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## Use of Raman spectroscopy for analyzing edible vegetable oils

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### ABSTRACT

Vegetable oils provide high nutritional value in the human diet. Specifically, extra virgin olive oil is one of the main ingredients of the Mediterranean diet, which is among the healthiest of eating practices. This article reviews the use of Raman spectroscopy for analyzing edible vegetable oils including olive oil. Although the spectra for edible vegetable oils are similar, they exhibit some differences which, however small, enable their discrimination. Thus, Raman spectra allow one to determine the degree of unsaturation of oils. This property is correlated with the iodine value but much faster and simpler to obtain. The degree of unsaturation can be used to classify and authenticate oils, which is especially useful with high-quality oils. In fact, adulteration with mixtures of more inexpensive oils can be easily detected by Raman spectroscopy. This technique additionally allows some minor components present in unsaponifiable matter to be identified. Fats in general and vegetable oils in particular, are prone to oxidation. Thus, double bonds in them are oxidized to form triglycerides. Vegetable oils are widely used for frying and Raman spectroscopy allows for their oxidative stability against heating at the usual frying temperatures to be assessed.

### KEYWORDS

Raman spectroscopy; edible oil; vegetable oil; degree of unsaturation; oxidation of oils

### Introduction

In recent years, use of spectroscopic techniques in general, and Raman spectroscopy in particular, in Food Chemistry has grown dramatically (1–3). Edible vegetable oils have been the subject of a number of applications of Raman spectroscopy, often in combination with chemometric methods (4, 5). Advances in this field of analysis have been promoted by the availability of increasingly powerful Raman spectrometers and the development of fiber optic probes.

Vegetable oils are among the main components of the human diet, whether as they are or as ingredients of processed foods such as margarine, bakery products, cakes, ice cream, or precooked foods (6). Also, many vegetable oils are used by the pharmaceutical, cosmetic or even biodiesel production industry (7).

Extracting vegetable oils from plants is usually a simple process typically involving pressing fruits or seeds (e.g., olives to obtain olive oil) or extraction with an organic solvent such

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as hexane (8,9). However, extraction with supercritical carbon dioxide (10) and centrifugation (11) are more environmentally clean than conventional solvent extraction. Although in most cases the extract must be refined to increase its oil quality, virgin olive oil, which is obtained from *Olea europea* L. fruits—a typical crop in Mediterranean countries—, requires no refining. Olive oil is the main source of fats in the Mediterranean diet, which has been associated with a decreased incidence of coronary heart disease and some tumors. These properties have made virgin olive oil one of the most widely studied edible oils. In the last few decades, spectroscopic techniques have become very powerful tools for analyzing not only virgin olive oil, but also all other types of vegetable oils.  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectroscopies (12–14), and FT-IR spectroscopy have been used in addition to Raman spectroscopy for this purpose.

This article reviews existing applications of Raman spectroscopy to the analysis of edible vegetable oils, the detection of adulteration and various manipulations, and the determination of minor components.

## Raman spectra for edible vegetable oils

The earliest application of Raman spectroscopy to oil analysis was reported in 1936 by Ch. V. Jogarao (17), who recorded the spectra for various vegetable oils including groundnut and coconut oil. This author assigned several bands in the spectra and used them to determine the formula of the main component of ghee. Early Raman determinations involved exposing samples for at least 48 h and recording two spectra—one with a quinine sulphate solution to quench the fluorescence and the other without it. Also, Raman spectrometers were expensive and largely custom-made. Despite the good results initially obtained, Raman spectroscopy was never again applied to oil analysis until the 1970s, where it was used to assess the *cis/trans* isomer composition of oils from the bands at  $1656\text{ cm}^{-1}$  and  $1670\text{ cm}^{-1}$  (18). This allowed stretching vibrations in  $\text{C}=\text{C}$  and  $\text{C}=\text{O}$  bonds, and scissoring vibrations in  $\text{CH}_2$  groups, for a series of fatty esters and triglycerides in different conformations to be assigned (see Table 1). In fact, the wavenumbers obtained for double bonds in a *cis* configuration (oleates, linoleates, and linolenates) and a *trans* configuration (elaidates and linoelaidates) were used to estimate the proportions of *cis* and *trans* isomers in variably hydrogenated soybean oils, thereby demonstrating the potential of Raman spectroscopy for determining isomer ratios in edible oils. In the years that followed, the bands in the Raman spectra for most edible vegetable oils were unequivocally assigned. Thus, the bands in the

**Table 1.** Raman bands (in  $\text{cm}^{-1}$ ) for reference esters and triglycerides.

| Compound            | $\nu_{\text{C}=\text{C}}$ | $\nu_{\text{C}=\text{O}}$ | $\nu_{\text{CH}_2}$ |
|---------------------|---------------------------|---------------------------|---------------------|
| Methyl oleate       | 1655.1                    | 1744                      | 1440                |
| Triolein            | 1653.8                    | 1747                      | 1438                |
| Methyl elaidate     | 1669.0                    | 1744                      | 1439                |
| Trielaidin          | 1668.5                    | 1748                      | 1438                |
| Methyl linoleate    | 1656.4                    | 1741                      | 1439                |
| Trilinoleate        | 1656.4                    | 1747                      | 1440                |
| Methyl linoelaidate | 1671.5                    | 1742                      | 1437                |
| Trilinoelaidin      | 1670.5                    | 1741                      | 1438                |
| Methyl linolenate   | 1657.0                    | 1741                      | 1438                |
| Trilinolenin        | 1656.4                    | 1745                      | 1440                |

Table 2. Raman bands for an edible oil.

| Wavenumber (cm <sup>-1</sup> ) | Assignment                                                    |
|--------------------------------|---------------------------------------------------------------|
| ~1270                          | In plane = C-H deformation in a unconjugated <i>cis</i> C = C |
| ~1305                          | In phase methylene twisting motion                            |
| ~1440                          | CH <sub>2</sub> scissoring deformation ( $\delta_{CH_2}$ )    |
| ~1660                          | C = C stretching ( <i>cis</i> )                               |
| ~1670                          | C = C stretching ( <i>trans</i> )                             |
| ~1750                          | C = O stretching in an ester                                  |
| ~2855                          | Symmetric aliphatic C-H stretch (CH <sub>2</sub> groups)      |
| ~2926                          | Asymmetric aliphatic C-H stretch (CH <sub>2</sub> groups)     |
| ~2970                          | Symmetric aliphatic C-H stretch (CH <sub>3</sub> groups)      |
| ~3015                          | Asymmetric olefinic C-H stretch (=CH groups)                  |

regions 800–1800 and 2800–3050 cm<sup>-1</sup> were found to suffice for authentication purposes (19). The Raman bands for a number of vegetable oils have now been reported and found to differ little between oils (4,19–22). Table 2 shows the wavenumbers of the main bands for edible oils (20) and Figure 1 a typical spectrum for extra virgin olive oil. As can be seen, the signal-to-noise ratio was very good across the spectral region and the different stretching ( $\nu$ ) and bending vibrations ( $\delta$ ) were all quite well defined. As can be concluded from Figure 1, the areas of interest for the study of an oil by Raman spectroscopy are two: 2750–3100 cm<sup>-1</sup> and 800–1800 cm<sup>-1</sup>. In fact, the spectral differences between different vegetable oils are very small. In Figure 2, these two spectral regions for different oils are shown and, as mentioned, the differences between them are slight.

For this reason, it is often necessary to use the statistical analysis of the spectral data. Among these methods, principal component analysis (PCA) is a very powerful tool to

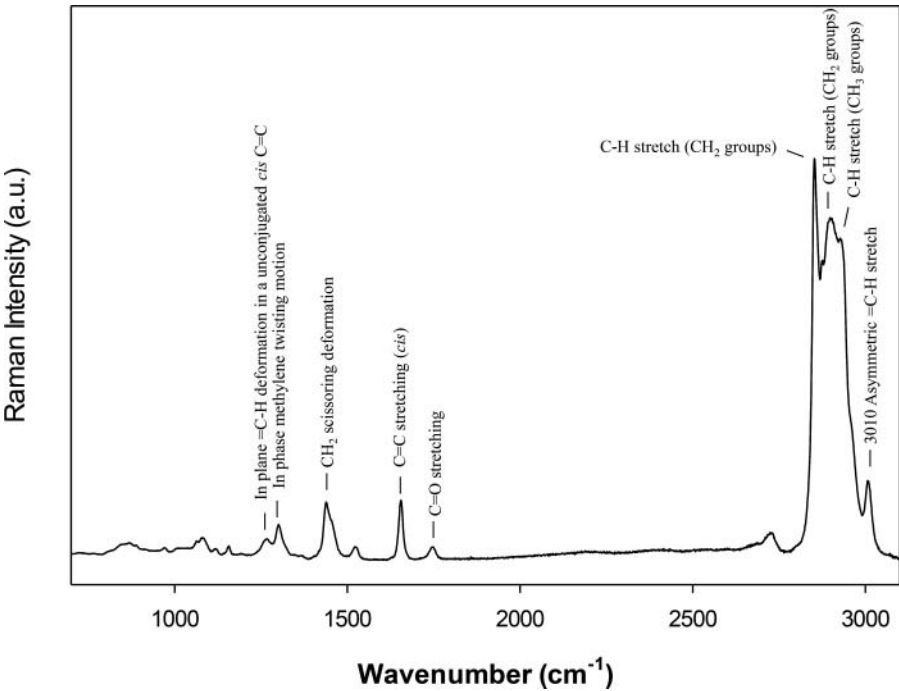


Figure 1. Typical Raman spectrum for an extra virgin olive oil.

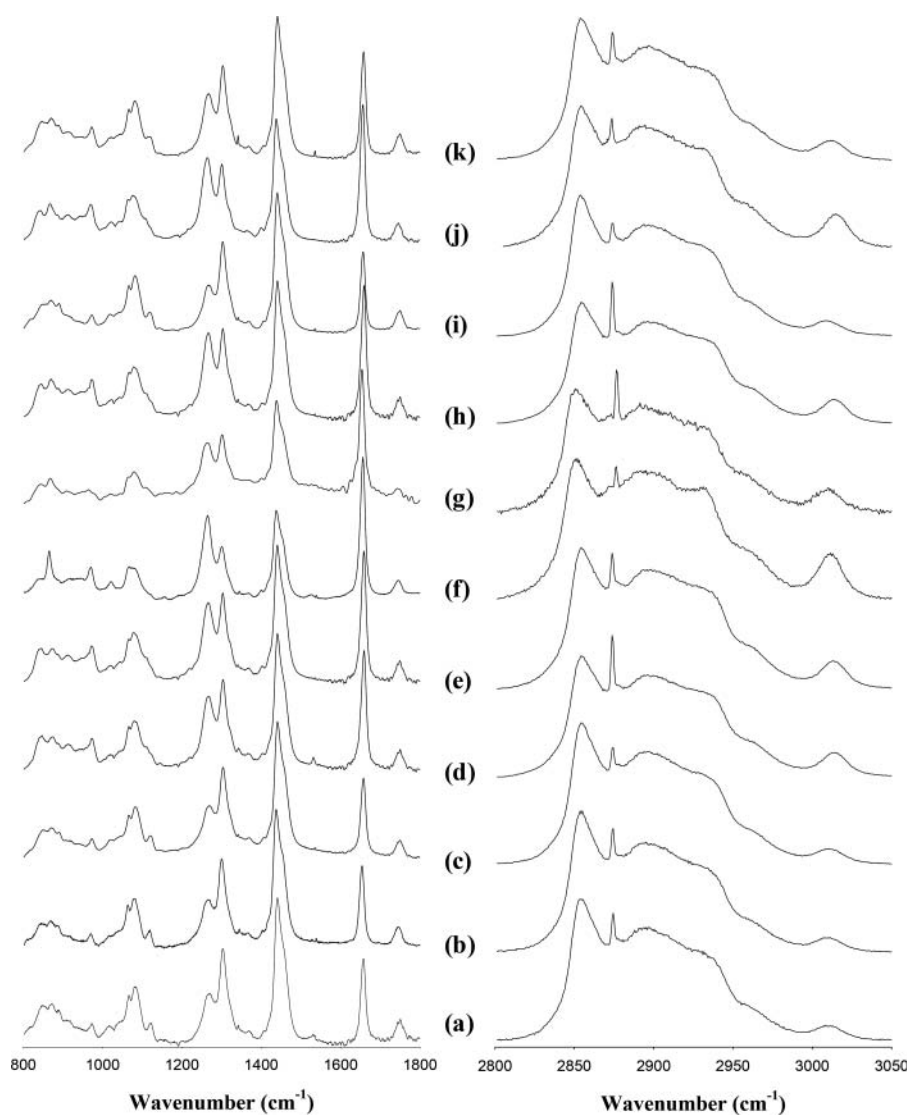


**Figure 2.** Raman spectra of different vegetable oils: (a) extra virgin olive; (b) refined olive; (c) pomace olive; (d) corn; (e) sunflower; (f) linseed; (g) wheat; (h) soy; (i) macadamia; (j) walnut; and (k) pecan nut.

differentiate the spectra. For example, the application of this PCA method has allowed the classification of more than 100 oils and fats from its Raman spectral characteristics (18). The discrimination of virgin olive oil from other edible oils, and the detection and quantification of virgin olive oil adulteration, can be achieved by this technique and the PCA analysis (5).

Based on the foregoing, Raman spectroscopy is a very promising tool for analytical purposes as it allows large numbers of samples to be measured in a short time; also, it is non-destructive and requires no sample preparation. Moreover, Raman spectra exhibit well-defined bands for vibrational transitions, which is highly useful for structural elucidation. In combination with chemometric treatments, Raman spectroscopy is a highly powerful tool for extracting quantitative chemical information from even complex matrices. Recent

instrumental advances have undoubtedly helped to rapidly expand the industrial applications of this technique. For example, Raman spectroscopy has for some time now been successfully used to assess total unsaturation in oils, classify oils and fats, detect adulteration in virgin olive oil, determine minor components, and evaluate oxidative stability, particularly in olive oil. The sections that follow discuss the most salient uses of this technique for the previous purposes.

### Assessing unsaturation of, and classifying and authenticating, vegetable oils by Raman spectroscopy

One of the earliest uses of Raman spectroscopy for the analysis of edible oils was the determination of the degree of unsaturation (DU), which represents the number of double bonds present in fatty acid chains and can be calculated from the combined proportions of mono-unsaturated acids plus two times the proportion of di-unsaturated acids plus three times the proportion of tri-unsaturated acids, all as determined by gas chromatography. Most official methods for measuring unsaturation in oils are based on the iodine value, which represents the amount of iodide ion taken up by 100 g of oil. This value is determined either instrumentally (e.g., by gas chromatography (23)) or with a chemical method such as that of Wijs (24). However, these “traditional” methods are subject to some limitations and have been increasingly superseded by others including some based on Raman spectroscopy (20,21,25–28).

In a recent study, Ruiz et al. (27) used Raman spectroscopy to determine the degree of unsaturation of 12 vegetable oils including extra virgin olive oils and correlated their results with the corresponding iodine values as determined with a standard method. Table 3 shows the DU values obtained. A plot of DU against the signal intensity for stretching vibrations in  $=C-H-$  bonds, which appeared in the zone from  $3009-3013\text{ cm}^{-1}$  for all oils, exhibited excellent correlation and so did iodine values. These results confirm that Raman spectroscopy is an accurate tool for assessing unsaturation in vegetable oils and an effective alternative to the “traditional” methods. The signal at  $1658\text{ cm}^{-1}$  in the spectra for all oils, which was assigned to *cis*  $C=C$  bonds, provided DU values that were also highly correlated with the iodine values.

Similarly, good correlation between the iodine value and the degree of unsaturation as determined from Raman spectra had previously been observed in other hydrogenated fats

**Table 3.** Degree of unsaturation (DU) and iodine value (IV) of various oils studied elsewhere (26).

| Oil                  | DU    | IV    |
|----------------------|-------|-------|
| Extra virgin olive 1 | 92.1  | 81.4  |
| Extra virgin olive 2 | 91.0  | 81.0  |
| Extra virgin olive 3 | 95.1  | 83.4  |
| Extra virgin olive 4 | 91.7  | 81.1  |
| Pomace olive         | 93.8  | 82.2  |
| Refined olive        | 92.0  | 81.4  |
| Corn                 | 142.7 | 118.8 |
| Sunflower            | 153.9 | 124.3 |
| Macadamian           | 87.6  | 79.8  |
| Pecan                | 119.6 | 96.5  |
| Walnut               | 171.8 | 134.0 |
| Brazilian nut        | 122.0 | 106.9 |

(20,21). Thus, by using standards of fatty esters, Sadeghi-Jorabchi et al. (21) confirmed such correlation and found that the frequency of the stretching vibration of C = C bonds was  $13\text{ cm}^{-1}$  greater in *cis* isomers than in *trans* isomers. The most substantial differences among hydrogenated fats were those in the  $1200\text{--}1800\text{ cm}^{-1}$  region. Obviously, hydrogenation reduces total unsaturation in oil and decreases the *cis* C = C signal in its Raman spectrum as a result. However, as revealed by the presence of a shoulder at  $1666\text{ cm}^{-1}$ , hydrogenation also increases the signal for *trans* monoenes.

The joint use of Raman spectroscopy and chemometric methods has enabled the determination of DU in vegetable oils and its correlation with the iodine value. Thus, iodine values estimated by Raman spectroscopy were found to be comparable to those provided by the AOCS certified titration method albeit with the added advantages of expeditiousness and the need for no toxic reagent (28).

Authenticity is one of the most important quality attributes of food (particularly, high-quality vegetable oils, which are usually much more expensive than lower-grade oils). Most oil authentication methods use gas chromatography. In recent years, however, chemometric processing of spectroscopic data has been increasingly used for this purpose. Thus, Baeten et al. (19) used FT-Raman spectra to classify oils and fats of animal and vegetable origin. Applying chemometric methods to the Raman bands for stretching vibrations in C = C and = C-H- bonds allowed them to classify oils and fats into three groups with a high content in polyunsaturated (PUFA), monounsaturated (MUFA), and saturated fatty acids (SFA), respectively. They determined not only the degree of unsaturation of the oils and fats, but also the proportion of each fatty acid class.

In a subsequent study, Baeten et al. (29) used the FT-Raman technique to distinguish virgin olive oil from other edible oils. This required calibrating the Raman spectrometer in order to quantify linolenic acid in the oils. Olive oil contains very little linolenic acid—less than 1%—; therefore, the presence of higher proportions of this acid provides unequivocal evidence of adulteration with other (soybean, corn) oils. In addition, the Raman technique allows the degree of adulteration to be estimated.

Similar studies have been conducted by other authors. In one, Korifi et al. (31) successfully classified 92.3% of French oils from various protected designations of origin by chemometric processing of their Raman spectra. Also, Ruiz et al. (22) used Raman spectroscopy to discriminate oils with a high added value such as those of macadamia and pecan nuts from lower-quality oils such as those of corn and sunflower. Discrimination was easily accomplished by examining the bands for C = C and = C-H- bond vibrations (i.e., by comparing the degree of unsaturation of the oils). The Raman technique also proved useful for detecting adulteration of macadamia oil (DU = 87.65) with corn oil (DU = 142.71) or sunflower oil (DU = 153.90), which resulted in measurable changes in the previous bands and allowed the extent of adulteration to be assessed.

### Use of Raman spectroscopy for assessing oxidation in vegetable oils

Oxidation of fats in general, and olive oil in particular, not only degrades their quality but also can adversely affect food safety and consumers' health. Vegetable oils undergo oxidation of double bonds in fatty acid chains via a free radical mechanism. The process targets the carbon atom in  $\alpha$  to the double bond and produces an allyl hydroperoxide as illustrated for oleic acid in Figure 3 (32). As a result, hydrocarbon chains retain their double bonds but the

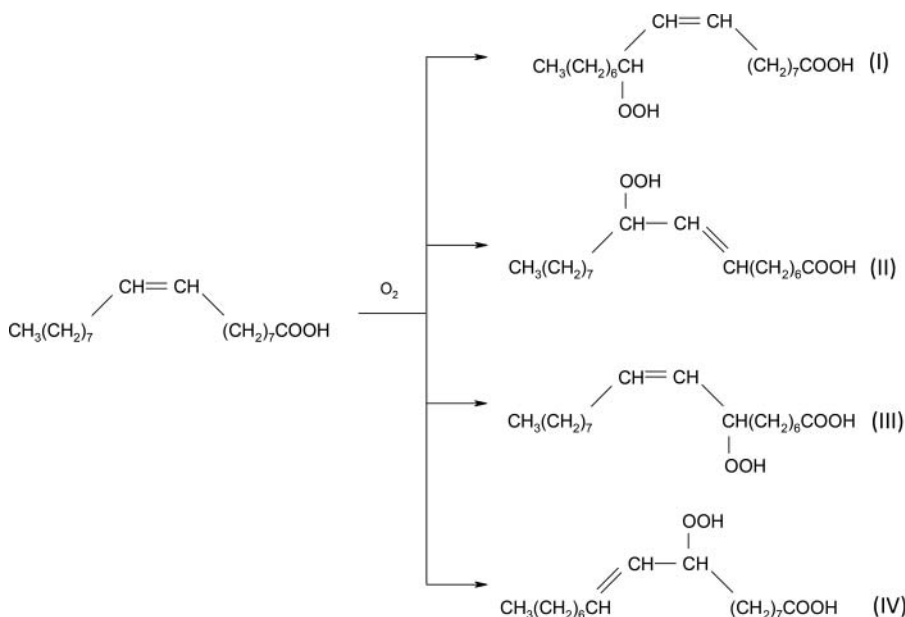


**Figure 3.** Allyl hydroperoxides formed by oxidation of oleic acid.

bonds may undergo positional and configurational changes. In a subsequent step, the resulting hydroperoxides are further oxidized to aldehydes, ketones and esters (see Table 4).

The European Union has set the maximum tolerated degree of oxidation of extra virgin olive oil in terms of parameters  $K_{232}$  and  $K_{270}$ , which represent the absorbance at 232 and 270 nm, respectively, in EC Commission Regulation 1989/3. The official method for determining the peroxide value of an oil involves two steps. In the first step, peroxides are reacted with excess iodide ion; in the second, the resulting iodine is titrated with a standard solution of sodium thiosulphate. As stated above, the degree of unsaturation of an oil is measured in terms of its iodine value, which is the amount, in grams, taken up by 100 g of oil.

Some authors have used physical (33–37) and spectroscopic methods (NMR (38–40) and IR spectroscopy (41–43), mainly) to examine degradation in various vegetable oils upon heating in the air. Recently, Raman spectroscopy was also successfully used to monitor lipid oxidation and assess oxidative stability in vegetable oils. The thermal decomposition of a

**Table 4.** Decomposition products of oleate hydroperoxides.

| Hydroperoxide<br>(from Figure 1) | Decomposition products                                                               |
|----------------------------------|--------------------------------------------------------------------------------------|
| I                                | Decanal + Methyl 8-oxooctanoate 2-Undecenal + Methyl heptanoate                      |
| II                               | Nonanal + Methyl 9-oxononanoate 2-Decenal + Methyl octanoate                         |
| III                              | Octane + 1-octanol + Methyl-10-oxo-8-decenoate<br>Nonanal + Methyl 9-oxononanoate    |
| IV                               | Heptane + 1-heptanol + Methyl 11-oxo-9-decenoate<br>Octanal + Methyl 10-oxodecanoate |

vegetable oil alters its characteristic Raman bands (44). Thus, it gives rise to a new band (a shoulder) at ca.  $1640\text{ cm}^{-1}$  which has been assigned to degradation products (aldehydes mainly); also, it decreases the band at  $1526\text{ cm}^{-1}$ , which is associated to stretching vibrations of  $\text{C}=\text{C}$  bonds, and those at  $3105\text{ cm}^{-1}$ , and  $1267\text{ cm}^{-1}$ .

In a pioneering study in this field, Muik et al. (45) examined chemical changes in edible oils including virgin olive oil upon heating at  $160^\circ\text{C}$  for 9 h. Prior to heating, the Raman spectrum for virgin olive oil exhibited a band at  $1525\text{ cm}^{-1}$  that was assigned to  $\text{C}=\text{C}$  stretching vibrations in highly conjugated compounds such as carotenoids. Because these compounds disappear during the refining process, they should only be present in virgin olive oil. The large number of conjugated double bonds in carotenoids impart them antioxidant properties. Because heating for 90 min caused the band at  $1525\text{ cm}^{-1}$  to disappear, this length of time was taken to be the stability period for virgin olive oil. Other oils exhibited different stability times (e.g., 60 min in sunflower oil and 120 min in corn oil) which the authors ascribed to the presence of tocopherols added as antioxidants. The greatest differences between the Raman spectra for the six oils studied were those in the bands for  $\text{C}=\text{C}$  stretching vibrations, which appeared at 1620, 1680, and  $1655\text{ cm}^{-1}$ . The ratio of the difference between the areas under the former two to the intensity of the latter,  $A_{1620-1680}/I_{1655}$ , was used to successfully monitor the oxidation of vegetable oils because it represented the formation of conjugated compounds (dienes and trienes) and *cis-trans* isomerization during the oxidation process. Virgin olive oil was that exhibiting the lowest ratio at any heating time, the other five oils (peanut, rapeseed, corn, sunflower, and safflower) exhibiting similar results in this respect after 7 h of heating. A subsequent study by Muik et al. (46) using two-dimensional Raman spectroscopy in combination with chemometric methods confirmed these results.

Guzmán et al. (47) also used chemometric methods jointly with Raman spectra to assess oxidative stability in olive oil. The most salient changes occurred below  $1800\text{ cm}^{-1}$ , particularly at  $1267\text{ cm}^{-1}$  (symmetric rocking of *cis*  $\text{C}=\text{C}$  bonds),  $1302\text{ cm}^{-1}$  (in-phase twisting of methylene groups),  $1442\text{ cm}^{-1}$  (scissoring of methylene groups,  $\delta_{\text{CH}_2}$ ),  $1655\text{ cm}^{-1}$  (stretching of *cis*  $\text{C}=\text{C}$  bonds), and  $1747\text{ cm}^{-1}$  (stretching of  $\text{C}=\text{O}$  bonds in esters).

More recently, Ruiz et al. (27) assessed the oxidative stability of four extra virgin olive oils, an olive pomace oil, and a mixture of virgin and refined olive oil (i.e., the three commercially allowed olive oil grades, of which extra virgin oil is the most expensive). As shown in Figure 4a for the  $2995\text{--}3040\text{ cm}^{-1}$  region, the Raman spectra for the six oils before heating were very similar. The  $2800\text{--}3050\text{ cm}^{-1}$  region exhibited five bands at ca.  $2855$ ,  $2874$ ,  $2894$ ,  $2932$ , and  $2969\text{ cm}^{-1}$  typical of  $\text{C-H}$  stretching vibrations in methyl and methylene groups in addition to a sixth band above  $3000\text{ cm}^{-1}$  corresponding to  $=\text{C-H-}$  stretching vibrations. Obviously, these bands should be largely due to the major fatty acid in the oils: oleic acid (see Table 5). Heating the oils at  $190^\circ\text{C}$  for 2 or 5 h altered the band at ca.  $3010\text{ cm}^{-1}$  for  $=\text{C-H-}$  bonds and that at ca.  $2874\text{ cm}^{-1}$  for  $\text{C-H}$  bonds in  $\alpha$  with respect to the double bonds. However, it was the band at  $3010\text{ cm}^{-1}$  which best allowed the effect of the thermal treatment to be monitored—those around  $2874\text{ cm}^{-1}$  were more difficult to normalize. As can be seen from Figures 4b and 4c, heating for 2 or 5 h decreased that band in all oils, albeit to a smaller extent in extra virgin olive oil. These results were ascribed to its containing increased amounts of antioxidants such as carotenoids and tocopherols.

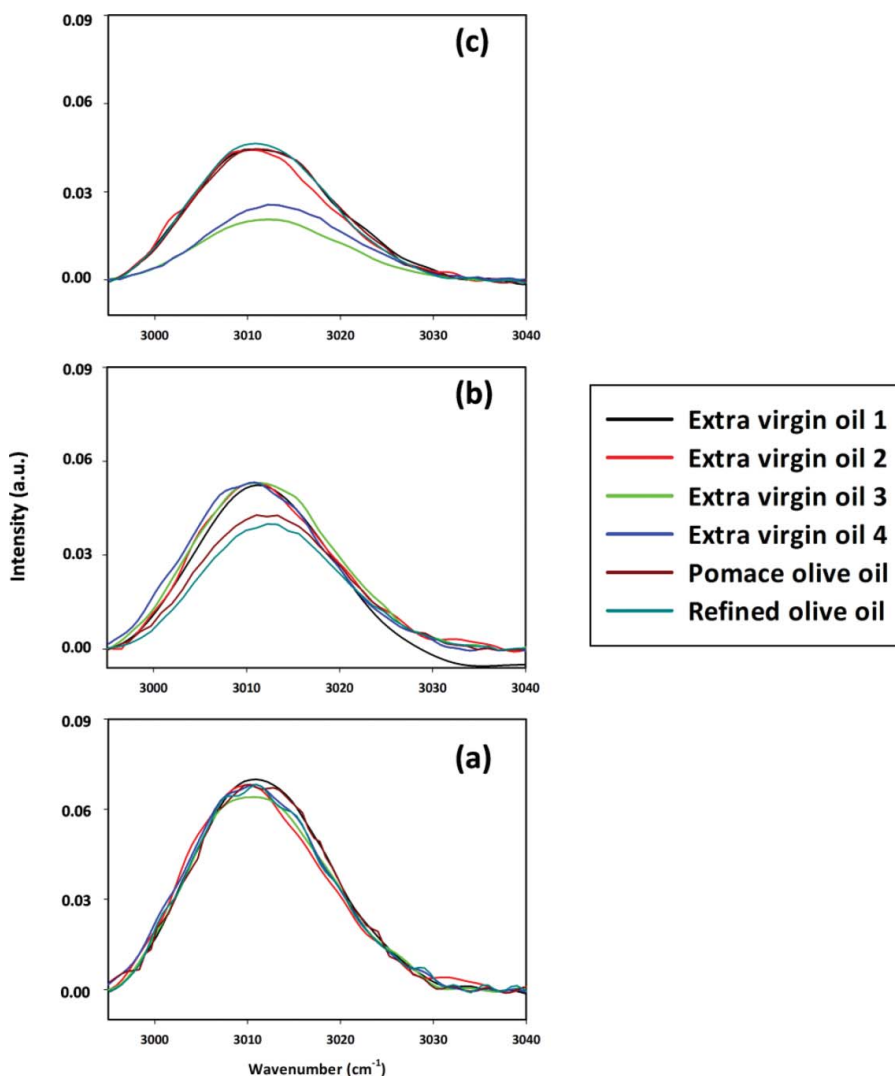


**Figure 4.** Raman bands in the  $2995\text{--}3040\text{ cm}^{-1}$  region for olive oils without heating (a) and after heating at  $190^\circ\text{C}$  for 2 h (b) or 5 h (c).

### Use of Raman spectroscopy for detecting adulteration in olive oil

Extra virgin olive oil is highly appreciated for its sensory and nutritional properties, which make it 3–5 times more expensive than the more common edible vegetable oils and has propitiated its adulteration with lower-grade oils degrading its quality.

At present, vegetable oils—extra virgin olive oil obviously included—are authenticated and adulteration detected by using liquid or gas chromatography, frequently coupled to mass spectrometry. This affords the determination and quantitation not only of fatty acids (48,49), but also of minor components such as sterols or tocopherols (50,51), as well as the detection of pesticides (49,52,53)—the presence of which rules out award of an organic production label. These techniques are expensive and use large amounts of reagents and

**Table 5.** Fatty acid composition of various olive oils.

| Oil            | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 |
|----------------|-------|-------|-------|-------|-------|
| Extra virgin 1 | 11.2  | 2.4   | 78.4  | 5.5   | 0.6   |
| Extra virgin 2 | 11.3  | 3.2   | 78.0  | 5.0   | 0.7   |
| Extra virgin 3 | 10.6  | 3.1   | 75.2  | 8.6   | 0.6   |
| Extra virgin 4 | 9.4   | 3.0   | 82.6  | 3.7   | 0.5   |
| Pomace         | 9.6   | 3.7   | 77.3  | 6.9   | 0.7   |
| Refined        | 10.7  | 3.4   | 72.7  | 6.0   | 0.5   |

solvents, two shortcomings which have raised interest in the development of alternative methods requiring no sample manipulation in order to dispense with the use of reagents and solvents, and make the process more economical. One such technique is Raman spectroscopy, which is increasingly being used to authenticate virgin olive oil (54–56).

Baeten et al. (57) were the first to use Raman spectroscopy to detect adulteration in virgin olive oil. This required prior calibration of the spectrometer with a virgin olive oil standard supplied with variable amounts of trilinolein. Other virgin olive oil samples were adulterated with pomace, soybean, and corn oil in variable proportions. Although the authors admitted that their methodology still required some refining, statistical processing of the Raman results allowed the degree of adulteration of the virgin olive oil samples to be estimated.

Yang and Irudayaraj (58) compared the efficiency of Raman spectroscopy with that of near- and mid-infrared spectroscopy in detecting adulteration of extra virgin olive oil with pomace oil. Although they succeeded in quantifying the amount of pomace oil contained in the extra virgin olive oil with the three techniques, Raman spectroscopy provided the highest correlation coefficient and the lowest standard error of prediction. These results further testify to the potential of the Raman technique for oil authentication.

One other problem faced by the olive oil industry is the absence of an official method of analysis for certifying adulteration with peanut oil. Raman spectroscopy has been successfully used in combination with chemometric analysis to this end (59,60).

### Use of Raman spectroscopy for determining minor components of vegetable oils

As stated in a previous section, Raman spectroscopy is useful for analyzing the triglyceride fraction, which is the most abundant in vegetable oils. Also, it allows other minor components such as those in unsaponifiable matter (viz., hydrocarbons, sterols, terpene alcohols, polyphenols) to be examined (61). Usually, determining these compounds, which account for 0.2–2% of the composition of vegetable oils, requires their prior isolation. Thus, the usual method for quantifying sterols and terpene alcohols in olive oil involves gas chromatography of the oil fraction previously isolated by preparatory thin layer chromatography (Commission Regulation (EEC) No. 2568/91). Alkanols, squalene and tocopherols can also be identified by gas chromatography (62,63). The Raman-based determination of unsaponifiable matter also requires prior extraction, which, however, can be easily and rapidly accomplished by using a number of methods including some endorsed by the European Union (Official Journal of European Union, March 29, 1995, Regulation 69/3–69/4). For example, Baeten et al. (64) applied chemometric methods to the Raman spectra for squalene and

various components of the sterol and terpene fractions in order to discriminate between olive oil and seed oils, and also between virgin olive oils from different olive varieties.

Paira-Martins et al. (65) conducted a pioneering structural study of the polyphenol fraction of olive oil based on Raman spectroscopy and density functional theory (DFT). In this way, they succeeded in identifying the preferential conformations of hydroxytyrosol and its metabolites, the secoiridoid oleuropein, and the oleuropein aglycones 3,4-DHPEA-EA and 3,4-DHPEA-EDA.

In another study, Muik et al. (66) used Raman spectroscopy in combination with partial least squares (PLS) regression for the direct determination of the fatty acid content of olive oils and olives. For this purpose, they compared the spectra for pure oleic acid, olive oil and milled olives. All spectra were similar and contained the typical strong bands at  $1267\text{ cm}^{-1}$  ( $\delta = \text{C-H}$  deformation),  $1302\text{ cm}^{-1}$  (in-phase methylene twisting),  $1442\text{ cm}^{-1}$  ( $\delta_{\text{CH}_2}$ ),  $1655\text{ cm}^{-1}$  ( $\nu_{\text{C}=\text{C}}$ ),  $1747\text{ cm}^{-1}$  ( $\nu_{\text{C}=\text{O}}$ , only in oil and olives),  $2852\text{ cm}^{-1}$  ( $\nu_{\text{CH}_2}$  sym), and  $2970\text{ cm}^{-1}$  ( $\nu_{\text{CH}_3}$  sym). Milled olives additionally exhibited a band at  $1604\text{ cm}^{-1}$  that was assigned to stretching of aromatic rings in lignin—a major constituent of olive kernels. The  $1200\text{--}1800\text{ cm}^{-1}$  and  $1000\text{--}1800\text{ cm}^{-1}$  regions were used to quantify free fatty acids in olive oil, using PLS regression. Although the results were less accurate than those obtained with the official method, they showed that the Raman technique is useful for fast, on-line quality control of oil production. Also, the Raman methodology is much more expeditious than the official method for olives as the only pretreatment needed is milling the fruits.

A similar methodology was used by El-Abassy et al. (67) to determine the free fatty acid content of extra virgin olive oils. They used the  $945\text{--}1600\text{ cm}^{-1}$  spectral region, which included the band for carotenes because they found these compounds to be statistically significant for predicting the content in fatty acids—in fact, carotenes exhibited the highest PLS correlation coefficients.

## Conclusions

This work demonstrates that Raman spectroscopy is a valuable and useful technique for determining the degree of unsaturation of a vegetable oil. In combination with statistical analysis, this spectroscopy allows to classify and authenticate certain oils. Also, Raman spectroscopy can be used to monitor the oxidative decomposition of olive oil by effect of heating and the presence of oxygen. Finally, the detection of adulterants and the determination of minor components of vegetable oils can be achieved by Raman spectroscopy.

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