

# A Short Review on Infrared Spectroscopy for Functional Compounds Evaluation in Olive Oil

R.RamaDevi

Department of chemistry ,Santhiram Engineering college Nandyal.

## Abstract

Olive oil is a liquid fat obtained from the fruit of *Olea europaea*, a plant belonging to the *Oleaceae* family, which is widely cultivated and diffused in the Mediterranean area. It is largely produced and used since antiquity. It is mainly used and consumed as food but also as key ingredient in a wide variety of cosmetic products, e.g., to moisturize and nourish dry skin. In the last few decades, olive oil has received much attention as compared to the other seed-obtained oils as well as to the animal fats due to many functional compounds with positive effects on health. To maintain the genuine picture of olive oil, it is essential to assure its authenticity and quality. The presence of bioactive compounds, which characterize the olive oil owing to their antioxidant properties, can be assessed by spectroscopic and chromatographic methods. Currently, spectroscopic techniques combined with chemometric data analysis represent one of the most promising detection methods in the food sector. They offer rapid, versatile, and inexpensive data collection and analyses. The main advantages include the limited and simple sample preparation and the possibility to get spectra directly from the production line. Infrared spectroscopy (mid- and near-infrared) coupled to chemometrics is considered as powerful, fast, accurate, and nondestructive analytical tool for rapid and precise determination of the bioactive compounds content, as well of their bioactivities, i.e., antioxidant properties. These techniques represent a valid alternative to the existing conventional methods of analysis, e.g., based on chromatography and mass spectrometry. Indeed, the present review focuses on the application of infrared spectroscopy for functional compounds evaluation in olive oil.

## 1. Introduction

Olive oil is a high-quality natural product, typical from the Mediterranean area, where it has been produced for millennia, and it is obtained from the fruits of *Olea europaea* L, [1–4]. The quality of the starting matrix, i.e., the olives, is a crucial parameter to obtain a high-quality product [5]. The quality can be affected, for example, by fungal occurrence and mycotoxin contamination. In particular, the contamination with microfungi capable of producing secondary metabolites (mycotoxins) that potentially affect health is a growing source of concern [6–12]. Consequently, considerable research interest towards more environmentally compatible, field-deployable, and sustainable fungi control systems was prompted over the last couple of years [13–15].

The process of extracting the oil from the seeds is a crucial step as well. There are various methods for this purpose and they can give different yield and quality grades [16]. As an example, extra-virgin olive oil (EVOO) is obtained using mechanical pressure devices. The lower-grade products are typically obtained from a variety of extraction methods including high-pressure extraction, solvent extraction, heat treatment, esterification, and refining. The lower-grade olive oils (e.g., lampante olive oil, pomace oil, refined pomace oil, and refined olive oil) are often characterized by a comparatively high acidity. For instance, according to the current regulations, the acidity of extra-virgin olive oil must not exceed 0.8%, while olive oil and lampante olive oil contain 2% and >2%, respectively. Refining processes lower the acidity to about 0.3%. In blends of virgin and refined olive oils the free acidity is typically about 1%. Olive pomace oil obtained by extraction with a solvent also has acidity around 1%, while the refined olive pomace oil can have an acidity of only 0.3%.

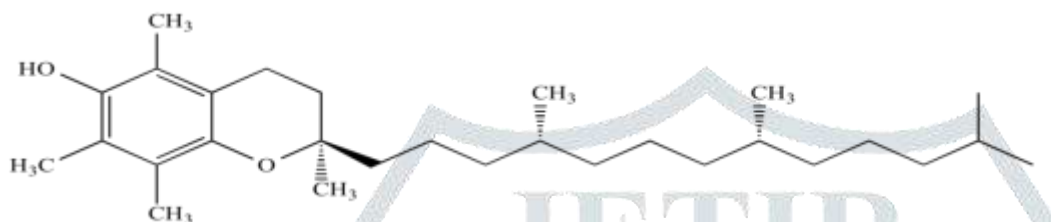
EEC Regulation 29/2012 determines the compulsory and optional information to include on olive oil labels, supplementing the general rules relating to foodstuffs and their marketing in EEC Regulation 2000/2013. The chemical composition of olive oil is characterized mainly by triacylglycerols, monoacylglycerols, diacylglycerols, and free fatty acids [17, 18], as well as fatty acid derivatives (i.e., phospholipids and waxes) [19, 20]. Moreover, there is a diverse group of compounds such as sterols, aliphatic alcohols, chlorophylls, carotenoids, and hydrocarbons [21–23]. The bioactive compounds in olive oil play an imperative role in the authentication and genuineness, quality and purity, traceability, and more importantly to the health point of view [24–33].

Olive oil is a highly interesting product from the nutraceutical perspective [34–42]. It exhibits sensory properties due to the presence of substances with functional activity, such as carotenoids, chlorophylls, tocopherols, and phenolic compounds. These substances are often missing in other common seed-based oils [43]. Moreover, olive oil is characterized by high levels of natural antioxidants, which are associated to potential beneficial properties to humans [44–49]. Phenolic compounds are the main bioactive compounds in olive oil and can be divided into lipophilic and hydrophilic phenols. As reported by El Riachy et al. [50], lipophilic phenols such as tocopherols [51] can be commonly found in other vegetable oils. On the contrary, most hydrophilic phenols found in olive oil are exclusive and/or characteristic of the *Olea europaea* species giving rise to a chemotaxonomic peculiarity [52].

A recent work by Garcia and co-workers [53] divided the phenolic compounds with biological activity into four groups: simple phenols, secoiridoid derivatives, lignans, and flavones. Oleuropein, hydroxytyrosol, tyrosol, and ligstroside are examples of specific phenols, which are usually present in olive oil. In addition, caffeic acid, vanillic acid, and syringic acid are characteristic components of olive oil [54, 55]. The phenolic composition and its total concentration in olive oil have an impact on the characteristic taste, which is bitter and pungent. It depends on different factors, mainly on the degree of ripening of the fruit, the cultivar, the

geographic origin [56–61], and the processing [62, 63]. Phenolic compounds are important antioxidants and act as radical scavengers in biological systems, consequently diminishing the lipid oxidation rate [64]. Phenolic compounds are also responsible for the olive oil stability during storage because of their antioxidant activity. With reference to the tocopherols in olive oil,  $\alpha$ -tocopherol is the major constituent while  $\beta$ - and  $\gamma$ -tocopherols are present in minor quantities [65, 66]. Figure 1 shows the chemical structure of  $\alpha$ -tocopherol.

**Figure 1** The structure of  $\alpha$ -tocopherol.



The greenish color of virgin olive oil is due to the presence of chlorophyll pigments. Amongst the chlorophyll compounds, pheophytin A is found in high amounts in olive oils. On the contrary, lutein and  $\beta$ -carotene are the major “yellow” coloring pigments responsible for the yellowish color of virgin olive oil. Due to the antioxidant nature and pro-oxidant activity of carotenoids and chlorophylls, respectively, they both play a crucial role in the oxidative stability during storage [67]. Moreover, phenols and tocopherols, together with carotenoids, provide excellent oxidative stability to olive oils and have a synergic anticarcinogenic and antioxidant activity.

The main lipid components of olive oil are unsaturated fatty acids such as mono-(oleic) and di-glycerides (linoleic acid). Both are bioactive compounds and play a major role in human health [68]. Besides this, triterpene squalene is also considered as a functional compound in the unsaponifiable fraction of olive oil. The content of such functional compounds is contributing to or even determining the quality and positive sensory properties especially in extra-virgin olive oil.

Many instrumental analytical methods have been developed for the determination of functional compounds in olive oil, mainly using mass spectrometry and chromatography. However, most of these methods are expensive, time-consuming, nonenvironmentally friendly and also may require highly qualified staff for the instrumental analyses. On the contrary, spectroscopic methods such as ultraviolet, visible, near-infrared, and mid-infrared spectroscopy have been successfully employed as rapid, nondestructive, real-time techniques capable of on/offline monitoring of various quality parameters of olive oil. Their benefits include minimum sample preparation and good measurement accuracy with less or no waste generation. This review article

presents an updated overview of the infrared spectroscopic methods in combination with chemometric data analysis to determine the functional compounds in olive oil.

## 2. Basics of Infrared Spectroscopy (Theory and Instrumentation)

### 2.1. Theoretical Principles

Infrared (IR) and near-infrared (NIR) spectroscopy are based on the absorption of photons by molecules. Absorption can take place when the energy of the photon to be absorbed matches the energy difference between two molecular rotovibrational energy levels. The mid-infrared spectral range is normally specified as  $2.5\text{--}50\ \mu\text{m}$  ( $200\text{--}4000\ \text{cm}^{-1}$ ), where the fundamental vibrational modes are located. On the contrary, the near-infrared covers the range  $0.75\text{--}2.5\ \mu\text{m}$  ( $4000\ \text{to}\ 13333\ \text{cm}^{-1}$ ) containing overtone and combination bands. A general requirement for allowing the absorption of a photon is that an oscillating dipole moment be produced during the vibrational motion of the molecule. This prerequisite can be expressed mathematically by the following equation: where  $\mu$  is the dipole moment and  $q$  is the normal coordinate. When this equation is fulfilled, the molecule or a specific vibrational mode is called IR-active.

The observed absorbance  $A$ , i.e., negative logarithmic transmission (intensity  $I$  of the light transmitted divided by the incident intensity  $I_0$ ) of a sample of thickness  $d$  is given by the Beer–Lambert law: with the extinction coefficient  $\varepsilon$ , which depends on the wavelength  $\lambda$ , and the concentration  $c$  of absorbing molecules. The IR spectrum eventually comprises the individual contributions from all the different vibrations originating from all the molecules in the sample. Consequently, it represents a true molecular fingerprint [69–71].

### 2.2. Instrumentation

As mentioned above, IR and NIR spectroscopy are absorption-based methods and hence the radiation needs to match the energy difference of the molecular transitions of interest. In other words, sources and detectors in the mid- and near-infrared are required. In the near-IR, Xenon and tungsten lamps are typically used as light sources and the detectors are commonly based on indium gallium arsenide (InGaAs) semiconductor materials. This allows us to perform spectroscopic analysis at wavelengths up to about  $2.5\ \mu\text{m}$ . The common silicon-based detectors that can be found in digital cameras are not suitable at wavelengths longer than  $1.1\ \mu\text{m}$ . Typical sources of mid-infrared radiation are glowing black-body radiators. Thermal detectors as well as semiconductors based on MCT and  $\text{LiTaO}_3$  are suitable for detecting the radiation.

Spectral dispersion of the signal is normally achieved by an interferometric analysis employing a Michelson interferometer. The obtained interferogram is then Fourier transformed to yield the spectrum in the frequency domain giving rise to the common name Fourier-transform infrared (FTIR) spectroscopy. Most commercial instruments are FTIR spectrometers.

There is a variety of approaches to achieve an interaction between the radiation and the sample. The most common method is the transmission measurement. The sample is placed in-between two transmissive plates, and the transmitted intensity in the infrared region is recorded as a function of wavelength. Choosing the appropriate sample thickness, however, is crucial in this approach. A very thick sample will result in strong lines being saturated since almost all the photons around the peak center wavelength are absorbed. Such peaks manifest as broadened bands without the typical Gaussian or Lorentzian shape. This may affect the data evaluation. For liquids, typical sample thicknesses lie in the range 25–500  $\mu\text{m}$ . Therefore, it is needless to say that the preparation of the sample is crucial as even dust particles may have significant influence. Common window materials include KBr,  $\text{CaF}_2$ , ZnSe, and NaCl, which are transmissive for the IR radiation. Conventional borosilicate or fused silica quartz glasses cannot be employed.

The method referred to as attenuated total reflection (ATR) FTIR spectroscopy is easier to use. In such an experiment, the IR radiation is propagating in a transmissive high-refractive-index material (often ZnSe or a diamond crystal), called the internal reflection element (IRE). The sample has a lower refractive index and is in contact with the surface of the crystal. The radiation undergoes total internal reflection at the angle  $\alpha$  at this surface, so that the evanescent field can interact with the sample. As a consequence, the reflected beam is attenuated and carries the spectroscopic information.

Both approaches are illustrated schematically in Figure 2. An advantage of the transmission arrangement lays in the well-defined sample thickness, which is given simply by the distance between the plates where the sample is placed. On the opposite, in ATR experiments, the effective path length depends on the penetration depth,  $d_p$ , of the evanescent wave. This is a complex function of the wavelength and must be taken into account when ATR FTIR spectra are interpreted quantitatively [72]. The penetration depth is approximately 1/5 of the wavelength and thus consists on order of a few micrometers in the mid-infrared wavelength spectrum. The resulting effective path length,  $d_{\text{eff}}$ , is usually the actual parameter of interest. It represents the corresponding sample thickness that would lead to the same absorption in a transmission experiment [73]. It is not a physical quantity and therefore it cannot be determined in a straightforward manner [72–74].

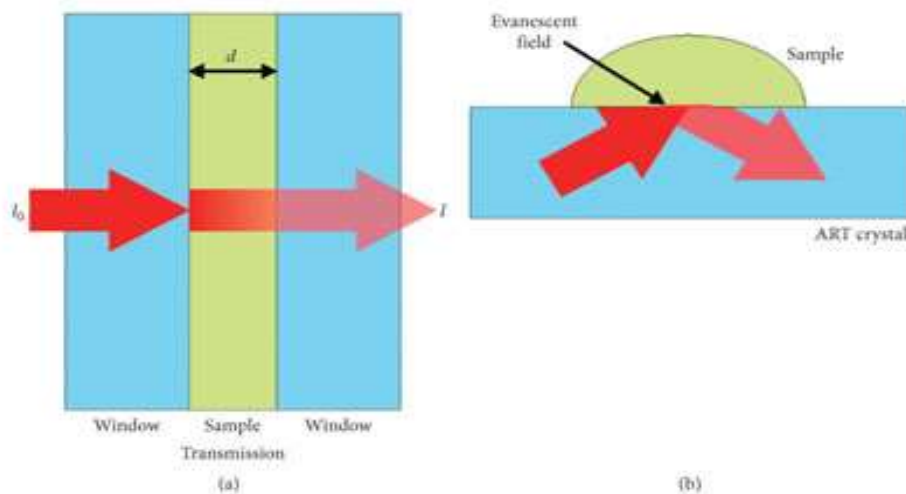


FIGURE 2: Schematic concepts of transmission and ATR spectroscopy. (a) Transmission. (b) ATR.

In the analysis of food and food products like olive oil, the ATR approach is highly beneficial since it does not require sample preparation and hence the measurements can be performed very quickly. The measurement time per sample is typically significantly below one minute. The data analysis depends on the information desired. Chemometric approaches are the methods of choice when the aim is to quantify constituents such as unsaturated fatty acids or other bioactive compounds. Cross-correlation techniques can be applied to compare the recorded spectrum with a library in order to find out whether or not the sample is genuine. This is often sufficient in order to identify adulterated and counterfeit products. Example applications will be discussed in more detail in the following. In order to give an impression, the FTIR spectra of 17 different olive oil samples from a local supermarket in Bremen, Germany, are displayed in Figure 3. The individual spectra are very similar to each other. This highlights the potential of FTIR spectroscopy for the authentication of olive oils. The most prominent signature at  $1745\text{ cm}^{-1}$  is the characteristic C=O stretching vibration, which can be assigned to the fatty acids and ester groups.

### 3. Application of Infrared Spectroscopy to Olive Oil: Focus on Functional Compounds Evaluation

For many years, analytical methods such as TLC, HPLC, GC-MS, and LC-MS/MS were the gold standard to identify and quantify the bioactive compounds (phenols, squalene, tocopherol, and fatty acids) in olive oils [75, 76]. Nowadays, as pointed out previously, the evaluation of bioactive compounds should be integrated in a multidisciplinary study approach for food research. This calls for the combination of advanced and innovative analytical techniques and sophisticated statistical methods for the data analysis in food science [77–79]. NIR and MIR techniques are rapidly emerging in this field. The bioactive compounds and antioxidant properties can be predicted by the spectroscopic methods. In particular, infrared spectroscopy coupled to chemometrics is considered as a powerful, fast, accurate, and nondestructive analytical tool for rapid and precise determination of the content of bioactive compounds as well as their bioactivities, i.e., antioxidant properties. It represents a versatile alternative to the conventional methods. Further advantages in the context of food analysis include the rapid and inexpensive acquisition of data with

nil or minimal sample preparation and the possibility to record spectra at or even in the production line. Besides a lot of studies on adulteration and discrimination of olive oil as well as monitoring contaminations, process or recovery by infrared spectroscopy [80–90], the focus here is on the evaluation of functional components.

### 3.1. Fatty Acids

Being the main constituents of olive oils, fatty acids and their triglycerides dominate the spectra of olive oils. Mailer [91] collected 216 olive oil samples throughout the Australian olive-growing areas to develop a NIR spectroscopy calibration for a range of quality parameters including the major fatty acids in olive oil. Multiple correlation coefficients squared ( $R^2$ ) for minor fatty acids such as stearic acid (0.86) and linolenic acid (0.85) were reported relatively low because of the very narrow concentration range, while the major fatty acids oleic (0.99) and linoleic (1.00) provided high levels of accuracy compared with the reproducibility of the reference method.

It has been reported that authentication of virgin olive oil is time-consuming and requires expertise in the use of sophisticated instruments. To overcome this issue, Galtier et al. [92] evaluated the chemometric treatment of NIR spectra for the assessment of fatty acids and triacylglycerols in French virgin olive oil samples ( $n = 125$ ) for their classification (PLS1-DA) into geographically closed registered designations of origin (RDOs). The chemometric treatments of NIR spectra revealed that regression vectors of each RDO were correlated to one or more specific components with similar results obtained by conventional techniques according to their cultivar compositions. For the very first time, Maggio et al. [93] reported a rapid method to determine the fatty acid profile of olive oils using FTIR spectroscopy with the ATR sampling technique as described in Section 2. Calibration models were built using PLS regression. The fatty acid calibration models (oleic acid, linoleic acid, SFA, MUFA, and PUFA) were constructed in the spectral range from 3033 to 700  $\text{cm}^{-1}$  using PLS. The values obtained for the correlation coefficient for all models were in the range of 0.96–0.99. The LODs of the FTIR-chemometry methods were reported for oleic acid 3.0%, linoleic acid 0.5%, saturated fatty acids 1.3%, monounsaturated fatty acids 0.3%, and polyunsaturated fatty acids 0.3%, with recovery rates in the range 98–103%. The proposed method showed results comparable to the one obtainable by using the official method, but with the advantage of being more rapid and less expensive. Another study by Gurdeniz et al. described the classification and quantification of Turkish extra-virgin olive oil on the basis of fatty acids by using mid-infrared spectroscopy in combination with chemometric methods [94]. The PCA successfully distinguished the southern olive oil samples from the northern samples, while PLS detected a correlation between the spectral data and the fatty acid profile. The values obtained for the correlation coefficient were 0.83, 0.97, 0.97, 0.83, and 0.69 for stearic, oleic, linoleic, arachidic, and linolenic acids, respectively. Dupuy et al. [95] investigated the potential use of NIR and MIR spectroscopy for the quantitative analysis of triacylglycerols and fatty acids for the identification

of the RDO of extra-virgin olive oils. PLS and PLS-DA regressions were separately applied to the two spectral ranges. MIR spectroscopy was found to perform better. It was further reported that RDO identification by DA using a multiblock method showed proficiency.

The quantification of oxidized fatty acids in virgin olive oil using mid-infrared spectroscopy coupled with multiple linear regression has been described by Lerma-García et al. [96]. For the prediction of oxidized fatty acid, the entire mid-infrared region was divided into 25 subregions. The subregions affected by oxidation were identified using multiple linear regression with a cube root data transformation that successfully predicted oxidized fatty acids. The value obtained for correlation coefficient was 0.944 with a prediction error of 17%. Uncu and Ozen [97] studied some important quality parameters (oxidative stability, color pigments, fatty acid profile, and phenolic composition) of olive oils by FTIR spectroscopy as one of the vibrational spectroscopic methods: it is possible to predict successfully the oxidative stability, the major fatty acids (palmitic (0.87), oleic (0.94), and linoleic acid (0.97), saturated (0.91), monounsaturated (0.94), and polyunsaturated fatty acids (0.97)), and the chlorophyll content and some phenolic compounds of the samples.

Casale et al. [98] authenticated the Italian protected designation of origin (PDO) extra-virgin olive oil Chianti Classico using UV-vis, NIR and MIR spectroscopy coupled with a chemometric data analysis. Unequal class models (UNEQ) and soft independent modelling of class analogy (SIMCA) along with PLS were employed individually and jointly on the fatty acid composition data. PLS successfully predicted the oleic and linoleic acid content in the extra-virgin olive oils. Similarly, Inarejos-García et al. [99] investigated the degree of unsaturation of virgin olive oil by NIR spectroscopy. They reported an excellent performance of the multivariate algorithms in particular for the fatty acid profile ( $r$ -multiple coefficient of determination = 0.84–0.96). In a different approach, Valli et al. [100] pointed out new parameters for the detection of low quality of extra-virgin olive oil. These parameters were based on the total amount of fatty acids methyl and ethyl esters  $\Sigma$  (FAMEs + FAEEs) and their ratio (FAEEs/FAMEs) applying PLS to MIR spectra. The spectroscopic data showed a good agreement between predicted and actual reference values of calibration data sets. The limit of detection and quantification was reported to be 8.8 and 29.3 mg·kg<sup>-1</sup> for  $\Sigma$  (FAMEs + FAEEs) with  $R^2$  (0.98). On the contrary, the ratio of FAEEs/FAMEs showed  $R^2$  (0.83).

Recently, the European Union regulation for olive oil set a value of fatty acids ethyl ester contents (35 mg·kg<sup>-1</sup>) in extra-virgin olive oils. To evaluate the fatty acid alkyl esters in extra-virgin olive oil, Cayuela [101] recently developed predictive models for the determination of total fatty acid alkyl esters based on NIR spectroscopy. The external validation exercise of FAAEs, FAEEs, and FAMEs using the NIR spectrometer showed SEP values of 33.6 and 54.5, 25.6, and 67.2, 18.7 with  $r=0.91$  and 0.93, 0.89, and 0.88, 0.92, respectively, for two validation sets.



### 3.2. Phenolic Compounds, Tocopherols, and Squalenes

Several studies applied chemometrics to spectroscopic data in order to develop models and predict the content of minor components/functional compounds such as phenolic compounds and tocopherols in olive oils [1, 102, 103]. The recent chapter of Lagouri et al. [102] summarizes the optical UV-Vis-NIR-MIR spectroscopic tools and chemometrics used for the monitoring of olive oil functional compounds. Fatty acids, phenols, tocopherols, carotenoids, and squalene were considered with a focus on the bioactivity, chemistry, and processing. Inarejos-García et al. [99] investigated minor components, sensory characteristics, and quality of virgin olive oil by NIR spectroscopy. Their multivariate algorithms allowed reasonably accurate measurements, in particular of hydroxytyrosol derivatives ( $r$ -multiple coefficient of determination = 0.86–0.88) and C6 alcohols ( $r$ -multiple coefficient of determination = 0.69–0.80). FTIR spectroscopy, on the contrary, was used by Gouvinhas et al. [104]. They carried out a chemometric analysis of FTIR spectra from extra-virgin olive oils of three cultivars from Portugal (cultivars “Cobranco,” “Galega,” and “Picual”) at different maturation stages. To create a model for the discrimination of the olive oil samples, principal component analysis and discriminant analysis were utilised. The need for such sophisticated data analysis can also be seen in Figure 3, which shows that the spectra of olive oils are very similar. For the quantification of individual compounds, Gouvinhas et al. [104] developed regression-based calibration and validation models. This allowed the determination of the total phenolic content, *ortho*-diphenols, flavonoids, and antioxidant activity during the ripening process. For *ortho*-diphenols and flavonoids, the slope  $R^2$  and the RMSE were higher than 0.98 and lower than 0.03, respectively, whereas the phenolic content had the lowest multiple coefficient of determination in the calibration model ( $R^2 = 0.94$ ). The same authors reported a slope  $R^2$  and the RMSE of 0.93 and 0.04, respectively, for the determination of the antioxidant activity. Their quality measures became worse when data were cross validated ( $R^2 = 0.86$ , RMSECV = 0.05).

Mora-Ruiz et al. [105] applied NIR and MIR spectroscopy to study polar phenolic compounds of virgin olive oil and their impact on the oil quality. They obtained satisfactory multivariate test set validation algorithms for total polar phenolic (TPP) compounds ( $r$ -coefficient of determination = 0.91), hydroxytyrosol and tyrosol secoiridoid derivatives (HtyrSec, TyrSec;  $r = 0.91$  and  $0.92$ , respectively) by NIR spectroscopy. Moreover, the authors pointed out that, in contrast to the NIR data, the chemometric analysis of the MIR spectra gave no satisfactory validation models ( $r = 0.43$ ,  $0.54$ , and  $0.66$  for HtyrSec, TyrSec, and TPP). This was unexpected as the calibration algorithms for MIR actually gave an even better correlation than NIR ( $r > 0.96$  for all the polar phenolics studied). On the contrary, the optimization of phenolic compounds extraction from EVOO was achieved applying a response surface methodology as proposed by Fratoddi et al. [106].

Also, very good MIR results for the quantification of virgin olive oil phenolic compounds were reported by Hirri et al. [107]. They obtained values for the correlation coefficient and the root mean square errors of prediction of 0.99 and 0.11, respectively. This study underlined also that the spectral region in the range 3050–600  $\text{cm}^{-1}$  was useful for predicting the total polyphenol content. Bellincontro et al. [108] also focused on the total phenols. They developed a portable NIR-AOTF tool for the on-field and nondestructive measurement of specific and total phenols in olives for oil production. Models were developed for the main phenolic compounds (e.g., oleuropein, verbascoside, and 3,4-DHPEA-EDA) and total phenols by PLS. The results in terms of  $R^2$  for the calibration, prediction, and cross validation ranged between 0.930 and 0.998, 0.874 and 0.942, and 0.837 and 0.992, respectively. A recent preliminary study of Trapani et al. [109] applied cost-saving NIR based on a discrete filter system for the rapid measurement of total phenolic content and oleuropein of olive fruits, in addition to the moisture, oil, and sugar. Although PLS models built for the latter ones were satisfactory, the instrument did not prove itself suitable for obtaining predictive models for phenolic compound contents. This is probably because the necessary wavelengths in the section of absorbance from 1100 to 1400 nm were not covered. Further studies in this direction are necessary and requested for enabling the possible application and use.

Concerning tocopherols, Cayuela and García [110] recently reported the classification of olive oils based on  $\alpha$ -tocopherol and total tocopherol content using NIR spectroscopy. Other studies utilised pigments such as carotenoids and chlorophyll derivatives in extra-virgin olive oil as parameters/index of authenticity and quality. Pigments in oil matrices can be identified and quantified by chromatographic techniques after a pretreatment of the samples such as extraction and/or saponification, or by spectroscopic techniques without pretreatment of the samples [111]. Another work by the same authors quantified the main pigments, i.e.,  $\beta$ -carotene, lutein, pheophytin A, and pheophytin B, in several extra-virgin olive oils produced from a blend of three cultivars (Moraiolo, Frantoio, and Leccino) typical of Tuscany (Italy) in three different years. For this purpose, they employed a method based on the mathematical analysis of the near-ultraviolet-visible absorption spectra of the oils [112]. Besides a good prediction of fatty acid ethyl ester and wax, Uncu et al. [113] quantified lutein, pheophytin A, and their derivatives and total xanthophylls by FTIR + UV/vis with a range of 0.71–0.85, of 0.70–0.84, and RPD = 1.5–2.5 values but the prediction of the rest of the pigments were poor ( $=0.60$ – $0.76$ ,  $:0.42$ – $0.62$ , and RPD = 1.2–1.5). Along the same line, Borello and Domenici compared two near-UV/vis spectroscopic techniques for the determination of pigments, i.e., total carotenoids, and total chlorophyll derivatives in virgin and extra-virgin olive oils. Although UV/vis spectroscopy is very tempting for analysing the pigments, NIR and MIR spectroscopy typically gives a more specific molecular fingerprint of oil. Cayuela and García developed rapid NIR techniques using PLS analysis for measuring the squalene content of olive oil. The external validation exercise for estimating squalene was performed with the squared validation regression coefficient ( $r^2$ ) 0.83 and residual predictive deviation of 2.31.

### 3.3. Antioxidants

The assessment of interactions between natural active compounds and other food matrix components represent the bioactivity of a food. The first indicator of potential health benefits is commonly given by the antioxidant properties. The comprehension of concerted and synergistic actions, of antagonist interactions, or of no effect of biologically active compounds should be considered in this context. The same is true for the factors influencing the overall effects such as the peculiar combination of antioxidants and the structure of the food matrix [77]. Lu and Rasco reviewed and summarized the recent applications of infrared spectroscopy for the development of models for the prediction of antioxidant properties in food, in addition to qualitative and quantitative analysis of antioxidant compounds. It is also worthy of mentioning the review article of Cozzolino who concluded that infrared spectroscopy is a versatile analytical tool for the quantitative determination of antioxidant properties in agricultural products, foods, and plants.

Cerretani et al. reported the rapid FTIR determination of phenolics and antioxidant activity of olive oil, in addition to water. For total phenols, good results were obtained considering the spectrum from 3610 to 816  $\text{cm}^{-1}$ , while the range from 3707 to 1105  $\text{cm}^{-1}$  was most suitable for ABTS+. Satisfactory LOD values were achieved by the FTIR-chemometry approach: 12.5 (mg gallic acid/kg oil) for total phenols and 0.76 (mmol Trolox/kg oil) for ABTS+. The authors concluded that the results obtained with the chemometric data analysis are comparable to those obtained using the official reference method. This highlights the potential of the spectroscopic technique as an alternative to the standard procedure for routine analysis or control at-line of production processes.

### 4. Conclusion

NIR and MIR spectroscopy coupled with chemometric data analysis has shown considerable potential for the determination of functional compounds present in olive oil. Recent applications have demonstrated to perform the assay of antioxidant activity (tocopherols, phenols, and squalene), to allow the discrimination of geographical origins, to facilitate the assessment of sensory attributes, and to determine the fatty acid profile, which is characteristic of the oil matrix. IR spectroscopic methods represent an interesting alternative to the conventional analytical methods as they do not require a pretreatment of the samples. Moreover, they offer a high sample throughput and significant time saving. In addition, these techniques are considerably more environmentally friendly since no solvents or carrier gases are used. These new approaches based on mid- and near-IR absorption spectroscopy are therefore interesting alternatives, for example, for rapid screening purposes.

In the future, IR spectroscopic methodology in combination with chemometric data evaluation will possibly overtake the conventional methodologies. In some cases, this may require the change of the regulatory frameworks, which are currently naming only conventional analytical tools for certain official purposes.

However, the convincing performance of the spectroscopic approaches with respect to authentication and comprehensive quantitative analysis will certainly make a strong case in the regulatory and political processes. Without a doubt, the methods reviewed in this paper will soon be extensively used in the oil processing industries as a routine method to evaluate the characteristic parameters of olive oil.

### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

### **References**

1. M. Casale and R. Simonetti, “Review: near infrared spectroscopy for analysing olive oils,” *Journal of Near Infrared Spectroscopy*, vol. 22, no. 2, pp. 59–80, 2014. View at: [Publisher Site](#) | [Google Scholar](#)
2. D. Boskou, “Olive fruits, table olives, and olive oil bioactive constituents,” in *Olive and Olive Oil Bioactive Constituents*, D. Boskou, Ed., pp. 1–30, AOCS Press, Urbana, IL, USA, 1st edition, 2015. View at: [Google Scholar](#)
3. F. P. Zito, B. Polese, L. Vozzella et al., “Good adherence to mediterranean diet can prevent gastrointestinal symptoms: a survey from Southern Italy,” *World Journal of Gastrointestinal Pharmacology and Therapeutics*, vol. 7, no. 4, pp. 564–571, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
4. C. M. Lăcătușu, E. D. Grigorescu, M. Floria, A. Onofriescu, and B. M. Mihai, “The mediterranean diet: from an environment-driven food culture to an emerging medical prescription,” *International Journal of Environmental Research and Public Health*, vol. 16, no. 6, p. 942, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
5. L. Conte, A. Bendini, E. Valli et al., “Olive oil quality and authenticity: a review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future,” *Trends in Food Science & Technology*, 2019, In press. View at: [Publisher Site](#) | [Google Scholar](#)
6. A. Srobarova, J. A. Teixeira da Silva, G. Kogan, A. Ritieni, and A. Santini, “Beauvericin decreases cell viability of wheat,” *Chemistry and Biodiversity*, vol. 6, pp. 1208–1215, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
7. P. Mikušová, A. Šrobárová, M. Sulyok, and A. Santini, “Fusarium fungi and associated metabolites presence on grapes from Slovakia,” *Mycotoxin Research*, vol. 29, no. 2, pp. 97–102, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
8. R. Bhat and K. R. N. Reddy, “Challenges and issues concerning mycotoxins contamination in oil seeds and their edible oils: updates from last decade,” *Food Chemistry*, vol. 215, pp. 425–437, 2017. View at: [Publisher Site](#) | [Google Scholar](#)

9. K. Otto, A. Santini, and S. Oancea, “Recent aflatoxin survey data in milk and milk products: a review,” *International Journal of Dairy Technology*, vol. 70, no. 3, pp. 320–331, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
10. P. Mikusová, A. Ritieni, A. Santini, G. Juhasová, and A. Srobárová, “Contamination by moulds of grape berries in Slovakia,” *Food Additives & Contaminants Part A*, vol. 27, no. 5, pp. 738–747, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
11. A. Santini, G. Meca, S. Uhlig, and A. Ritieni, “Fusaproliferin, beauvericin and enniatins: occurrence in food—a review,” *World Mycotoxin Journal*, vol. 5, no. 1, pp. 71–81, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
12. P. Mikušová, A. Santini, A. Ritieni, J. Pavlokin, and A. Šrobárová, “Berries contamination by microfungi in Slovakia vineyard regions: impact of climate conditions on microfungi biodiversity,” *Revista Iberoamericana de Micologia*, vol. 29, no. 3, pp. 126–131, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
13. A. Cimmino, A. Andolfi, M. C. Zonno et al., “Chenopodolin: a phytotoxic unrearranged ent-pimaradiene diterpene produced by *Phoma chenopodolica*, a fungal pathogen for *Chenopodium album* biocontrol,” *Journal of Natural Products*, vol. 76, no. 7, pp. 1291–1297, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
14. A. Medina, S. Mohale, N. I. P. Samsudin, A. Rodriguez-Sixtos, A. Rodriguez, and N. Magan, “Biocontrol of mycotoxins: dynamics and mechanisms of action,” *Current Opinion in Food Science*, vol. 17, pp. 41–48, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
15. M. F. Abdallah, M. Ameye, S. De Saeger, K. Audenaert, and G. Haesaert, “Biological control of mycotoxigenic fungi and their toxins: an update for the pre-harvest approach,” in *Fungi and Mycotoxins—Their Occurrence, Impact on Health and the Economy as Well as Pre- and Postharvest Management Strategies*, P. Berka Njobeh, Ed., Intech Open, London, UK, 2018. View at: [Google Scholar](#)
16. S. Vichi, A. Romero, J. Tous, and J. Caixach, “The activity of healthy olive microbiota during virgin olive oil extraction influences oil chemical composition,” *Journal of Agricultural and Food Chemistry*, vol. 59, no. 9, pp. 4705–4714, 2011. View at: [Publisher Site](#) | [Google Scholar](#)
17. S. Cicerale, X. A. Conlan, A. J. Sinclair, and R. S. J. Keast, “Chemistry and health of olive oil phenolics,” *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 3, pp. 218–236, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
18. A. Salvo, A. Rotondo, G. L. La Torre, N. Cicero, and G. Dugo, “Determination of 1,2/1,3-diglycerides in Sicilian extra-virgin olive oils by <sup>1</sup>H-NMR over a one-year storage period,” *Natural Product Research*, vol. 31, no. 7, pp. 822–828, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
19. L. Schwingshackl, A. M. Lampousi, M. P. Portillo, D. Romaguera, G. Hoffmann, and H. Boeing, “Olive oil in the prevention and management of type 2 diabetes mellitus: a systematic review and meta-analysis of

- cohort studies and intervention trials,” *Nutrition and Diabetes*, vol. 7, no. 4, p. e262, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
20. E. Alves, M. R. M. Domingues, and P. Domingues, “Polar lipids from olives and olive oil: a review on their identification, significance and potential biotechnological applications,” *Foods*, vol. 7, no. 7, p. 109, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
21. E. M. Yubero-Serrano, J. Lopez-Moreno, F. Gomez-Delgado, and J. Lopez-Miranda, “Extra virgin olive oil: more than a healthy fat,” *European Journal of Clinical Nutrition*, 2018, In press. View at: [Publisher Site](#) | [Google Scholar](#)
22. G. Serreli and M. Deiana, “Biological relevance of extra virgin olive oil polyphenols metabolites,” *Antioxidants*, vol. 7, no. 12, p. 170, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
23. V. Francisco, C. Ruiz-Fernández, V. Lahera et al., “Natural molecules for healthy lifestyles: oleocanthal from extra virgin olive oil,” *Journal of Agricultural and Food Chemistry*, vol. 67, no. 14, pp. 3845–3853, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
24. D. L. García-González, R. Aparicio-Ruiz, and R. Aparicio, “Virgin olive oil—chemical implications on quality and health,” *European Journal of Lipid Science and Technology*, vol. 110, no. 7, pp. 602–607, 2008. View at: [Publisher Site](#) | [Google Scholar](#)
25. M. Aparicio-Soto, M. Sánchez-Hidalgo, M. Á. Rosillo, M. L. Castejón, and C. Alarcón-de-la-Lastra, “Extra virgin olive oil: a key functional food for prevention of immune-inflammatory diseases,” *Food & Function*, vol. 7, no. 11, pp. 4492–4505, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
26. S. Rigacci and M. Stefani, “Nutraceutical properties of olive oil polyphenols. An itinerary from cultured cells through animal models to humans,” *International Journal of Molecular Science*, vol. 17, no. 6, p. 843, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
27. M. Piroddi, A. Albin, R. Fabiani et al., “Nutrigenomics of extra-virgin olive oil: a review,” *Biofactors*, vol. 43, no. 1, pp. 17–41, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
28. B. Saibandith, J. P. E. Spencer, I. R. Rowland, and D. M. Commane, “Olive polyphenols and the metabolic syndrome,” *Molecules*, vol. 22, no. 7, p. 1082, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
29. A. Alkhatib, C. Tsang, and J. Tuomilehto, “Olive oil nutraceuticals in the prevention and management of diabetes: from molecules to lifestyle,” *International Journal of Molecular Science*, vol. 19, no. 7, p. 2024, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
30. A. M. Borzì, A. Biondi, F. Basile, S. Luca, E. S. D. Vicari, and M. Vacante, “Olive oil effects on colorectal cancer,” *Nutrients*, vol. 11, no. 1, p. 32, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
31. M. Crespo, J. Tomé-Carneiro, A. Dávalos, and F. Visioli, “Pharma-nutritional properties of olive oil phenols. Transfer of new findings to human nutrition,” *Foods*, vol. 7, no. 6, p. 90, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
32. A. Foscolou, E. Critselis, and D. Panagiotakos, “Olive oil consumption and human health: a narrative review,” *Maturitas*, vol. 118, pp. 60–66, 2018. View at: [Publisher Site](#) | [Google Scholar](#)

33. O. Garcia-Martinez, C. Ruiz, A. Gutierrez-Ibanez, R. Illescas-Montes, and L. Melguizo-Rodriguez, "Benefits of olive oil phenolic compounds in disease prevention," *Endocrine, Metabolic and Immune Disorders Drug Targets*, vol. 18, no. 4, pp. 333–340, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
34. A. Santini, E. Novellino, V. Armini, and A. Ritieni, "State of the art of ready-to-use therapeutic food: a tool for nutraceuticals addition to foodstuff," *Food Chemistry*, vol. 140, no. 4, pp. 843–849, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
35. R. Andrew and A. A. Izzo, "Principles of pharmacological research of nutraceuticals," *British Journal of Pharmacology*, vol. 174, no. 11, pp. 1177–1194, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
36. A. Santini and E. Novellino, "Nutraceuticals—shedding light on the grey area between pharmaceuticals and food," *Expert Review of Clinical Pharmacology*, vol. 11, no. 6, pp. 545–547, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
37. A. Santini, S. M. Cammarata, G. Capone et al., "Nutraceuticals: opening the debate for a regulatory framework," *British Journal of Clinical Pharmacology*, vol. 84, no. 4, pp. 659–672, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
38. A. Santini, G. C. Tenore, and E. Novellino, "Nutraceuticals: a paradigm of proactive medicine," *European Journal of Pharmaceutical Sciences*, vol. 96, pp. 53–61, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
39. P. Daliu, A. Santini, and E. Novellino, "From pharmaceuticals to nutraceuticals: bridging disease prevention and management," *Expert Review of Clinical Pharmacology*, vol. 12, no. 1, pp. 1–7, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
40. A. Durazzo, "Extractable and non-extractable polyphenols: an overview," in *Non-Extractable Polyphenols and Carotenoids: Importance in Human Nutrition and Health. Food Chemistry, Function and Analysis*, No. 5, F. Saura-Calixto and J. Pérez-Jiménez, Eds., p. 37, Royal Society of Chemistry, London, UK, 2018. View at: [Google Scholar](#)
41. A. Durazzo, L. D'Addezio, E. Camilli et al., "From plant compounds to botanicals and back: a current snapshot," *Molecules*, vol. 23, no. 8, p. 1844, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
42. A. Durazzo and M. Lucarini, "A Current shot and rethinking of antioxidant research strategy," *Brazilian Journal of Analytical Chemistry*, vol. 5, no. 20, pp. 9–11, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
43. F. Aouidi, N. Dupuy, J. Artaud et al., "Rapid quantitative determination of oleuropein in olive leaves (*Olea europaea*) using mid-infrared spectroscopy combined with chemometric analyses," *Industrial Crops and Products*, vol. 37, no. 1, pp. 292–297, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
44. M. Jansen and J. Birch, "Composition and stability of olive oil following partial crystallization," *Food Research International*, vol. 42, no. 7, pp. 826–831, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
45. E. N. Frankel, "Chemistry of extra virgin olive oil: adulteration, oxidative stability, and antioxidants," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 10, pp. 5991–6006, 2010. View at: [Publisher Site](#) | [Google Scholar](#)

46. E. N. Frankel, "Nutritional and biological properties of extra virgin olive oil," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 3, pp. 785–792, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
47. M. Celano, V. Maggisano, S. M. Lepore, D. Russo, and S. Bulotta, "Secoiridoids of olive and derivatives as potential adjuvant drugs in cancer: a critical analysis of experimental studies," *Pharmacological Research*, vol. 142, pp. 77–86, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
48. J. M. Moran, O. Leal-Hernandez, R. Roncero-Martin, and J. D. Pedrera-Zamorano, "Antitumor perspectives of oleuropein," *Journal of Food Science*, vol. 84, no. 3, p. 384, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
49. P. Przychodzen, R. Wyszowska, M. Gorzynik-Debicka, T. Kostrzewa, A. Kuban-Jankowska, and M. Gorska-Ponikowska, "Anticancer potential of oleuropein, the polyphenol of olive oil, with 2-methoxyestradiol, separately or in combination, in human osteosarcoma cells," *Anticancer Research*, vol. 39, no. 3, pp. 1243–1251, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
50. M. El Riachy, F. Priego-Capote, L. León, L. Rallo, and M. D. Luque de Castro, "Hydrophilic antioxidants of virgin olive oil. Part 1: hydrophilic phenols: a key factor for virgin olive oil quality," *European Journal of Lipid Science and Technology*, vol. 113, no. 6, pp. 678–691, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
51. C. L. Huang and B. E. Sumpio, "Olive oil, the mediterranean diet, and cardiovascular health," *Journal of the American College of Surgeons*, vol. 207, no. 3, pp. 407–416, 2008. View at: [Publisher Site](#) | [Google Scholar](#)
52. J. Chandran, N. Nayana, and P. Nisha, "Phenolics in vegetable oils," in *Compounds in Food: Characterization and Analysis Food Analysis & Properties, Chapter 21*, L. M. L. Nollet and J. A. Gutierrez-Urbe, Eds., p. 407, Taylor & Francis Group, Abingdon, UK, 2018. View at: [Google Scholar](#)
53. A. García, E. Rodríguez-Juan, G. Rodríguez-Gutiérrez, J. J. Rios, and J. Fernández-Bolaños, "Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs)," *Food Chemistry*, vol. 197, pp. 554–561, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
54. B. Bayram, B. Ozcelik, G. Schultheiss, J. Frank, and G. Rimbach, "A validated method for the determination of selected phenolics in olive oil using high-performance liquid chromatography with coulometric electrochemical detection and a fused-core column," *Food Chemistry*, vol. 138, no. 2-3, pp. 1663–1669, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
55. G. Cioffi, M. S. Pesca, P. De Caprariis, A. Braca, L. Severino, and N. De Tommasi, "Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity," *Food Chemistry*, vol. 121, no. 1, pp. 105–111, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
56. C. Conde, S. Delrot, and H. Gerós, "Physiological, biochemical and molecular changes occurring during olive development and ripening," *Journal of Plant Physiology*, vol. 165, no. 15, pp. 1545–1562, 2008. View at: [Publisher Site](#) | [Google Scholar](#)



57. A. Gómez-Rico, M. D. Salvador, and G. Fregapane, "Virgin olive oil and olive fruit minor constituents as affected by irrigation management based on SWP and TDF as compared to  $ET_c$  in medium-density young olive orchards (*Olea europaea* L. cv. Cornicabra and Morisca)," *Food Research International*, vol. 42, no. 8, pp. 1067–1076, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
58. R. Amarowicz, R. Carle, G. Dongowski et al., "Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods," *Molecular Nutrition and Food Research*, vol. 53, no. 2, pp. S151–S183, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
59. O. Köseoğlu, D. Sevim, and P. Kadiroğlu, "Quality characteristics and antioxidant properties of Turkish monovarietal olive oils regarding stages of olive ripening," *Food Chemistry*, vol. 212, pp. 628–634, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
60. V. Di Stefano and M. G. Melilli, "Effect of storage on quality parameters and phenolic content of Italian extra-virgin olive oils," *Natural Product Research*, vol. 21, pp. 1–9, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
61. J. Giacometti, Č. Milin, F. Giacometti, and Z. Ciganj, "Characterisation of monovarietal olive oils obtained from croatian cvs. drobnica and buza during the ripening period," *Foods*, vol. 7, no. 11, p. 188, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
62. R. Romano, A. Giordano, L. Le Grottaglie et al., "Volatile compounds in intermittent frying by gas chromatography and nuclear magnetic resonance," *European Journal of Lipid Science and Technology*, vol. 115, no. 7, pp. 764–773, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
63. D. Naviglio, R. Romano, F. Pizzolongo et al., "Rapid determination of esterified glycerol and glycerides in triglyceride fats and oils by means of periodate method after transesterification," *Food Chemistry*, vol. 102, no. 1, pp. 399–405, 2007. View at: [Publisher Site](#) | [Google Scholar](#)
64. D. Boskou, M. Tsimidou, and G. Blekas, "Olive oil composition," in *Olive Oil: Chemistry and Technology*, D. Boskou, Ed., AOCS Press, Champaign, IL, USA, 2006. View at: [Google Scholar](#)
65. V. N. Kamvissis, E. G. Barbounis, N. C. Megoulas, and M. A. Koupparis, "A novel photometric method for evaluation of the oxidative stability of virgin olive oils," *Journal of AOAC International*, vol. 91, no. 4, pp. 794–801, 2008. View at: [Google Scholar](#)
66. R. Ambra, F. Natella, S. Lucchetti, V. Forte, and G. Pastore, " $\alpha$ -Tocopherol,  $\beta$ -carotene, lutein, squalene and secoiridoids in seven monocultivar Italian extra-virgin olive oils," *International Journal of Food Sciences and Nutrition*, vol. 68, no. 5, pp. 538–545, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
67. E. Psomiadou and M. Tsimidou, "Pigments in Greek virgin olive oils: occurrence and levels," *Journal of the Science of Food and Agriculture*, vol. 81, no. 7, pp. 640–647, 2001. View at: [Publisher Site](#) | [Google Scholar](#)
68. P. Viola and M. Viola, "Virgin olive oil as a fundamental nutritional component and skin protector," *Clinics in Dermatology*, vol. 27, no. 2, pp. 159–165, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
69. B. Schrader, *Infrared and Raman Spectroscopy*, VCH Verlagsgesellschaft, Weinheim, Germany, 1995.

70. P. R. Griffiths and J. A. De Haseth, *Fourier Transform Infrared Spectrometry*, Wiley, Weinheim, Germany, 2nd edition, 2007.
71. H. Haken and H. C. Wolf, *Molecular Physics and Elements of Quantum Chemistry*, Springer, Berlin, Germany, 1995.
72. J. Kiefer, N. H. Rasul, P. K. Ghosh, and E. Von Lieres, “Surface and bulk porosity mapping of polymer membranes using infrared spectroscopy,” *Journal of Membrane Science*, vol. 452, pp. 152–156, 2014. View at: [Publisher Site](#) | [Google Scholar](#)
73. L. A. Averett, P. R. Griffiths, and K. Nishikida, “Effective path length in attenuated total reflection spectroscopy,” *Analytical Chemistry*, vol. 80, no. 8, pp. 3045–3049, 2008. View at: [Publisher Site](#) | [Google Scholar](#)
74. N. J. Harrick and F. K. du Pré, “Effective thickness of bulk materials and of thin films for internal reflection spectroscopy,” *Applied Optics*, vol. 5, no. 11, pp. 1739–1743, 1966. View at: [Publisher Site](#) | [Google Scholar](#)
75. C. I. G. Tuberoso, I. Jerković, M. Maldini, and G. Serreli, “Phenolic compounds, antioxidant activity, and other characteristics of extra virgin olive oils from Italian autochthonous varieties Tonda di Villacidro, Tonda di Cagliari, Semidana, and Bosana,” *Journal of Chemistry*, vol. 2016, Article ID 8462741, 7 pages, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
76. D. Trombetta, A. Smeriglio, D. Marcoccia et al., “Analytical evaluation and antioxidant properties of some secondary metabolites in northern Italian mono- and multi-varietal extra virgin olive oils (EVOOs) from early and late harvested olives,” *International Journal of Molecular Sciences*, vol. 18, no. 4, p. 797, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
77. A. Durazzo, “Study approach of antioxidant properties in foods: update and considerations,” *Foods*, vol. 6, p. E17, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
78. A. Durazzo, J. Kiefer, M. Lucarini et al., “An Innovative and Integrated Food Research Approach: spectroscopy applications to milk and a case study of a milk-based dish,” *Brazilian Journal of Analytical Chemistry*, vol. 5, no. 18, pp. 12–27, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
79. A. Durazzo, J. Kiefer, M. Lucarini et al., “Qualitative analysis of traditional Italian dishes: FTIR approach,” *Sustainability*, vol. 10, no. 11, p. 4112, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
80. M. de Luca, W. Terouzi, G. Ioele et al., “Derivative FTIR spectroscopy for cluster analysis and classification of Morocco olive oils,” *Food Chemistry*, vol. 124, no. 3, pp. 1113–1118, 2011. View at: [Publisher Site](#) | [Google Scholar](#)
81. A. P. la Mata, A. Dominguez-Vidal, J. M. Bosque-Sendra, A. Ruiz-Medina, L. Cuadros-Rodríguez, and M. J. Ayora-Cañada, “Olive oil assessment in edible oil blends by means of ATR-FTIR and chemometrics,” *Food Control*, vol. 23, no. 2, pp. 449–455, 2012. View at: [Publisher Site](#) | [Google Scholar](#)

82. A. Rohman and Y. B. Che Man, "Quantification and classification of corn and sunflower oils as adulterants in olive oil using chemometrics and FTIR Spectra," *Scientific World Journal*, vol. 2012, Article ID 250795, 6 pages, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
83. O. Jović, T. Smolić, I. Primožič, and T. Hrenar, "Spectroscopic and chemometric analysis of binary and ternary edible oil mixtures: qualitative and quantitative study," *Analytical Chemistry*, vol. 88, no. 8, pp. 4516–4524, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
84. M. De Luca, D. Restuccia, M. L. Clodoveo, F. Puoci, and G. Ragno, "Chemometric analysis for discrimination of extra virgin olive oils from whole and stoned olive pastes," *Food Chemistry*, vol. 202, pp. 432–437, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
85. A. M. Jiménez-Carvelo, M. T. Osorio, A. Koidis, A. González-Casado, and L. Cuadros-Rodríguez, "Chemometric classification and quantification of olive oil in blends with any edible vegetable oils using FTIR-ATR and Raman spectroscopy," *LWT*, vol. 86, pp. 174–184, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
86. K. Georgouli, J. Martinez Del Rincon, and A. Koidis, "Continuous statistical modelling for rapid detection of adulteration of extra virgin olive oil using mid infrared and Raman spectroscopic data," *Food Chemistry*, vol. 217, pp. 735–742, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
87. M. H. abadi Sherahi, F. Shahidi, F. Tabatabai Yazdi, and S. M. Bagher Hashemi, "Effect of *Lactobacillus plantarum* on olive and olive oil quality during fermentation process," *LWT—Food Science and Technology*, vol. 82, pp. 572–580, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
88. I. Durán Merás, J. Domínguez Manzano, D. Airado Rodríguez, and A. Muñoz de la Peña, "Detection and quantification of extra virgin olive oil adulteration by means of autofluorescence excitation-emission profiles combined with multi-way classification," *Talanta*, vol. 178, pp. 751–762, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
89. L. Hadhoum, G. Burnens, K. Loubar, M. Balistrrou, and M. Tazerout, "Bio-oil recovery from olive mill wastewater in sub-/supercritical alcohol-water system," *Fuel*, vol. 252, pp. 360–370, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
90. O. Uncu and B. Ozen, "A comparative study of mid-infrared, UV-Visible and fluorescence spectroscopy in combination with chemometrics for the detection of adulteration of fresh olive oils with old olive oils," *Food Control*, vol. 105, pp. 209–218, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
91. R. J. Mailer, "Rapid evaluation of olive oil quality by NIR reflectance spectroscopy," *Journal of the American Oil Chemists' Society*, vol. 81, no. 9, pp. 823–827, 2004. View at: [Publisher Site](#) | [Google Scholar](#)
92. O. Galtier, N. Dupuy, Y. Le Dréau et al., "Geographic origins and compositions of virgin olive oils determined by chemometric analysis of NIR spectra," *Analytica Chimica Acta*, vol. 595, no. 1-2, pp. 136–144, 2007. View at: [Publisher Site](#) | [Google Scholar](#)

93. R. M. Maggio, T. S. Kaufman, M. D. Carlo et al., “Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares,” *Food Chemistry*, vol. 114, no. 4, pp. 1549–1554, 2009. View at: [Publisher Site](#) | [Google Scholar](#)
94. G. Gurdeniz, B. Ozen, and F. Tokatli, “Comparison of fatty acid profiles and mid-infrared spectral data for classification of olive oils,” *European Journal of Lipid Science and Technology*, vol. 112, no. 2, pp. 218–226, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
95. N. Dupuy, O. Galtier, D. Ollivier, P. Vanloot, and J. Artaud, “Comparison between NIR, MIR, concatenated NIR and MIR analysis and hierarchical PLS model. Application to virgin olive oil analysis,” *Analytica Chimica Acta*, vol. 666, no. 1-2, pp. 23–31, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
96. M. J. Lerma-García, E. F. Simó-Alfonso, A. Bendini, and L. Cerretani, “Rapid evaluation of oxidised fatty acid concentration in virgin olive oil using Fourier-transform infrared spectroscopy and multiple linear regression,” *Food Chemistry*, vol. 124, no. 2, pp. 679–684, 2011. View at: [Publisher Site](#) | [Google Scholar](#)
97. O. Uncu and B. Ozen, “Prediction of various chemical parameters of olive oils with Fourier transform infrared spectroscopy,” *LWT—Food Science and Technology*, vol. 63, no. 2, pp. 978–984, 2015. View at: [Publisher Site](#) | [Google Scholar](#)
98. M. Casale, P. Oliveri, C. Casolino et al., “Characterisation of PDO olive oil Chianti Classico by non-selective (UV-visible, NIR and MIR spectroscopy) and selective (fatty acid composition) analytical techniques,” *Analytica Chimica Acta*, vol. 712, pp. 56–63, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
99. A. M. Inarejos-García, S. Gómez-Alonso, G. Fregapane, and M. D. Salvador, “Evaluation of minor components, sensory characteristics and quality of virgin olive oil by near infrared (NIR) spectroscopy,” *Food Research International*, vol. 50, no. 1, pp. 250–258, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
100. E. Valli, A. Bendini, R. M. Maggio et al., “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,” *International Journal of Food Science & Technology*, vol. 48, no. 3, pp. 548–555, 2013. View at: [Publisher Site](#) | [Google Scholar](#)
101. J. A. Cayuela, “Rapid NIR determination of alkyl esters in virgin olive oil,” *Grasas Y Aceites*, vol. 68, no. 2, p. 195, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
102. V. Lagouri, V. Manti, and T. Gimisis, “Optical nondestructive UV-Vis-NIR-MIR spectroscopic tools and chemometrics in the monitoring of olive oil functional compounds: bioactivity, chemistry and processing,” in *Book: Olives and Olive Oil as Functional Foods: Bioactivity, Chemistry and Processing. Chapter 11*, A. Kiritsakis and S. Fereidoon, Eds., Shahidi Wiley and Sons Ltd., Hoboken, NY, USA, 1st edition, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
103. N. Nenadis and M. Z. Tsimidou, “Perspective of vibrational spectroscopy analytical methods in on-field/official control of olives and virgin olive oil,” *European Journal of Lipid Science and Technology*, vol. 119, no. 1, Article ID 1600148, 2017. View at: [Publisher Site](#) | [Google Scholar](#)

104. I. Gouvinhas, J. M. M. M. de Almeida, T. Carvalho, N. Machado, and A. I. R. N. A. Barros, “Discrimination and characterisation of extra virgin olive oils from three cultivars in different maturation stages using Fourier transform infrared spectroscopy in tandem with chemometrics,” *Food Chemistry*, vol. 174, pp. 226–232, 2015. View at: [Publisher Site](#) | [Google Scholar](#)
105. M. E. Mora-Ruiz, P. Reboredo-Rodríguez, M. Desamparados Salvador et al., “Assessment of polar phenolic compounds of virgin olive oil by NIR and mid-IR spectroscopy and their impact on quality,” *European Journal of Lipid Science and Technology*, vol. 119, no. 1, Article ID 160009, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
106. I. Fratoddi, M. Rapa, G. Testa, I. Venditti, F. A. Scaramuzzo, and G. Vinci, “Response surface methodology for the optimization of phenolic compounds extraction from extra virgin olive oil with functionalized gold nanoparticles,” *Microchemical Journal*, vol. 138, pp. 430–437, 2018. View at: [Publisher Site](#) | [Google Scholar](#)
107. A. Hirri, M. Bassbasi, S. Souhassou, F. Kzaiber, and A. Oussama, “Prediction of polyphenol fraction in virgin olive oil using mid-infrared attenuated total reflectance accessory-mid-infrared coupled with partial least squares regression,” *International Journal of Food Properties*, vol. 19, no. 7, pp. 1504–1512, 2016. View at: [Publisher Site](#) | [Google Scholar](#)
108. A. Bellincontro, A. Taticchi, M. Servili, S. Esposito, D. Farinelli, and F. Mencarelli, “Feasible application of a portable NIR-AOTF tool for on-field prediction of phenolic compounds during the ripening of olives for oil production,” *Journal of Agricultural and Food Chemistry*, vol. 60, no. 10, pp. 2665–2673, 2012. View at: [Publisher Site](#) | [Google Scholar](#)
109. S. Trapani, M. Migliorini, L. Cecchi et al., “Feasibility of filter-based NIR spectroscopy for the routine measurement of olive oil fruit ripening indices,” *European Journal of Lipid Science and Technology*, vol. 119, no. 6, Article ID 1600239, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
110. J. A. Cayuela and J. F. García, “Sorting olive oil based on alpha-tocopherol and total tocopherol content using near-infra-red spectroscopy (NIRS) analysis,” *Journal of Food Engineering*, vol. 202, pp. 79–88, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
111. C. Lazzerini, M. Cifelli, and V. Domenici, “Pigments in extra-virgin olive oil: authenticity and quality,” in *Products from Olive Tree, Chapter 6*, D. Boskou and M. L. Clodoveo, Eds., pp. 99–114, InTech, Rijeka, Croatia, 1st edition, 2016. View at: [Google Scholar](#)
112. C. Lazzerini and V. Domenici, “Pigments in extra-virgin olive oils produced in Tuscany (Italy) in different years,” *Foods*, vol. 6, no. 4, p. 25, 2017. View at: [Publisher Site](#) | [Google Scholar](#)
113. O. Uncu, B. Ozen, and F. Tokatli, “Use of FTIR and UV-visible spectroscopy in determination of chemical characteristics of olive oils,” *Talanta*, vol. 201, pp. 65–73, 2019. View at: [Publisher Site](#) | [Google Scholar](#)