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Research Article

Surface-Enhanced Raman Spectroscopy for Monitoring Extravirgin Olive Oil Bioactive Components

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Olive oil is the main fat source of the Mediterranean diet. This seasoning ingredient is highly appreciated for its unique taste, functional properties, and positive impact on human health. The determination of chemical composition is a demanding task in order to fully characterize this precious food product, ensure its quality, and prevent fraudulent practices. Among innovative techniques proposed for the oil analysis, surface-enhanced Raman spectroscopy (SERS) can be an extremely useful tool for olive oil characterization. In this frame, we have investigated five noncommercial olive oils produced in different parts of South Italy by using a commercial Raman microspectroscopy apparatus and home-made signal-enhancing SERS substrates. A wavelet-based data analysis has allowed us to efficiently remove the background and the noise from the acquired spectra. The analysis of these SERS spectra has enabled the quantification of the relative contents of carotene, oleic acid, and phenols. These relative contents differ in the examined samples. In addition, SERS response in the lipid region has indicated differences in the relative abundance of saturated fatty acids. The present results confirm the validity of the SERS technique as a rapid, nondestructive, and reliable analytical technique for identifying olive oil bioactive components.

1. Introduction

Virgin olive oils (VOOs) are extracted from olive fruit (*Olea europaea* L.) by mechanical or other physical processes, without any alteration of the oil. The contribution of VOO to human health is considered very relevant. The risk factors of some chronic and degenerative pathologies can be reduced by a regular consumption of extravirgin olive oil, thanks to its high content of polyphenol compounds.

Olive oils are classified as extravirgin olive oil (EVOO), VOO, and olive oil. EVOO is characterized by a maximum content of oleic acid less than 0.8 g per 100 g and VOO by a maximum content of oleic acid less than of 2 g per 100 g, and olive oil is allowed to have more than 2 g of oleic acid per 100 g. EVOO represents the best quality product of the olive oil category.

The most important producer countries of olive oil are Spain, Italy, Greece, Tunisia, and Turkey. VOO is the main fat source of the Mediterranean diet and is well known for its high nutritional and taste quality. Several factors can affect VOO composition such as olive variety, ripening degree, geographical origin, agronomic and climatic conditions, extraction process, and storage conditions. Among vegetable oils, EVOO is very rich in monounsaturated fatty acid but its peculiarity concerns also minor components as polyphenols, sterols, tocopherols, vitamins E and K, and volatile compounds [1–3]. The EVOO commercial fraud is becoming more and more common. Adulterations with cheaper oil (soybean, sunflower, hazelnut, corn, and refined olive oil) or false certifications are illicit practices to make an easy profit. Because of the impact of this issue, both on the market and sometimes on the human health, a great effort is being devoted to develop and apply efficient methods and approaches enabling to evidence the occurrence of these illicit practices. Very sensitive techniques have been employed to determine EVOO chemical composition and evaluate its authenticity, to quantify vegetable adulterants or degradation compounds, such as nuclear magnetic resonance, gas

chromatography, and liquid chromatography with mass spectrometry [1–3]. In recent years, optical methods have acquired popularity as powerful techniques for studying liquid samples with various biomolecules, demonstrating their ability in the detection of target molecules and the study of the molecules themselves [4–8]. In particular, Raman microspectroscopy (μ-RS) has also been used as a very sensitive method to characterize olive oils. It offers many advantages because it can provide information on chemical bonds, conservation state, and possible adulteration without complicate or time-expensive procedures. In fact, some literature reports are available about the use of μ-RS for detecting EVOO geographical origin [9], cultivar [10], and thermal stability [11, 12]. However, the main attractive application of μ-RS in this field is the possibility to be used for developing rapid methods enabling to get oil composition for adulteration detection [13–19]. In some cases, the use of μ-RS for EVOO analysis is hampered by a huge fluorescent signal that hides the Raman response, a problem that cannot be completely solved by the use of low-energy light excitation (infrared laser), as mainly done in the last years [14, 20]. In addition, in some cases, the Raman signal of the molecules of interest is very low. Surface-enhanced Raman spectroscopy (SERS) has been fruitfully used for materials with low Raman signal [21–23] and high fluorescence background [24]. This technique is based on the use of metallic nano-objects (such as Au or Ag nanoparticles, nanorods, and so on) or nanopatterned substrates characterized by a well-suited surface plasmon resonance (i.e., oscillations of surface electrons of metal nanostructures induced by the impinging electromagnetic radiation), thanks to the interaction among molecules, nanomaterials, and light. In fact, the electromagnetic (EM) effect, related to the presence of the plasmon resonance, and the chemical effect, resulting from the interaction between molecules with Raman active modes and the nanostructures, concurrently work in SERS for giving rise to the enhancement of the otherwise weak Raman signals [25, 26]. Actually, the interaction between molecules and nanomaterials changes the cross section of the Raman effect and sometimes also of the fluorescence emission, thus resulting also in a reduction of the fluorescence signal [24].

Recently, SERS has shown to offer good potentiality for EVOO adulteration detection [27, 28] and authentication [29]. Some of us have recently performed a preliminary investigation on Italian EVOOs [30], obtaining promising results about the usefulness of the approach. Boosted by these results, a deeper study of the composition of five EVOO noncommercial samples from different sites of the southern part of Italy by using SERS has been performed and reported in this paper. A commercial Raman microspectroscopy apparatus has been employed for the study along with home-made signal-enhancing substrates, made of home-made gold nanoparticles seeded on a glass surface [30]. The analysis of the obtained clear Raman spectra has enabled the quantification of the relative content of carotene, oleic acid, and phenols. Our results indicate that SERS can be a valuable tool for investigating the content of particular significant components such as carotene, oleic acid, and phenols that are particularly useful to reduce the risk factors of some chronic and degenerative pathologies as suggested by many studies available in [31, 32]. The chosen approach is very promising for the realization of sensing methods well suited for routinely investigation of commercial samples.

2. Materials and Methods

2.1. Nanoparticles Preparation. Gold nanoparticles were obtained by conventional citrate reduction method [33, 34]. A 0.01% $HAuCl_4$ solution was reduced by 1% of sodium citrate with vigorous stirring at near boiling temperature. GNP size was controlled by adjusting the amount of sodium citrate. Solutions at various concentrations of GNPs with an expected diameter in the range of 20–70 nm were prepared. In this work, GNPs with an average size of 30 ± 4 nm were used. The dimension of the GNPs was inferred from their light absorbance spectrum, showing a significant plasmon resonance absorption band [33]. A drop of the GNP solution was placed on a microscope glass and left to dry for some hours, thus obtaining a SERS substrate. A small amount of the olive oil sample to analyze was thus deposed on the dried GNP solution.

2.2. Samples. Five noncommercial EVOOs were investigated. They were produced in three different Italian regions of the south of Italy, namely, Basilicata (from the locality of Chiaromonte), Puglia (Alberobello and Triggiano), and Campania (Avella and Bellona) and directly obtained by the producers. Samples were prepared by dropping a small amount of each investigated EVOO on the naked glass layer (samples for Raman measurements) and on a SERS substrate (samples for SERS measurements).

2.3. Experimental Setup. For the acquisition of the EVOO SERS spectra, a Jobin-Yvon system from Horiba ISA, equipped with a TriAx 180 monochromator and a liquid nitrogen cooled charge-coupled detector, was used. The employment of a1800 grooves/mm grating enables to obtain a spectral resolution of 4 cm^{-1}. The spectra were recorded in the air at room temperature using a 17 mW He-Ne laser source (wavelength 632.8 nm). The spectrum accumulation time was 300 s. The laser light was focused to a 2 μm spot size on the sample through an Olympus microscope with a 50× optical objective. For each investigated EVOO, spectra from five different samples were acquired and three acquisitions were collected for each of them. The same apparatus was used to acquire preliminary Raman spectrum of the investigated EVOOs by inspecting EVOO drops on a bare glass surface (i.e., samples for Raman measurements).

2.4. Data Analysis. Background signal and noise were reduced by using a data treatment based on wavelet algorithm [35]. The spectrum signal was decomposed in terms of the sum of different wavenumber-scaled elementary functions (namely, wavelets), and a hierarchical representation of the spectrum was thus obtained. Starting from the decomposed

signal, the spectrum was reconstructed removing the low- and high-frequency components due to the background and the noncorrelated noise, respectively. This data treatment method allows a reliable quantitative individuation of spectral detail also in the case of very weak signals, and it has been applied successfully to the Raman analysis of human single cell [36], human tissue [37], and fluids [38, 39].

A schematic description of this wavelet-based data elaboration is shown in Figure 1. In particular, Figure 1(a) shows the as-measured spectrum (in the 500–1800 cm^{-1} spectral range) to be processed using direct wavelet transform (DWT) algorithm that allows decomposing the spectrum itself in components at different scales. The signal is then reconstructed excluding high-scaled (Figure 1(b)) and low-scaled (Figure 1(c)) components. The spectrum resulting after this preprocessing procedure is shown in Figure 1(d).

The spectra resulting after the preprocessing procedure were then analyzed in terms of a sum of Lorentzian functions by using a best-fitting routine of GRAMS/AI (2001, Thermo Electron) program, which is based on the Levenberg–Marquardt nonlinear least-square methods.

The quantification of the relative contents of carotene, oleic acid, and phenols was obtained by calculating the ratio between the area of the peak assigned to the component and the sum of the areas of all the peaks in Raman spectrum.

3. Results and Discussion

The Raman spectra of all the investigated EVOO samples were preliminarily taken for comparison by performing measurements on the samples deposited on bare glass slides. These spectra are dominated by a huge broad mode due to fluorescence, as expected because of the fluorescence due to the chlorophyll content of natural oil [40, 41]. In SERS spectra, i.e., spectra collected from samples placed on the SERS substrate, the fluorescence signal persists but the Raman signal is enhanced, as expected because of the occurrence of SERS mechanisms [21]. A representative SERS spectrum of investigated EVOOs in the 500–1800 cm^{-1} spectral range is shown in Figure 2(a) and compared with the spectrum obtained by conventional micro-Raman spectroscopy on the same sample. The spectra reported in Figure 2 have been obtained by using as acquisition times 8 s (no longer times are allowed due to detector saturation) for conventional Raman spectroscopy and 300 s for SERS, respectively. The elaborated signals obtained after the subtraction of the background signal are reported in Figure 2(b), where SERS spectrum evidences different contributions. It is worth to note that the SERS effect implies not only a significant enhancement of the Raman signal but also an attenuation of the fluorescence contribution allowing longer acquisition times and a consequent improvement of the Raman-fluorescence signal ratio. This confirms the need for using the described preprocessing procedure in order to get information on vibrational features of the samples.

In Figure 3, a representative SERS spectrum from an EVOO sample is shown in the 500–1800 cm^{-1} spectral range together with the results of the deconvolution procedure performed in terms of a sum of Lorentzian functions. The obtained features are in agreement with the EVOO known Raman features even if some slight shifts are observed as usually obtained in the SERS spectrum [24]. The main contributions from vibrations of chemical bonds of triglycerides were individuated at wavenumber shifts equal to 1070 cm^{-1} and 1119 cm^{-1} (C-C stretching), 1265 cm^{-1} (in-plane C-H deformation in unconjugated *cis*-double bond), 1441 cm^{-1} (related to the deformation vibration of the C-H group), and 1629 cm^{-1} (C=C stretching). These modes are generally found in all kinds of vegetable oils [14] even if their relative intensity can change significantly depending on the oil origin. The EVOO mainly consists of monounsaturated oleic acid (i.e., with only one C=C double bond), different from other vegetable oils that have a high content of linoleic acid characterized by a second C=C double bond. The weak intensity found for the 1265 cm^{-1} mode is expected because this mode is related to *cis*-(=C-H) vibration [14]. A peculiar property of the EVOO is a significant content of phenolic compounds and carotene, which are thought to endow it with many of health benefits [41, 42]. The Raman contribution of these two components is clearly observed. Carotene is mainly related to chlorophyll and has Raman modes at 1002, 1150–1170, and 1525 cm^{-1} [13]. The mode at 1002 cm^{-1} can be also assigned to phenylalanine, an amino acid present in many proteins and in a moderate amount in olive oil. The Raman mode at 616 and 1237 cm^{-1} is due to phenolic compounds [43, 44]. The broad peak centred at about 820–880 cm^{-1} is argued to be related to cellulose content, due to a limited filtering of samples [45, 46]. Finally, the Raman peak at 1350 cm^{-1} is probably related to the CH_3 bond vibrations of the oleic acid, even if blue-shifted with respect to the value of 1300–1320 cm^{-1} reported in the literature [47–49]. Generally, the Raman spectra of oils do not exhibit this mode, while it has a relevant intensity in the spectra that we measured, probably due to the enhancement effect of SERS. A prominent mode at 1317 cm^{-1} is actually observed in SERS data of oleic acid in [49].

The assignments of the main detected SERS contributions are listed in Table 1, in agreement with the references therein reported. The spectral positions of SERS modes do not differ significantly from those found in Raman response of oleic acid and of EVOO [11, 21]. Conversely, mode intensities do not agree in some cases. In particular, SERS modes at about 1079 and 1440 cm^{-1} are noticeably weak compared to conventional Raman spectroscopy signals. The enhancement of the signal due to SERS is a selective mechanism strongly depending on the bond configuration and orientation, as a consequence some modes can be penalized even if they have a relatively strong intensity in conventional Raman spectrum. This selectivity in the enhancement of Raman scattering is primarily due to the SERS dependence on the distance and orientation of moieties with respect to the nanoparticle surface [21].

In Figure 4, representative SERS spectra for the examined EVOO samples produced in three different regions of the South Italy, namely, in Basilicata (from the locality of Chiaromonte), in Puglia (Alberobello and Triggiano), and in Campania (Avella and Bellona), are shown in the 500–1750 cm^{-1} spectral range. In particular, spectra obtained

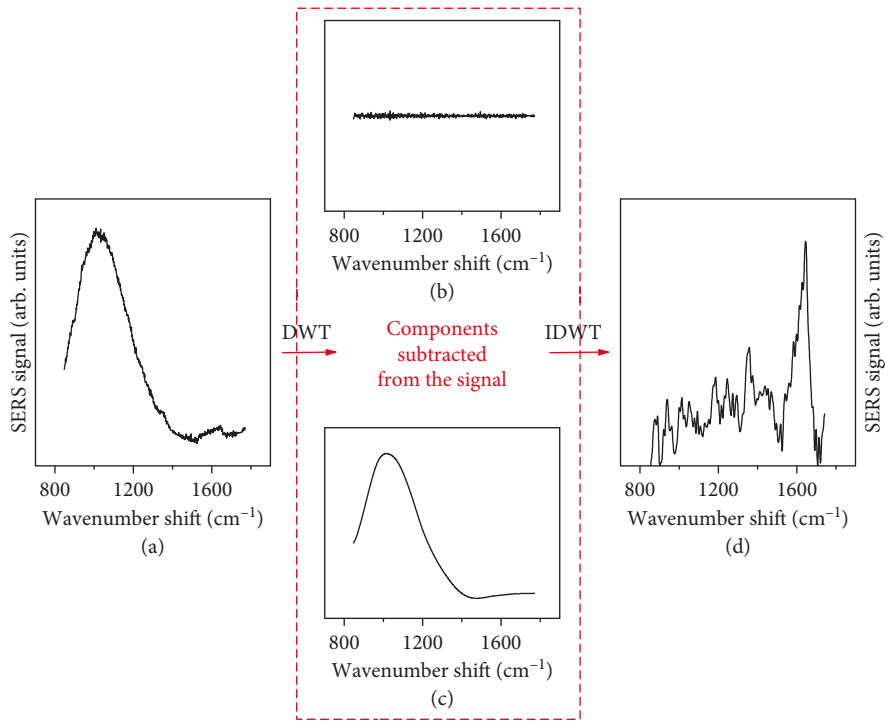

Figure 1: Schematic description of the data elaboration procedure based on the wavelet algorithm. The measured spectrum of an olive oil sample is reported in (a) panel. The signal exhibits a strong fluorescence signal centred at 1017 cm^{-1}, corresponding to a wavelength of about 675 nm. Using direct wavelet transform (DWT) algorithm, the signal has been decomposed in components at different scales. The signal has been reconstructed by excluding high-scaled ((b) panel) and low-scaled ((c) panel) components. The resulting signal is shown in (d) panel.

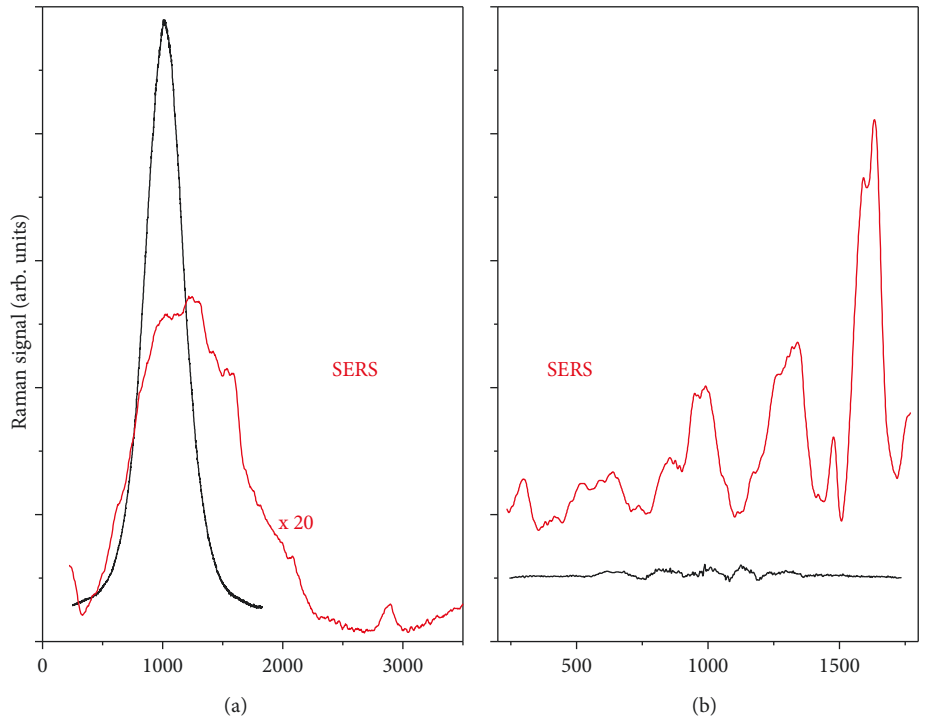

Figure 2: EVOO Raman signal measured by conventional micro-Raman spectroscopy (black lines) and SERS signal (red lines). In (a) panel, the raw data (SERS signal is reported magnified 20 times with respect to Raman signal) are reported and in (b) panel, the elaborated signals are shown. An acquisition time of 8 s has been used for the Raman signal and 300 s for SERS.

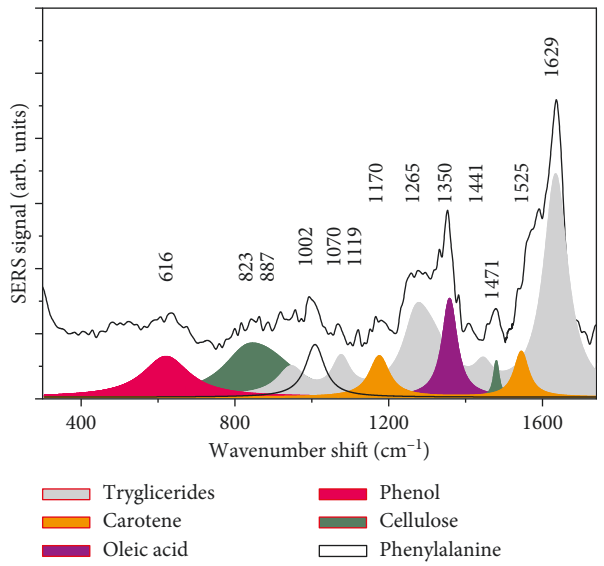

Figure 3: SERS spectrum of EVOO on a GNP seed/glass substrate after background signal subtraction. The main spectrum components are indicated in different colours.

Table 1: Main Raman peaks observed in the spectra with assignments in accordance with the data reported in the literature. s, strong; m, medium; w, weak; vw, very weak.

Peak position (cm^{-1})	Mode	Assignment	Ref.
616 m	In-plane phenyl ring deformation	Phenol compounds	[43]
823; 887 m	α-Anomers and α-glycosides	Cellulose	[44]
1002 m	Symmetric phenyl ring breathing	Protein; carotenoids	[13]
1070 w	C-C stretching out-of-phase	Triglycerides	[20]
1119 vw	C-C stretching in-phase	Triglycerides	
1150–1170 m	C-C stretching	Carotene	[13]
1237 m	Phenyl ring and CO group motion	Phenol	[43]
1265 m	Symmetric rock in *cis* double bond (=C-H)	Triglycerides	[13, 14]
1350 s	In-phase methylene twisting deformation vibration	Oleic acid	[46, 47]
1441 w	(C-H bend) (CH_2) methylene scissor deformations	Triglycerides	[14]
1471 w	O-H, CH_2	Cellulose	[46]
1525 m	C-C stretching	Carotene	[13]
1629 s	Stretching (C=C) cis	Triglycerides	[50]
2865 m	C-H sym. stretching (CH_2)	Triglycerides	[48]
2904 m	C-H sym. stretching (CH_3)	Triglycerides	
2931 m	C-H asym. stretching (CH_2)	Triglycerides	
2971 m	C-H asym. stretching (CH_3)	Triglycerides	

analyzing EVOO from (a) Triggiano, (b) Avella, (c) Chiaromonte, (d) Bellona, and (e) Alberobello is shown. As is evident, they show similar behavior along with clear differences among them regarding the characteristics of some specific features. We focused our attention on the differences among the spectra regarding the labeled features, i.e., contributions at 1150 and 1525 cm^{-1} (carotene), at 1237 cm^{-1} (phenol), and at 1350 cm^{-1} (oleic acid), since they enable to obtain information about the relative content of the specific molecules that are also of peculiar relevance for EVOO characteristics. Accordingly, these features are adopted for an estimation of the relative amount of the main components in the EVOO samples. In particular, the relative weights of the sum of the intensity areas of the Raman modes that can be respectively attributed to carotene, oleic acid, and phenol are reported in Figure 5. Differences in the oil composition are observed. For instance, the phenol compound content is negligible in the EVOO from Triggiano (Figure 5(a)), of some per cent in oil from Avella and Chiaromonte (Figures 5(b) and 5(c), respectively) and maximum (12%) in EVOO from Bellona (Figure 5(d)) and Alberobello (Figure 5(e)). At the opposite, the EVOOs from Triggiano, Avella, and Chiaromonte have a carotene content higher than what detected for EVOOs from Bellona and Alberobello. The peculiar taste of each high-quality EVOO derives from many factors related to local terrain composition, climatic conditions, and cultivation modality. Aging effects can also modify the relative composition of the oil. However, the present results seem to indicate a correlation between the carotene content and the components related to oleic acid and phenols, the content in carotene being related to an increase in oleic acid and phenols. Because these two

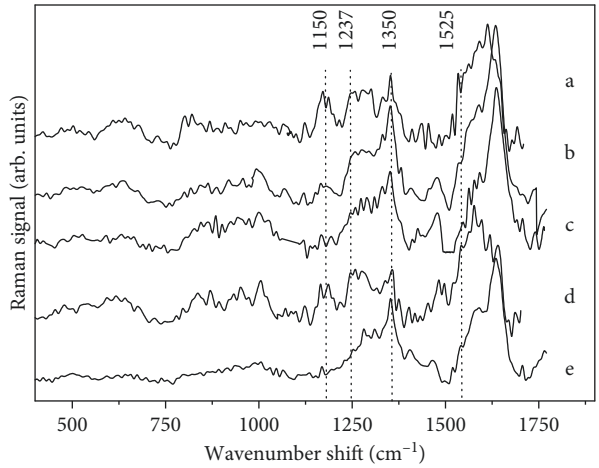

FIGURE 4: Representative SERS spectra from noncommercial EVOO samples from different sites (a) Triggiano, (b) Avella, (c) Chiaromonte, (d) Bellona, and (e) Alberobello in 500–1750 cm^{-1} region.

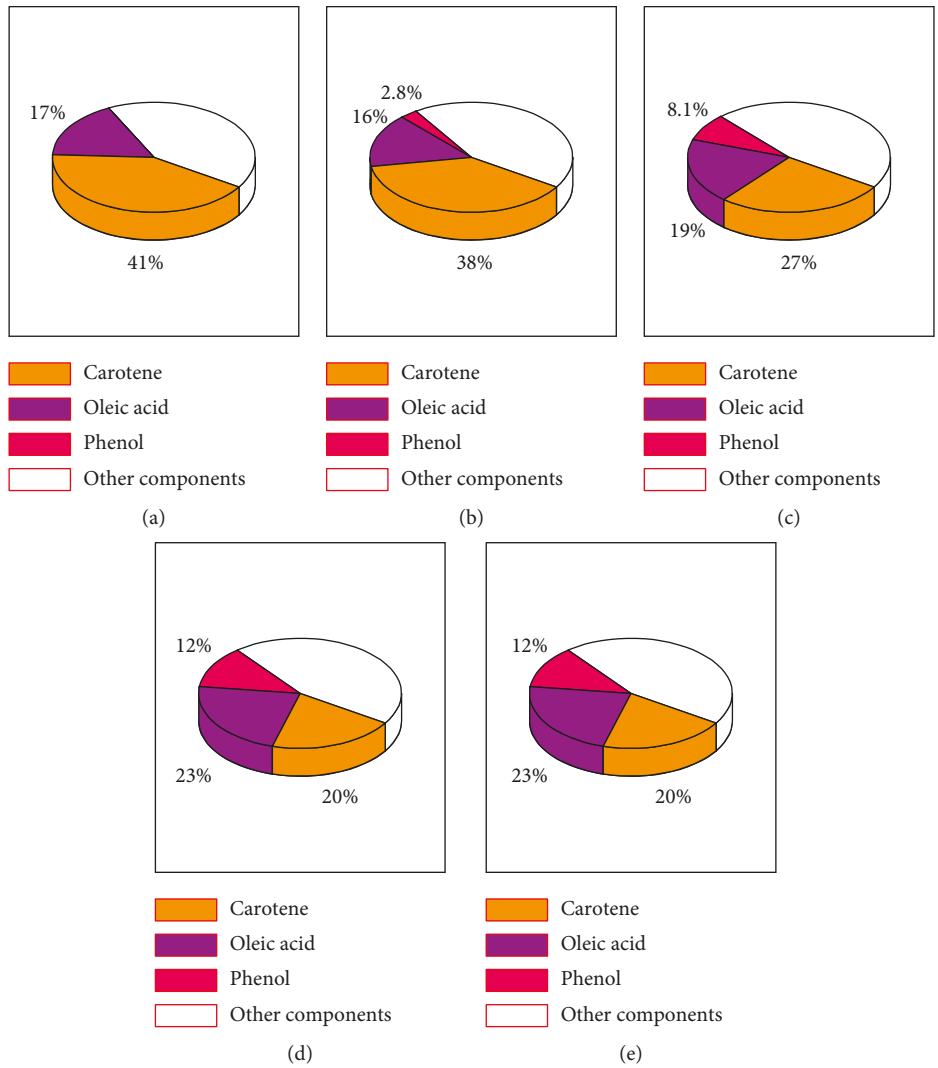

FIGURE 5: Relative intensities of Raman mode assigned to carotene, oleic acid, and phenol components for noncommercial EVOO samples from different sites (a) Triggiano, (b) Avella, (c) Chiaromonte, (d) Bellona, and (e) Alberobello.

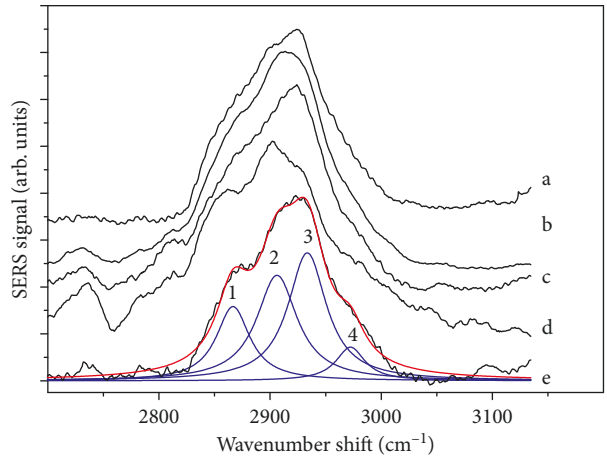

FIGURE 6: Representative SERS spectra from noncommercial EVOO samples from different sites (a) Triggiano, (b) Avella, (c) Chiaromonte, (d) Bellona, and (e) Alberobello in 2700–3100 cm^{-1} region due to lipid contribution. The results of deconvolution procedure are also reported.

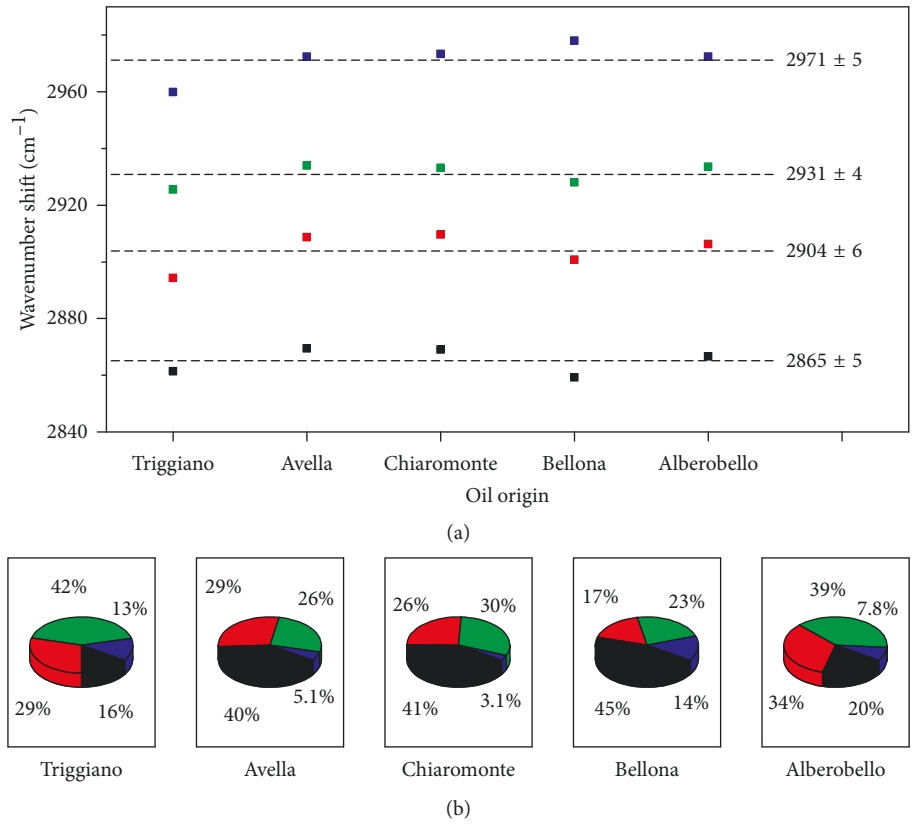

FIGURE 7: (a) Spectral position of the different contributions outlined by the deconvolution of SERS spectra in 2700–3100 cm^{-1} spectral range. (b) Relative contribution of the different components indicated as the ratio of the normalized peak areas (in percentage). The colour refers to symbols in (a) panel.

last components have a strong influence on the oil taste, the analysis provided by the Raman spectroscopy has interesting implications for the development of analytical control procedures in the production of high-quality EVOO.

In Figure 6, representative SERS spectra for the examined EVOO samples produced in (a) Triggiano, (b) Avella, (c) Chiaromonte, (d) Bellona, and (e) Alberobello are shown in the 2700–3100 cm^{-1} spectral range together with the results of the deconvolution procedure. As is evident, the main band located around 2920 cm^{-1} can be decomposed into four components that are related to located at ≈ 2865, 2904, 2931, and 2971 cm^{-1} mainly due to the C-H stretching modes of the CH_2 (symmetric stretch mode at about 2895 cm^{-1} and asymmetric one at around 2931 cm^{-1}) of the lipid chains of the

fatty acids and to the C-H stretching modes of the CH_3 (symmetric stretch mode at around 2904 cm^{-1} and asymmetric one at about 2971 cm^{-1}) of the lipid chains of the fatty acids. The detailed assignment is reported in Table 1. The wavenumber position of these contributions is little changing (the values of the peak positions are reported in Figure 7(a) even if they are constant within the errors). These bands are known to give information about the degree of saturation of the fatty acids present in the EVOOs [48]. The relative contribution of each of the four bands is obtained by calculating, for each spectrum, the ratio between the area of each band and the total area of the spectrum in the high-wavenumber region. The results in Figure 7(b) show that the relative weight of these features highly depends on the investigated samples. In three samples (EVOOs from Chiaromonte, Avella, and Bellona), the most abundant contribution is given by the 2865 cm^{-1} mode and the less abundant one is that from the 2971 cm^{-1} mode, which results almost negligible in two of these EVOOs (samples from Avella and Chiaromonte). In EVOOs from Triggiano and Alberobello, the most abundant contribute is given by the mode at around 2931 cm^{-1}. In some studies, the intensity of the 2865 cm^{-1} mode has been related to the presence of fatty acids with a high degree of saturation [48]. In this frame, the present results suggest that the EVOOs samples differ as for the relative abundance of saturated fatty acids (SFAs) that are of great importance for olive oil characterization.

4. Conclusions

SERS spectroscopy has been applied to the characterization of EVOO by using a commercial Raman microspectroscopy apparatus and home-made signal-enhancing SERS substrates. A wavelet-based data analysis allowed an efficient preprocessing of the acquired spectra. We examined five different noncommercial olive oils produced in different parts of South Italy, and we focused our attention to some specific spectral features assigned to components of relevant importance for EVOO characterization, i.e., SERS modes at 1150 and 1525 cm^{-1}(carotene), at 1237 cm^{-1} (phenol), and at 1350 cm^{-1} (oleic acid). The relative contents of carotene, oleic acid, and phenols determined from this SERS spectra analysis have shown significant differences in the examined samples. In addition, SERS response in the lipid region has also indicated that the relative abundance of saturated fatty acids differs in the various samples. The results have further proved that the home-made SERS substrates induced a large amplification of the Raman signal of the molecule of interest allowing a deep insight into the composition and molecular structure of the investigated EVOO samples.

This study has confirmed the validity of SERS as a rapid, nondestructive, and reliable analytical technique for identifying bioactive components, such as phenols and carotenoids. The chosen approach requiring a small quantity of the sample and no peculiar preparation procedures of the sample itself is very promising for the realization of sensing methods well suited for routine investigation of commercial samples.

Data Availability

The Raman spectra data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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