils (Gallardo-Guerrero et al., 2005). The effect of thermal abuse and lengthy storage on PPP formation was also determined in a different study (Anniva et al., 2006). Thermal degradation kinetics of carotenoids as well as chlorophylls were analyzed in detail in several studies (Aparicio-Ruiz & Gandul-Rojas, 2012; Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2010; Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2011). Decoloration kinetics of chlorophylls and carotenoids in virgin olive oil triggered by autoxidation were examined under varying time and temperature. The results indicated that chlorophylls were more stable to heat treatment due to the requirement of higher activation energy compared to carotenoids. Additionally, it was concluded that obtained kinetic models could be used to construct a mathematical model to predict the decoloration of chlorophyll and carotenoids pigments in olive oil in terms of time and temperature (Aparicio-Ruiz & Gandul-Rojas, 2014). In addition, chemical changes in thermoxidized virgin olive oil with respect to various parameters including pigments were monitored by fluorescence spectroscopy (Tena, Aparicio, & García-González, 2012). Photooxidation reaction of pigments especially chlorophyll was followed effectively through UV-visible spectroscopy in combination with artificial neural networks (Torrecilla, Vidal, Aroca-Santos, Wang, & Cancilla, 2015). In another study, effect of light exposure on functional compounds of olive oil such as vitamin E and chlorophyll was evaluated successfully by fluorescence spectroscopy (Díaz, Pega, Primrose, Sancho, & Nanni, 2019). These studies were based on investigating the effects of various storage conditions and time on the quality of olive oils similar to a study of Guillaume, Gertz, and Ravetti (2014). Effects of different factors such as environment, cultivar, storage conditions as well as time on several physico-chemical parameters including PPP were determined. The results showed that PPP *a* and 1,2-DAGs were good indicators for overall olive oil quality and freshness as well as storage history (Guillaume

et al., 2014). Recently, shelf life prediction was also investigated by using induction time, 1,2-DAGs, PPPs, and free fatty acids of olive oils (Guillaume & Ravetti, 2016).

The method for determination of the degradation products of the chlorophyll *a* (pheophytin *a*, *a'* and PPP) in olive oil was officially described by the German Society for Fat Science (Gertz & Fiebig, 2006b). The method was based on HPLC analysis with UV detector measurement after solid phase extraction of the olive oil samples and it was then adopted by the International Standards Organization (ISO, 2009b) as a quality measurement method (Li, Woodman, & Wang, 2015). PPPs content, ultimate degradation product of chlorophyll *a*, was calculated as ratio of PPP *a* to PPP *a* + pheophytin *a* + *a'* in terms of percentage with a limit up to 17% to grade an olive oil as extra virgin in official regulations. After official recognition of the PPPs content by some official bodies (Table 1), rapid determination of pigment composition has become more important. An alternative method based on HPLC analysis with fluorescence detection which is comparably less in cost and time was proposed (Li et al., 2015). In addition, amount of PPP *a* formed in olive oil during storage was tried to be predicted with promising results using a mathematical expression (Aparicio-Ruiz, Roca, & Gandul-Rojas, 2012). Prediction of extra virgin olive oil freshness correlated with PPPs content during storage was successfully accomplished using fluorescence spectroscopy (Aparicio Ruiz et al., 2017b). As a result, effectiveness of PPPs in shelf life determination was indicated. In addition, PPPs were recently proposed as adulteration determination criteria along with FAAEs, volatiles, and phenols for olive oils passing through deodorization process (Aparicio-Ruiz et al., 2017a).

Authentication of olive oils with respect to variety and geographical origin was also investigated in olive oil studies. Pigment content was useful in this type of applications because genetic as well as environmental conditions have significant effects on pigment content (Montealegre et al., 2010). In addition, it was found out that pigments could be correlated to other factors such as ripeness stage, geographic origin and cultivars (Lazzerini et al., 2017). Varietal characterization and differentiation of olive oil was performed by determining the content of some chlorophyll and carotenoid compounds (Cichelli & Pertesana, 2004). Discrimination based on harvest year was also accomplished by using main pigments of Italian olive oils (Lazzerini & Domenici, 2017). Furthermore, there is a trend of combining total



**Table 4**  
Recent studies in quality and authenticity determination of olive oils by using pigment content.

Aim	Main Findings	Reference
Photo and thermal-oxidation determination	Excitation-emission matrices of olive oil samples correlated to polyphenols, and chlorophyll and derivatives. All could discriminate non-irradiated and irradiated as well as non-heated and heated samples	Manzano, de la Peña, and Merás (2019)
Differentiation of virgin olive oils from a specific mill	Concentration of some key isoprenoids and color compounds ( $\beta$ - carotene, lutein, and pheophytin <i>a</i> ) could achieve differentiation (88%) between olive oils from a specific mill and other mills	Mapelli-Brahm, Hernanz-Vila, Stinco, Heredia, and Meléndez-Martínez (2018)
Quantification of binary and ternary mixtures of monovarietal olive oils	The artificial neural networks applied to visible spectroscopic data could be used to determine varietal quantifications based on the pigment profile of monovarietal extra virgin olive oils at 10% and 2.8% for the linear and non-linear models, respectively	Aroca-Santosa, Cancilla, Pérez-Pérez, Moral, and Torrecilla (2016)
Detection of possible fraud markers	Ultra-high-performance liquid chromatography tandem mass spectrometry coupled with two types of atmospheric pressure ionizations (chemical and photoionization) were found efficient in determining natural color pigments (carotenoids, chlorophylls and chlorophyll derivatives) as well as artificial ones (E141i) in olive oils. These methods were applicable in pigment profile identification as well as the detection of possible exogenous adulterants	Arrizabalaga-Larrañaga, Rodríguez, Medina, Santos, and Moyano (2019)
Cultivar differentiation	Five different Greek olive oil cultivars were successfully characterized and classified based on acidity, total chlorophylls and carotenoids, myristic, margaric, stearic, arachidic, and eicosenoic acids at a rate of 91.9% and 81.1% by using original and cross-validation methods, respectively	Karabagias et al. (2019)
Geographical differentiation	Geographical discrimination power of several chemical parameters (total phenol content, fatty acid and phenol profile, total carotene and chlorophyll content and oxidative stability) and mid-infrared spectroscopy on olive oils was investigated. It was found that combination of chemical parameters was better than mid-IR spectroscopy in classification of monovarietal olive oil obtained from geographically close regions of Turkey	Uncu and Ozen (2016)
Cultivar discrimination	Monovarietal extra virgin olive oils could be classified up to 94.4% according to their variety by using Raman spectroscopy highly correlated with both carotenoid and fatty acid composition of olive oils. Also, distinct Raman spectral bands could be used for the prediction of major fatty acids as well as lutein/ $\beta$ -carotene content ratio both known as quality parameters for olive oils	Portarena et al. (2019)
Geographical discrimination	Olive oil samples from PDO production areas of coastal Italy were analyzed in terms of their isotopic composition and carotenoid content with isotope ratio mass spectrometry and resonant Raman spectroscopy, respectively. The combination of isotopic and carotenoid data yielded a promising result with correct classification of 82% of olive oil samples with respect to geographical origin	Portarena, Baldacchini, and Brugnoli (2017)
Authentication	Detailed pigment profile including major and minor color compounds as well as their derivatives were successful in authentication of olive oils with respect to harvest year and geographical origin. On the other hand, UV-visible and FTIR spectroscopic techniques were reliable alternatives for the same purposes with the higher discriminatory power of FTIR alone and in combination	Uncu, Ozen, and Tokatli (2020)

chlorophyll and carotenoid contents with other chemical parameters for geographical and/or varietal classification instead of using only the pigment profile (Karabagias, Badeka, Casiello, Longobardi, & Kontominas, 2019; Karabagias et al., 2013; Taamalli, Gómez-Caravaca, Zarrouk, Segura-Carretero, & Fernández-Gutiérrez, 2010). It could be very hard to characterize an olive oil with a unique compositional marker by knowing that compositions of these markers are easily affected by the environmental conditions, the fruit ripening, and the extraction technology (Montealegre et al., 2010). Therefore, bringing together different markers to obtain the discriminatory information as much as possible by using chemometric tools could provide better results (Montealegre et al., 2010).

Pigment content of olive oil could also be susceptible to the alterations and frauds (Lazzerini et al., 2016). Illegal addition of artificial pigments to olive oil to prevent any color loss due to refining is still a common adulteration method. European regulations do not allow the addition of colorants to any oils and/or fats from animal or vegetable origin (Roca, Gallardo-Guerrero, Mínguez-Mosquera, & Gandul Rojas, 2010). Therefore, if any artificial color is detected in the olive oil this situation is considered as an adulteration. As a greenish colorant, copper complexes of chlorophyll known as E-141i, are obtained by solvent extraction from plant sources. The additive E-141i is produced by the addition of  $\text{Cu}^{+2}$  salts to the pigments in which the inner metal ion  $\text{Mg}^{+2}$  is replaced with the more stable  $\text{Cu}^{+2}$  causing the formation of copper-chlorophyll derivatives and it has been mostly used in the olive oil frauds due to its stable color characteristics during the

processing and storage (Gandul-Rojas et al., 2016; Lazzerini et al., 2016; Roca et al., 2010). The adulteration studies about color pigments in olive oils showed that Cu-pyropheophytin *a* was the major component among copper-chlorophyll derivatives (Gandul-Rojas et al., 2016). Naturally, almost none of these derivatives exist in olive oils; therefore, detection of the presence of any of these compounds reveals the adulteration of the oil (Gandul-Rojas et al., 2016). Several techniques are available to determine Cu-chlorophyll derivatives in olive oil and the majority of these methods are based on HPLC analysis with different detector systems (Fang et al., 2015; Roca et al., 2010). Capillary electrophoresis was also used for the same type of application (Del Giovine & Fabietti, 2005). Recently, some alternative techniques such as Raman spectroscopy (Lian et al., 2015) and other spectrophotometric measurements (Wang, Hou, & Hsieh, 2018) were also used to determine these compounds in a fast way without harming the environment. Other examples of recent application of pigments usage in olive oil authenticity and/or quality determination are presented in Table 4.

#### 4. Conclusion and future trends

In this review, several minor compounds of olive oils as pigments (including derivatives such as PPPs), DAGs with derivatives (comprising MCPDEs and GEs), and FAAEs were summarized through their latest applications in the olive oil field. FAAEs could provide detection of mildly refined olive oils in high quality olive oils if the adulterant initially has high FAAE content. DAGs, on the other hand, are effective

as freshness indicators for olive oil. MCPDEs and GEs as the derivatives of DAGs could be used in determination olive oil adulteration with softly deodorized oils. Pigment contents alone or in combination with other physicochemical parameters are generally successful in geographical and/or botanical origin determination. The weaknesses of these minor components as quality and authentication indicators can be compensated by using several parameters together which could increase the discrimination power and further research on the use of combined parameters could provide solutions to complex authentication problems.

Some of the minor components reviewed were not officially regulated by internationally recognized organizations such as IOC and EU whereas they are considered as quality and authenticity parameters by other countries. In order to cover all the aspects of olive oil identity, it could be a good starting point to adapt voluntary standards and encourage alternative environmentally friendly methods to analyze these standards.

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