# Interpretation of Fourier Transform Raman Spectra of the Unsaponifiable Matter in a Selection of Edible Oils

Vincent Baeten,† Pierre Dardenne,† and Ramón Aparicio*,‡

Quality Department, CRAGx, Chaussée de Namur 24, Gembloux 5030, Belgium, and Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, Sevilla 41012, Spain

The unsaponifiable matter of edible oils is a source of information for their characterization and authentication. FT-Raman spectroscopy has been applied with success to the determination of the spectra of the unsaponifiable matter of varietal olive oils as well as other refined and crude edible oils. The spectra of the major unsaponifiable series of compounds (squalene, sterolic, and terpenic alcoholic fractions), together with $\beta$-carotene and lutein, have been used to explain the most prominent bands found in the spectra of the unsaponifiable matter of 15 edible oil samples. The order of the scattering intensities of the varietal olive oils agrees with the results obtained by chromatography. An unsupervised multivariate statistical analysis of selected bands points out differences between olive oils and the other seed oils and also among varietal virgin olive oils.

**Keywords:** *Raman spectroscopy; olive oil; hazelnut oil; seed oils; unsaponifiable matter; squalene; carotenoids*

## INTRODUCTION

In recent decades, the high potential of spectroscopic techniques such as near-infrared or mid-infrared spectroscopy in food analysis has been clearly established. These techniques are widely used to quantify the major food compounds (water, protein, fat, etc.) and are now favored techniques in the grain, milk, and fat products industries (*1*, *2*). The usual explanation for the success of spectroscopic techniques is twofold. On the one hand, there are the analytical advantages as these are rapid, direct, and economic techniques that do not use any reagents. On the other hand, there are the instrumental advantages, including no or reduced sample preparation, the suitability for use at-, on-, and in-line, the compatibility with fiber optics, and the possibility of configuring the techniques as a "push-button" instrumentation (*3*−*5*).

The most frequent drawback with spectroscopic techniques is the difficulty of the chemical interpretation of the spectral data. Separative techniques such as chromatography generate information (a chromatogram) consisting mainly of well-resolved and separate peaks, that is, discrete information. However, spectroscopic techniques such as infrared and Raman generate continuous information (a spectrum) rich in both isolated and overlapped bands. Whereas in chromatography each peak is, in general, characteristic of a precise compound, in infrared and Raman spectroscopy the bands are the result of the vibration of one or more chemical bonds (e.g., C−H or C═C) present in the compounds that make up the sample (*6*). At present, it appears that the initial disadvantage of these spectroscopic techniques (i.e., the interpretation of the whole chemical information—the so-called fingerprint) has become their main advantage with the help of mathematical algorithms (*7*, *8*). These algorithms have allowed an improvement in precision and accuracy to levels not achieved before.

Using the whole, or selected, information contained in the spectra and the appropriate mathematical algorithms, the application of spectroscopic techniques to a new branch of food science—the assessment of product authenticity—is now possible. This is reflected in the myriad papers describing methods based on spectroscopic techniques for the authentication of food products (e.g., coffee, fat products, cereal, etc.) (*9*). These studies demonstrate the contribution of almost all possible chemical components in the infrared and Raman spectra of a food as the great advantage and feature of spectroscopic techniques in terms of detecting and quantifying adulteration.

Until now, the methodologies proposed for authenticating food products using spectroscopic techniques did not involve any pretreatment or extraction stages (*10*, *11*). For major food compounds, however, there is a limited application of authenticity assessment as it is possible to find an adulterant with similar composition (*12*), for example, virgin olive oil versus high oleic sunflower oil (*13*). In fact, minor compounds, which occur mainly in the unsaponifiable matter, are more characteristic of each fat and oil and hence more useful in authenticity tests (*14*). Sterols, alcohols, hydrocarbons, or other minor compounds have been used with success in the authentication of edible oils (*13*, *15*, *16*).

In an effort to improve the results of the methodology developed by Baeten et al. (*17*, *18*) for the authentication of virgin olive oil using FT-Raman spectroscopy, perspectives of the analysis of unsaponifiable matter using FT-Raman spectroscopy were studied. In virgin olive oil, this fraction is composed mainly of hydrocarbons (40−60%), sterols (20−30%), and terpenic and aliphatic alcohols (20−30%), in addition to minor components such as tocopherols and pigments (*19*). The choice of the

* Author to whom correspondence should be addressed (e-mail aparicio@cica.es).

† CRAGx.

‡ CSIC.

10.1021/jf010146x CCC: $20.00 
Published on Web 10/05/2001

Raman technique was justified by some decisive advantages over infrared spectroscopy with regard to the authentication of edible oils (*20*). Raman scattering arises from the changes in the polarizability of the electron distribution in the molecule, whereas infrared absorption requires a change of the dipole moment. The experience dictates that some vibrations, which are absent in the infrared technique, are strongly Raman active and vice versa. For example, the intensity of the symmetric stretching vibrations of the double bands is usually very weak in mid-infrared spectroscopy, while its Raman scattering band is very strong.

The present study describes the potential of combining extractive and spectroscopic techniques in the analysis of unsaponifiable matter. The application of this approach to the characterization and authentication of virgin olive oil is discussed. The unsaponifiable matter of seven virgin olive oils from different varieties and eight edible oils from other vegetable sources was analyzed using FT-Raman spectroscopy.

## MATERIALS AND METHODS

**Sampling.** A set of seven authentic virgin olive oils from Spain (Hojiblanca, Arbequina, and Picual), Italy (Coratina and Frantoio), and Greece (Koroneiki and Tzunnati), as well as olive pomace oil and refined olive (two) oil, was chosen from the SEXIA sample bank (*21*). Refined and crude hazelnut plus refined corn, high-oleic sunflower, and soybean oils, obtained from local retailers, were added to the sample set analyzed. $\beta$-Carotene and lutein were obtained from the Instituto de la Grasa (Sevilla, Spain).

**Chemical Analysis.** For the extraction of unsaponifiable matter the humid process (i.e., extraction of unsaponifiable matter from an aqueous or alcoholic solution of the soap) recommended by the European Union for olive oil analysis was used (*22*). A 5 g portion of the sample was saponified and extracted with diethyl ether. The extract was washed three times with distilled water. Anhydrous sodium sulfate was then added to dry the sample. The extract was filtered, and the solvent was evaporated before the unsaponifiable matter was dried under a nitrogen stream to ensure the elimination of any traces of solvent and water. The nonsaponified segment was resaponified and re-extracted when incomplete saponification was detected. The fractionation of the unsaponifiable components into sterols and alcohols was carried out by thin-layer chromatography on silica gel. The sterols and triterpenic alcohols were recovered from the plate by scraping their band and extracting them with diethyl ether (*23*). The unsaponifiable matter of each sample was prepared in duplicate and stored in a freezer before Raman analysis.

Squalene was isolated according to the modified process of the standard analysis of waxes (*23*). A low-polarity solvent (98.5 hexane/1.5 diethyl ether) was used in a silica gel column to separate squalene from esters of aliphatic alcohols and sterols esters (*23*). $\beta$-Carotene and lutein were obtained from spinach and alfalfa leaves according to the procedure described in ref *24*.

**Raman Analysis.** The Raman spectra were obtained on a Nicolet 910 FT-Raman spectrometer (Nicolet Analytical Instrument, Madison, WI). The detector was a nitrogen-cooled germanium detector, and the excitation beam was an Nd:YAG laser with an output at 1064 nm (9394 $cm^{-1}$). The laser power ranged from 0 to 1200 mW at the focus after filtering. All spectra were taken in the 180° backscattering refractive geometry.

The unsaponifiable matter was put into the in-house tube (*25*). A known amount of pure carbon tetrachloride ($CCl_4$) had previously been added to the unsaponifiable matter and to the various compounds and fractions analyzed, because some of these samples were solid or viscous and needed to be dissolved before their insertion into the cell. Carbon tetrachloride was used because it is a classic solvent for spectroscopic analysis (*26*); it does not react with the products analyzed and shows Raman scattered bands at low frequencies. Its specific Raman shifts are near 790, 762, 459, 314, and 218 $cm^{-1}$ (*6*), and they do not interfere with the most prominent Raman scattered bands of the analyzed compounds.

The spectrometer was linked to a Nicolet 680 D workstation using the Nicolet software version 3.20. The binary files of the nonconverted spectral data were transferred to a personal computer using the NIC2DOS program. The Spectra Calc program, version 2.2 (Galactic Industries Corp, St. Louis, MO), was then used for spectra manipulation and transformation.

**Statistical and Mathematical Analysis.** Univariate and multivariate analyses were performed using the Excel program version 7.0 (Microsoft Corp., Redmond, WA) and Statistica 6.0 (Statsoft, Tulsa, OK). The spectra were exported from the Spectral Calc software to the Excel software. The Excel software was used to convert the spectral data frequencies to Raman shifts according to the frequency of the excitation source (9394 $cm^{-1}$). The same software was used to carry out the normalization procedure, to perform the univariate statistical analysis, and to export the data to the Statistica software.

The unsupervised procedure of multidimensional scaling (MDS) was applied to analyze the similarities and dissimilarities among samples. It can be considered an alternative to factor analysis, the main goal of which is to detect meaningful underlying dimensions. This procedure was used, instead of principal component analysis, because MDS does not require that the underlying data be distributed as a normal multivariate and that the relationships be linear. MDS can be applied to any kind of distances or similarities, whereas factor analysis requires us to first compute a correlation matrix, which can result in a problem when, such as in this case, the number of selected bands is several times the number of samples (*27*). MDS is not so much an exact procedure as a way to rearrange samples in an efficient manner. The program actually moves the samples around in the space defined by the requested number of dimensions and checks how well the distances between samples can be reproduced by the new configuration. Squared Euclidean distance and Wards' linkage methods were used for this purpose by cluster analysis procedure, whereas the measure of the "goodness-of-fit" was carried by the stress algorithm.

The Statistica software was used to perform the cluster analysis and multidimensional scaling. Statistica and Matlab (The MathWorks) software were used to draw the graphics.

Parts of the spectra containing mainly the baseline variation were removed. The information of three regions—I (3089.9−2802.5 $cm^{-1}$), II (1705.2−1610.8 $cm^{-1}$), and III (1570.8−1250.1 $cm^{-1}$)—was retained and then normalized. The normalized values were calculated as follows: normalized scattering intensity at each frequency of the spectrum = (raw scattering intensity at frequency $x$ − mean of the scattering intensities of the spectrum)/standard deviation of the scattering intensities of the spectrum. The normalized scattering intensity values of the frequencies 3089.8, 1705.2, and 1570.8 $cm^{-1}$ were then subtracted from the scattering intensities measured between 3089.9 and 2802.5, between 1705.2 and 1610.8, and between 1570.8 and 1250.1 $cm^{-1}$, respectively.

## RESULTS AND DISCUSSION

The unsaponifiable matter of oil is usually defined as the set of compounds that do not react with sodium or potassium hydroxide and produce soaps that remain soluble in classic fat solvents (e.g., hexane and ether) after saponification (*23*). The unsaponifiable content of oil obviously depends not only on the biological origin but also on the chemical and physical treatments used to extract and refine the product as well as the solvent used in the unsaponifiable matter extraction. In general, the unsaponifiable content is between 0.2 and 2% for vegetable oils, although it can reach 10% in some fats such as shea butter (*19*).

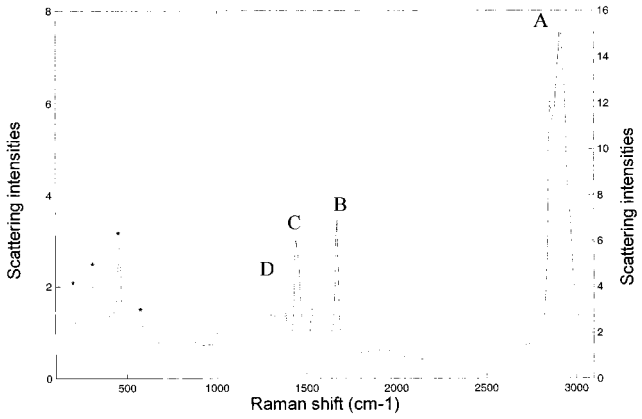


**Figure 1.** FT-Raman spectra of a virgin olive oil (dashed line) and its unsaponifiable matter (continuous line) (LAS ≅ 500 mW, RES = 4 $cm^{-1}$, SCAN = 200, smoothed data).

The composition of unsaponifiable matter is widely used in studies of the characteristics and authenticity not only of an oil or fat but also of other food products such as coffee (*9*). In this regard, the unsaponifiable matter is fractioned into several groups of constituents (e.g., hydrocarbons, sterols, diterpenic, triterpenic, and aliphatic alcohols, tocopherols, pigments) using chromatographic procedures such as column and thin-layer chromatography (TLC). The target group of constituents is then isolated and its composition determined using gas chromatography (sterols and hydrocarbons) or high-performance liquid chromatography (HPLC) (pigments and tocopherols). Following these procedures, it is possible to obtain accurate, useful, and relevant information for characterizing and authenticating edible oils (*13*, *14*, *16*). Although these procedures allow accurate information to be obtained on one or a series of determined compounds, they do not allow one to obtain global information on the fraction analyzed, let alone on the entire unsaponifiable matter. Moreover, they are time-consuming and destructive.

The potential of FT-Raman spectroscopy in the characterization and authentication of vegetable oils has been discussed in earlier papers (*8*, *17*, *18*, *28*). Figure 1 presents the FT-Raman spectrum (dashed line) of a virgin olive oil. The spectrum shows a series of bands with various Raman scattering intensities and shapes.

The FT-Raman spectrum of the unsaponifiable matter of the same virgin olive oil is also shown in Figure 1 (continuous line). In comparison to the spectrum of the virgin olive oil, the profile of the Raman scattered bands is very different. The asterisks indicate the Raman scattered bands characteristic of $CCl_4$. This chemical compound shows bands below 800 $cm^{-1}$ that do not interfere with the peaks of the unsaponifiable spectrum (*6*). From high to low Raman shifts, different comments can be made. The spectrum has been divided arbitrarily into five regions (A−E) corresponding to different vibrations of various groups of atoms.

Region A of the unsaponifiable matter spectrum presents a very different shape in comparison with that of the olive oil spectrum. In the spectrum of unsaponifiable matter, a weak bump is observed near 3005 $cm^{-1}$ and two intense Raman bands are observed near 2910 and 2855 $cm^{-1}$ (C−H stretching vibrations of the CH, $CH_2$, and $CH_3$ groups). The unsaponifiable matter spectrum does not show a band in the vicinity of 1750 $cm^{-1}$ (C═O stretching vibration). Region B (C═C stretching vibrations) shows a single intense scattered band. In comparison with the band in the olive oil spectrum (*18*), the peak is more intense and has shifted to a higher frequency (1670 versus 1655 $cm^{-1}$). This reveals the presence of a significant content of trans isomers in the unsaponifiable matter (*29*). Region C shows two peaks centered near 1530 and 1440 $cm^{-1}$, respectively. Whereas the latter peak is present in both unsaponifiable and olive oil spectra, the former is not visible in the virgin olive oil spectrum or in the spectrum of other edible oils and fats. Region D and part of region E contain a series of peaks with profiles and intensities very different from those in the olive oil spectrum. In the spectra of unsaponifiable matter, there are various bands in the vicinity of 1380, 1330, 1305, 1280, 1160, and 1005 $cm^{-1}$.

To assign part of the Raman scattered bands of the unsaponifiable FT-Raman spectrum, the spectra of various pure compounds (squalene, lutein, and $\beta$-carotene) and fractions (sterols and di- and triterpenic alcohols) were analyzed (Figure 2). It is worth noting that the unsaponifiable matter of a virgin olive oil is composed mainly of hydrocarbons, sterols, and diterpenic, triterpenic, and aliphatic alcohols (*23*). The hydrocarbon fraction (40−60% of the unsaponifiable matter of olive oil) included saturated and unsaturated (normal, branched, terpenic, and aromatic isomers) compounds (*30*, *31*). Squalene is the most important hydrocarbon in almost all edible oils (up to 90% of the hydrocarbon fraction). The concentration range is 2500−9250 $\mu$g/g for olive oils, whereas the content is lower, 16−370 $\mu$g/g, in the other vegetable oils (e.g., sunflower, canola, or peanut oil) (*32*). Figure 2b presents the FT-Raman spectrum of squalene. This compound shows intense Raman scattered bands in the vicinity of 2910, 2860, 1665, and 1435 $cm^{-1}$. These Raman shifts agree with the chemical structure of the squalene, a linear triterpenic polymer of isoprene rich in C═C bands (Figure 3a).

The sterolic fraction (20−30% of the unsaponifiable matter of olive oil) includes an extensive series of compounds with an analogous structure, that is, a tetracyclic nucleus (Figure 3b). The difference between the sterols lies in the number and position of double bands and in the nature of the side chain. In general, vegetable lipids include two to five major sterols with a series of minor sterols. The most abundant sterol in vegetable oils is $\beta$-sitosterol (*14*). In addition to $\beta$-sitosterol (75−93% of the sterolic fraction), we can find campesterol and $\Delta^5$-avenasterol as major sterols in olive oil (*19*). Figure 2c shows the FT-Raman spectrum of the sterolic fraction extracted from a virgin olive oil. The sterolic compounds show bands in the vicinity of 2960, 2940, 2870, 1670, 1440, and 1350 $cm^{-1}$.

Figure 2d presents the FT-Raman spectrum of the fraction (20−30% of the unsaponifiable fraction of olive oil) composed of the diterpenic, triterpenic, and aliphatic alcohols present in olive oil. The most important compounds in this fraction are hexacosanol, tetracosanol, and octacosanol (*23*). The spectrum displayed in Figure 2d shows peaks near 3075, 2960, 2930, 2895, 2870, 1730, and 1450 $cm^{-1}$.

Once the major series of unsaponifiable matter has been studied, there is enough information to explain the most important bands of the unsaponifiable matter of each of the edible oils described under Materials and Methods: seven varietal virgin olive oils, two refined olive oils, one olive pomace oil, one virgin hazelnut oil, and five refined seed oils (corn, hazelnut, soybean, and

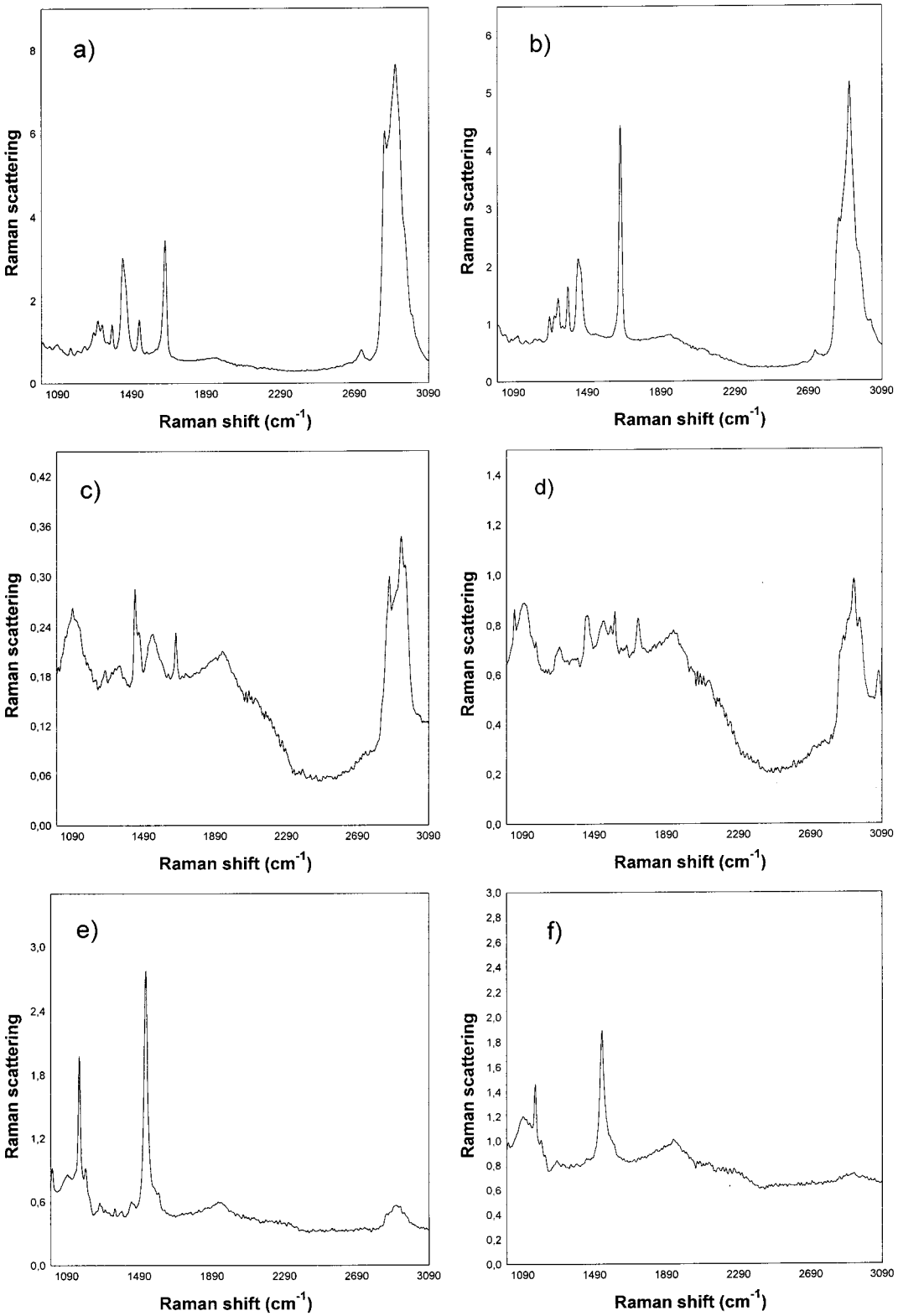


**Figure 2.** FT-Raman spectra of fractions and pure minor compounds included in virgin olive oil: (a) unsaponifiable matter; (b) squalene; (c) sterolic fraction; (d) alcoholic fraction; (e) $\beta$-carotene; (f) lutein (LAS $\cong$ 500 mW, RES = 4 $cm^{-1}$, SCAN = 200, smoothed data).

sunflower). Figure 4a displays the Raman scattering intensities observed near 1530 $cm^{-1}$, corresponding to the second band of region C (Figure 1). The unsaponifiable matter spectra of all refined oil samples do not show a band in this region, whereas those of the virgin olive oil sample present high scattering intensities. The spectra of the unsaponifiable matter of virgin (crude) hazelnut oil show a weak peak in the vicinity of this Raman shift and also olive pomace oil, although it is even weaker. The spectral study of the main compounds and fractions of the unsaponifiable matter (Figure 2b–d) does not explain the band centered near 1530 $cm^{-1}$. However, it is well-known that the refining process (including degumming, decoloration, and deodorization) partially reduces the unsaponifiable matter fraction, although no notable change is observed in the composi-

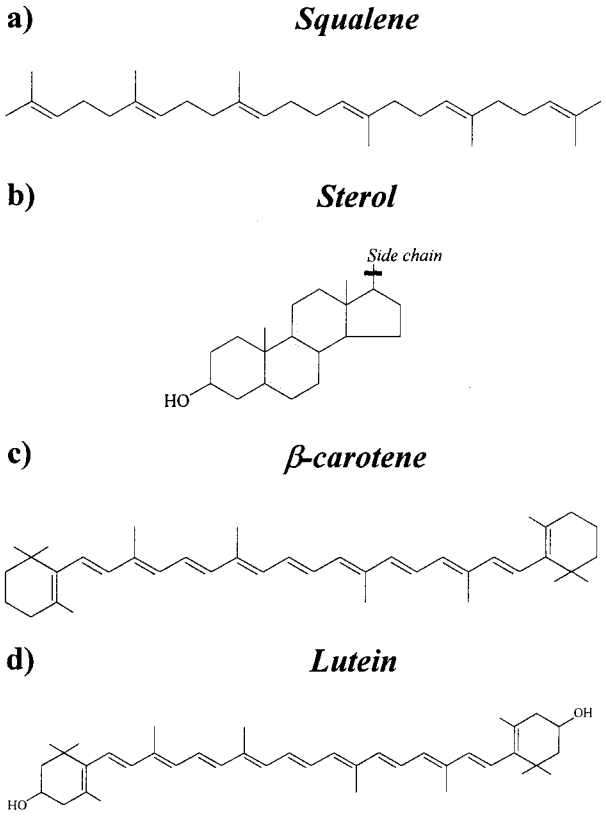


**Figure 3.** Chemical structures of various compounds found in the unsaponifiable matter of a virgin olive oil: (a) chemical structure of squalene; (b) common chemical structure of the whole family of sterols; (c and d) chemical structures of $\beta$-carotene and lutein, respectively.

tion of the major unsaponifiable fraction of refined oil (*33*). Consequently, it was necessary to take into account the minor compounds that are degraded during the refining process in order to explain the absence of a peak in the vicinity of 1530 $cm^{-1}$ (i.e., the pigments and the volatile compounds that are removed during the refining process). As the unsaponifiable fraction of refined oils showed a less intense color than the other samples, we decided to orient our work toward the study of pigments described in a virgin olive oil (*34*). Chlorophylls and carotenoids are the two families of major pigments found in olive oil. These compounds are present in virgin olive oil in concentrations included in the range of 1−10 and 5−10 mg/kg, respectively. The chlorophyll fraction is made up of chlorophylls *a* and *b* and pheophytins *a* and *b*. The carotenoid fraction is made up of xanthenes but mostly of $\beta$-carotene (30−60% of the carotenoid fraction) and lutein that, proceeding from the degradation of the chlorophyll, constitute 5−15% of the carotenoid fraction. The content depends on the olive oil variety, the olive ripeness used to obtain the oil, and the freshness of the oil. Concerning the refined oils, the pigment content of oils that have undergone refining is very low or even insignificant because of the loss during the decoloration process.

The $\beta$-carotene (Figure 2e) spectrum presents peaks centered near 1007.1, 1157.7, and 1525.9 $cm^{-1}$. Less intense bands are included inside the 800−1600 and 2800−3000 $cm^{-1}$ regions. The lutein (Figure 2f) spectrum shows two intense peaks centered near 1157.7 and 1529.7 $cm^{-1}$ and a weak peak near 1007.1 $cm^{-1}$ (Figure 2e). The bands centered near 1155 and 1005 $cm^{-1}$ show a scattering intensity order similar to that observed near 1530 $cm^{-1}$. These bands were observed in the unsaponifiable matter of all virgin olive oils with high scattering intensities in this region. Figure 4a shows the peaks between 1520 and 1535 $cm^{-1}$. Gerrard (*26*) reported that the bands of $\beta$-carotene were present near 1527 and 1158 $cm^{-1}$. Ozaki et al. (*35*) described the existence of these peaks in the FT-Raman spectra of fresh spinach leaf, mandarin peel, fresh leaf tea, egg yolk, and carrot. They stressed that FT-Raman spectroscopy is able to detect levels of $\beta$-carotene lower than 0.008%. Keller et al. (*36*) corroborated these results working with orange peel, carrot, and pure $\beta$-carotene. The intense bands observed in Figure 2e,f cannot be assigned to a traditional Raman scattering phenomenon as the bands near 1530 and 1160 $cm^{-1}$ are the resonance-enhanced bands of the C=C and C−C stretching modes, respectively, of the conjugated polyene system present in carotenoids (Figure 3c). These two groups constitute the chromophore that gives rise to the visible absorption of the carotenoid compounds. Other Raman-active vibrations in the molecule remain at their normal strength (e.g., bands observed in the 3000−2800 $cm^{-1}$ region of the $\beta$-carotene) (*26*). The degree of resonance enhancement depends on how close the laser wavelength is to the wavelength of the maximum absorption of the electronic transition. The intensity also depends on the nature of the electronic transition accounting for that absorption (*26*, *37*).

Focusing again on the spectra of the unsaponifiable matter of virgin olive oils presented in Figure 4a, these samples present an intense band in the vicinity of 1530 $cm^{-1}$. The scattering intensity order, from high to low, for these samples is Coratina > Picual, Hojiblanca > Tzunnati, Arbequina, Koroneiki, Frantoio. Using HPLC and appropriate extraction protocol, Gandul-Rojas and Mínguez-Mosquera (*34*) quantified total contents of carotenoids of 10.2, 9.2, and 4.5 mg/kg for Picual, Hojiblanca, and Arbequina varieties, respectively. The order of the FT-Raman scattering intensities observed near 1530 $cm^{-1}$ is in accord with these figures. Moreover, the band of unsaponifiable matter of the variety Picual has shifted slightly to a higher Raman shift than the bands corresponding to the varieties Hojiblanca and Arbequina. These differences are due to the differences in the ratio between lutein and $\beta$-carotene in the three varieties. In the variety Picual the ratio lutein/$\beta$-carotene is about 4.02−5.14; in the varieties Arbequina and Hojiblanca the same ratios are about 1.30 and 2.73, respectively (*34*). Figure 5 presents the bands near 1530 $cm^{-1}$ of the three virgin olive oil varieties and of the $\beta$-carotene and lutein. For the record, $\beta$-carotene has the maximum peak in the vicinity of 1525.9 $cm^{-1}$, whereas the maximum peak of the lutein is near 1529.7 $cm^{-1}$. The ratio lutein/$\beta$-carotene, quantified by HPLC, has been suggested (*34*) to differentiate varietal virgin olive oils.

The scattered band observed for each sample in the region between 1645 and 1665 $cm^{-1}$ is shown in Figure 4b. The spectra of virgin olive, refined olive, and olive pomace oil unsaponifiable matters present a maximum band in the vicinity of 1665 $cm^{-1}$. In contrast, the unsaponifiable matter of the other vegetable oils has a peak centered near 1655 $cm^{-1}$. The scattering intensities observed in this region are lower for the olive pomace, corn, hazelnut (virgin and refined), sunflower, and soybean oil samples than for the virgin and refined olive oils. The bands observed in the olive oil samples

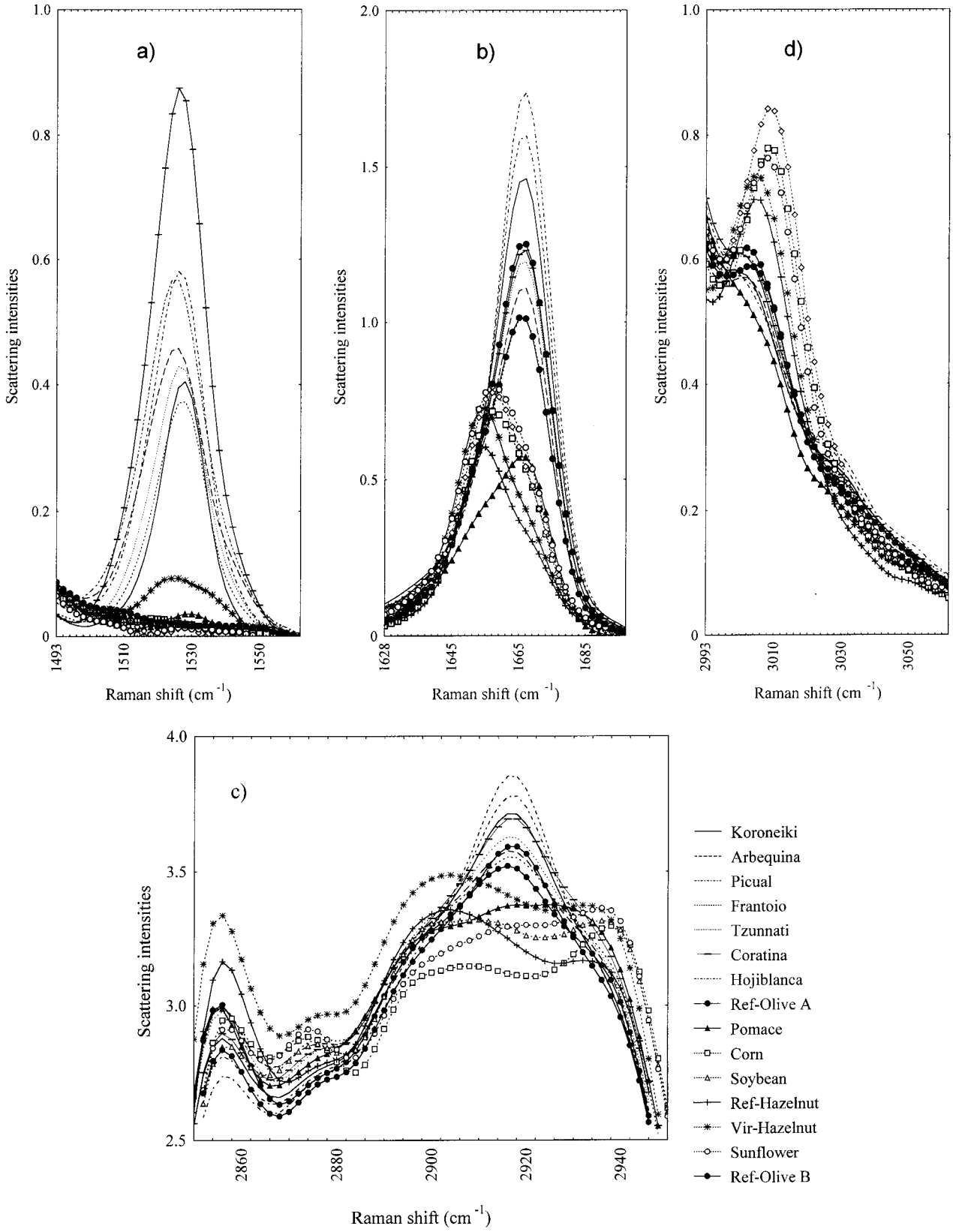


**Figure 4.** FT-Raman spectrum of an unsaponifiable fraction extracted from olive oils and other vegetable oils: (a) 1500−1560 $cm^{-1}$ region; (b) 1635−1690 $cm^{-1}$ region; (c) 2855−2940 $cm^{-1}$ region; (d) 3000−3055 $cm^{-1}$ region (LAS ≅ 500 mW, RES = 4 $cm^{-1}$, SCAN = 200, smoothed and normalized data). Ref, refined; Vir, virgin.

near 1665 $cm^{-1}$ are explained by the spectra of squalene discussed earlier (Figure 2b). In fact, the virgin olive oils, refined olive oils, and olive pomace oils have high contents of squalene (0.30−0.70% of the oil), whereas the other vegetable oils studied have lower contents (0.015−0.030% of the oil). The sample of olive pomace oil shows lower scattering intensities than the unsaponifiable matter of the other olive oils. This indicates that the temperature conditions and chemical reagents used for extracting olive pomace oil affect the content

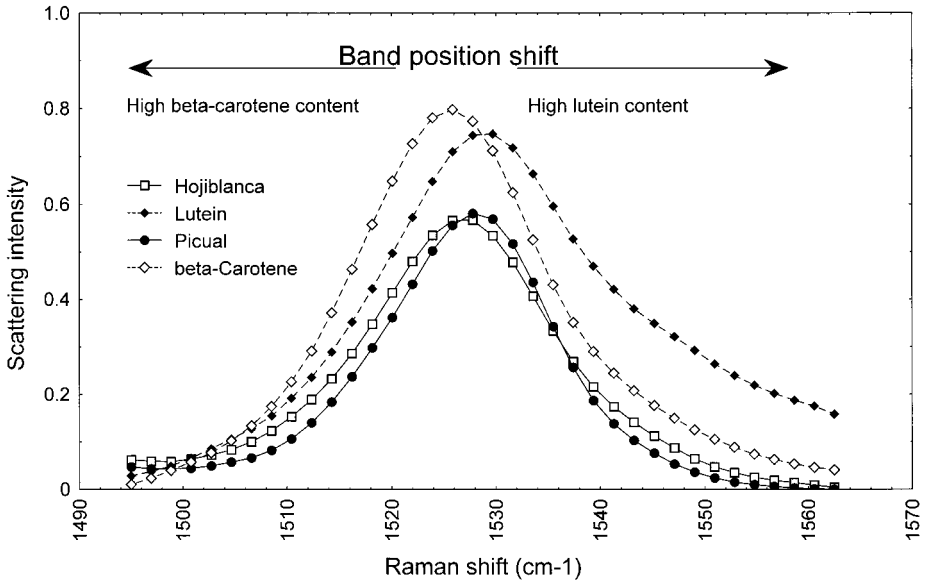


**Figure 5.** FT-Raman scattering band position observed in the 1500−1560 $cm^{-1}$ region of $\beta$-carotene and lutein (dashed lines) and Picual, Hojiblanca, and Arbequina varieties (continuous lines) (LAS ≅ 500 mW, RES = 4 $cm^{-1}$, SCAN = 200, smoothed and normalized data).

of squalene, which is lower in olive pomace oil than in virgin olive oil.

With regard to the spectra of the unsaponifiable matter near 1665 $cm^{-1}$ of the virgin olive oils, the following order of the scattering intensities was observed: Hojiblanca > Picual > Koroneiki > Coratina > Tzunnati > Arbequina > Frantoio. This order is the same as that observed near 2910, 1380, and 1330 $cm^{-1}$. This order agrees with the content of squalene in the Spanish varieties (*38*).

The scattering intensities observed near 1665 $cm^{-1}$ for the corn, soybean, sunflower, and hazelnut oil samples show a very weak bump compared to olive (virgin and refined) oil samples. This reveals the higher content of squalene in the first group (olive oil) than the second group (seed oils). The position of the band near 1655 $cm^{-1}$ for the unsaponifiable matter extracted from the corn, sunflower, soybean, and hazelnut (virgin and refined) oils reflects the presence in these samples of a significant content of unsaturated compounds rich in cis isomers. Bastic et al. (*32*) in their study on the unsaponifiable fraction of some vegetable oils underscore the presence of isoprenoidal polyolefin compounds in sunflower and soybean oils. More work is necessary to identify precisely the origin of this band and the composition of isoprenoidal polyolefin compounds in hazelnut oil and other vegetable oils.

In the region from 2940 to 2850 $cm^{-1}$ (Figure 4c), the unsaponifiable matter of olive (virgin and refined) oils shows intense scattering intensities near 2915 and 2855 $cm^{-1}$, whereas the spectra of the other edible oils show peaks centered near 2930, 2900, and 2855 $cm^{-1}$. Centered at 2910 $cm^{-1}$, the order of scattering intensities for the varietal olive oils is, from high to low intensities, Hojiblanca > Picual > Koroneiki, Coratina > Tzunnati > Arbequina, Frantoio, whereas the inverse order is observed near 2855 $cm^{-1}$. The band between 2900 and 2940 $cm^{-1}$ (Figure 4c) shows that olive pomace oil seems to be different from either olive oils (virgin and refined) or seed oils. In fact, olive pomace oil is obtained by treating the residual paste (pomace), a byproduct of the virgin olive oil production, with solvents (*39*). This means that the process is neither the physical means used for obtaining virgin olive oils nor exactly that used for refining crude vegetable oils.

To enhance the spectral features observed in the region from 2950 to 2850 $cm^{-1}$, Figure 6 presents the differential spectra of the unsaponifiable matter: scattering intensities of the olive and seed oils minus the scattering intensities of the olive pomace oil. The white zones represent positive differences (i.e., higher scattering intensities than in the spectrum of olive pomace), whereas dark zones represent negative difference (i.e., lower scattering intensities than in the spectrum of olive pomace). The unsaponifiable matter of olive oil (virgin and refined) shows positive differences in the regions 2820−2840, 2900−2920, and 3000−3020 $cm^{-1}$. In contrast, the unsaponifiable matter of the seed oils shows positive differences in the regions 2860−2880, 2940−2960, and 3000−3020 $cm^{-1}$. Table 1 summarizes the most important Raman bands observed in the analyzed unsaponifiable matter, where clear differences can be observed between both groups of edible oils.

Concerning the latter region, Figure 4d shows the Raman scattered bands in the vicinity of 3005 $cm^{-1}$. The spectrum of olive oil unsaponifiable matter (olive pomace, refined olive, and virgin olive oils) shows a weak bump centered near 3005 $cm^{-1}$, whereas the other spectra clearly show a band centered near 3010.8 $cm^{-1}$ (soybean, corn, and sunflower oils) or 3006.9 $cm^{-1}$ (virgin and refined hazelnut oils). An easy explanation for these bands could be that they indicate the presence of residual saponifiable matter in the unsaponifiable matter of samples, because this spectral region corresponds to =C−H stretching vibration, and good correlations ($R$ > 0.90) have been found for methyl oleate (3006 $cm^{-1}$) and methyl linoleate (3011 $cm^{-1}$) (*40*). However, the presence of fatty acid methyl esters in the unsaponifiable matter should be confirmed with a band at (or around) 1745 $cm^{-1}$, but not even a weak scattering intensity has been detected in all samples analyzed. The possible alternative is the presence of a very small amount of free fatty acids as a band at 1710 $cm^{-1}$ has been sometimes detected by FT-MIR (V. Baeten, unpublished data), and it corresponds to a C=O bond. This explanation does not hold for the samples where this

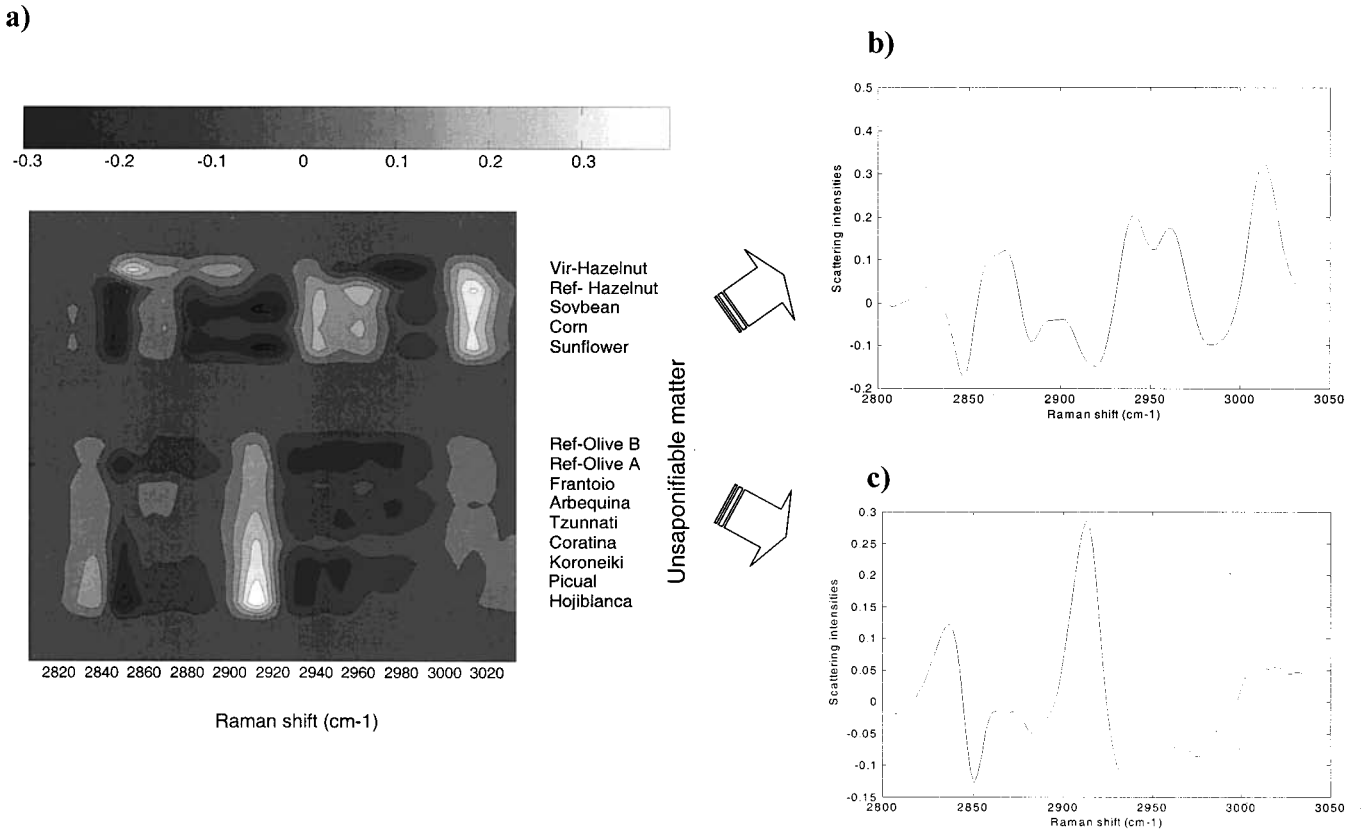


**Figure 6.** Spectral features observed in the 2800−3040 $cm^{-1}$ region of the FT-Raman spectra: (a) gradient of the ratio between the scattering intensities of olive pomace oil and the other edible oils; (b) mean spectra of the unsaponifiable matter of the seed oils; (c) mean spectra of the unsaponifiable matter of the olive (virgin and refined) oils (LAS ≅ 500 mW, RES = 4 $cm^{-1}$, SCAN = 200, smoothed and normalized data). Ref, refined; Vir, virgin.

**Table 1. FT-Raman Shift Positions ($cm^{-1}$) of Several Raman Bands of Different Unsaponifiable Fractions**

| | chemical group | | | | | | |
|---|---|---|---|---|---|---|---|
| | =CH | CH | CH | | CH | C=C | |
| virgin olive oil varieties | | | | | | | |
| Arbequina | 3005.0 | | 2912.4 | | 2854.6 | 1668.6 | 1527.8 |
| Hojiblanca | ≈3005 | | 2912.4 | | 2854.6 | 1668.6 | 1527.8 |
| Picual | ≈3005 | | 2914.4 | | 2854.6 | 1668.6 | 1527.8 |
| Koroneiki | ≈3005 | | 2912.4 | | 2854.6 | 1668.6 | 1529.7 |
| Tzunnati | 3003.1 | | 2912.4 | | 2854.6 | 1668.6 | 1527.8 |
| Coratina | ≈3005 | | 2914.4 | | 2854.6 | 1668.6 | 1527.8 |
| Frantoio | 3003.1 | | 2912.4 | | 2854.6 | 1668.6 | 1529.8 |
| refined olive oil samples | | | | | | | |
| A | 3005.0 | | 2912.4 | | 2854.6 | 1666.6 | |
| B | 3005.0 | | 2912.4 | | 2854.6 | 1668.6 | |
| olive pomace oil | ≈3003 | 2920.1 | 2914.4 | | 2854.6 | 1666.6 | 1531.6 |
| refined corn oil | 3010.8 | 2933.6 | 2904.7 | 2871.9 | 2856.5 | 1658.9 | |
| refined soybean oil | 3010.8 | 2931.7 | 2904.7 | 2873.9 | 2856.5 | 1658.9 | |
| refined sunflower oil | 3010.8 | 2931.7 | | 2871.9 | 2854.6 | 1658.9 | |
| virgin hazelnut oil | 3006.9 | 2927.9 | 2900.9 | | 2854.6 | 1657.0 | 1523.0 |
| refined hazelnut oil | 3006.9 | 2927.9 | 2900.9 | | 2854.6 | 1657.0 | |

band was not detected. The other alternative would be the presence of unsaturated hydrocarbons (*30*) or other minor compounds, rich in cis isomers, but this hypothesis will need further study.

The described univariate study shows, from a mathematical viewpoint, the high potential of the FT-Raman unsaponifiable spectra for classifying varietal virgin olive oils and distinguishing these from selected seed oils. Thus, a multivariate study was carried out with the unsupervised procedure of multidimensional scaling (MDS). This procedure was applied to the selected bands described in the text with the exception of the bands at 3005, 3006.9, and 3010.8 $cm^{-1}$, the presence of which has been discussed in the previous paragraph. MDS allows objects that are judged to be experimentally similar to one another to be represented as points close to each other in the results spatial map; objects judged to be dissimilar are represented as points distant from one another. The results of applying MDS to the selected bands is displayed in Figure 7, and this pictorial representation will allow us to study the characterization of the vegetable oils.

Figure 7 shows that the first two dimensions of MDS (stress, 0.05; alienation, 0.07) cluster the samples into basically two groups. The first dimension divides the samples into two groups, virgin olive oils (negative values of the first dimension) and refined vegetable oils (positive values of the first dimension). However, the

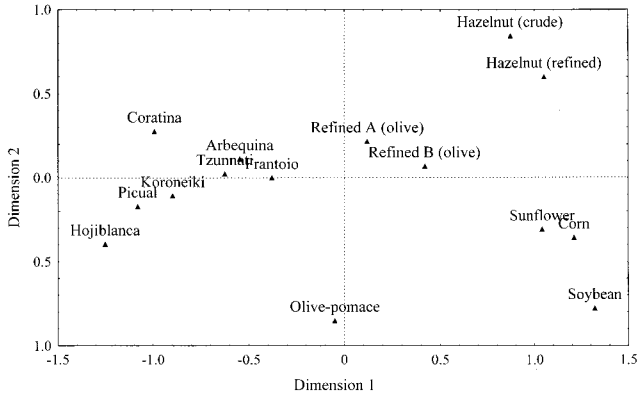


**Figure 7.** Results of applying the multidimensional scaling procedure to the unsaponifiable matter of samples. Only normalized selected bands (3089.9−3020.4, 2999.2−2802.5, 1705.2−1610.8, and 1570.8−1250.1 $cm^{-1}$) were used.

olive pomace oil (a crude oil) is within the former group despite the fact that it is not a varietal virgin olive oil, whereas the crude hazelnut oil (a seed oil) is in the latter group, although it is not refined. The second dimension slightly divided the virgin olive oils into oils obtained from black olives (Koroneiki, Picual, and Hojiblanca) and oils obtained mostly from green (or purple) olives (Arbequina, Coratina, and Tzunnati), suggesting the importance of the bands related to carotenoides and sterols (*21*, *34*). The second and fourth quadrants clearly cluster the refined vegetable oils into two groups: refined seed oils (sunflower, corn, and soybean) and refined olive oils plus hazelnut oils. The latter oils (second quadrant) have chemical compositions (fatty acids, sterols, and alcohols) so similar that the adulteration of olive oils with hazelnut oil is a current matter of research (*41*).

## LITERATURE CITED

(1) Osborne, B. G.; Fearn, T. *Near-Infrared Spectroscopy in Food Analysis*; Longman-Wiley: New York, 1986.

(2) Williams, P.; Norris, K. *Near-Infrared Technology in the Agricultural and Food Industries*; American Association of Cereals Chemists: St. Paul, MN, 1987.

(3) Wilson, R. H. Fourier transform mid-infrared spectroscopy for food analysis. *Trends Anal. Chem*. **1990**, *9*, 127−131.

(4) van de Voort, F. R. FTIR spectroscopy in edible oil analysis. *Int. News Fats, Oils Relat. Mater.* **1994**, *5*, 1038−1042.

(5) Scotter, C. N. G. Non-destructive spectroscopic techniques for the measurement of food quality. *Trends Food Sci. Technol.* **1997**, *8*, 285−292.

(6) Diem, M. *Introduction to Modern Vibrational Spectroscopy*; Wiley: New York, 1993.

(7) Martens, H.; Næs, T. *Multivariate Calibration*; Wiley: Chichester, U.K., 1989.

(8) Aparicio, A. Characterization: Mathematical procedures for chemical analysis. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J. L., Aparicio, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 285−353.

(9) Lees, M. Introduction. In *Food Authenticity: Issues and Methodologies*; Lees, M., Ed.; Eurofins Scientific: Nantes, France, 1998; pp 11−17.

(10) Singhal, R. S.; Kulkarni, P. R.; Rege, D. V. *Handbook of Indices of Food Quality and Authenticity*; Woodhead Publishing: Cambridge, U.K., 1997.

(11) Dennis, M. J.; Ashurst, P. R. An Introduction to Methods for Food Authentication. In *Analytical Methods of Food Authentication*; Ashurst, P. R., Dennis, M. J., Eds.; Chapman and Hall: Cambridge, U.K., 1998; pp 1−13.

(12) Dennis, M. J.; Ashurst, P. R. An Introduction to Food Authentication. In *Food Authentication*; Ashurst, P. R., Dennis, M. J., Eds.; Chapman and Hall: Cambridge, U.K., 1996; pp 1−14.

(13) Aparicio, A. Authentication. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J. L., Aparicio, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 491−520.

(14) Firestone, D.; Reina, R. J. Authenticity of Vegetable Oils. In *Food Authentication*; Ashurst, P. R., Dennis, M. J., Eds.; Chapman and Hall: Cambridge, U.K., 1996; pp 198−258.

(15) Aparicio, R. Characterization of Foods by Inexact Rules: The SEXIA Expert System. *J. Chemom*. **1988**, *3*, 175−192.

(16) Aparicio, R.; McIntyre, P. Oils and Fats. In *Food Authenticity: Issues and Methodologies*; Lees, M., Ed.; Eurofins Scientific: Nantes, France, 1998; pp 214−273.

(17) Baeten, V.; Meurens, M.; Morales, M. T.; Aparicio, R. Detection of virgin olive oil adulteration by Fourier Transform Raman spectroscopy. *J. Agric. Food Chem*. **1996**, *44*, 2225−2230.

(18) Baeten, V.; Hourant, P.; Morales, M. T.; Aparicio, R. Oil and fat classification by FT-Raman spectroscopy. *J. Agric. Food Chem*. **1998**, *46*, 2638−2646.

(19) Karleskind, A.; Wolff, J. P.; Guthmann, J. P. *Manuel des Corps Gras*; TEC & DOC: Paris, France, 1996.

(20) Baeten, V.; Aparicio, R. Possibilities Offered by Infrared and Raman Spectroscopic Techniques in Virgin Olive Oil Authentication. *Olivae* **1997**, *69*, 38−43.

(21) Aparicio, R.; Alonso, V. Characterization of Virgin Olive Oils by SEXIA Expert System. *Prog. Lipid Res.* **1994**, *33*, 29−38.

(22) EU. *Off J. Eur. Union* **1995**, March 29, Regulation L 69/3-69/4.

(23) Morales, M. T.; León-Camacho, M. Gas and Liquid Chromatography: Methodology Applied to Olive Oil. In *Handbook of Olive Oil: Analysis and Properties;* Harwood, J. L., Aparicio, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 159−207.

(24) Mínguez-Mosquera, M. I.; Gandul-Rojas, G.; Montaño-Asquerino, A.; Garrido-Fernández, J. Determination of clorophylls and carotenoids by high-performance liquid chromatography during olive lactic fermentation. *J, Chromatogr*. **1991**, *585*, 259−266.

(25) Baeten, V. Authentication of Virgin Olive Oil by Fourier Transform Raman Spectroscopy. Ph.D. Dissertation, Universitè Catholique de Louvain (Belgium), 1998.

(26) Gerrard, D. L. Organic and petrochemical applications of Raman spectroscopy. In *Analytical Raman Spectroscopy*; Grasselli, J. G., Bulkin, B. J., Eds.; Miller and Stace: New York, 1991; pp 275−325.

(27) Schiffman, S. S.; Reynolds, M. L.; Young F. W. *Introduction to Multidimensional Scaling: Theory, Methods and Applications*; Academic Press: London, U.K., 1981.

(28) Marigheto, N. A.; Kemsley, E. K.; Defernez, M.; Wilson, R. H. A comparison of mid-infrared and Raman spectroscopies for the authentication of edible oils. *J. Am. Oil Chem. Soc*. **1998**, *75*, 987−992.

(29) Grasseli, J. G.; Bulkin, B. J. *Analytical Raman Spectroscopy*; Wiley: New York, 1991.

(30) Lanzón, A.; Albi, T.; Cert, A.; Gracian, J. The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *J. Am. Oil Chem. Soc*. **1994**, *71* (3), 285−291.

(31) Guinda, A.; Lanzón, A.; Albi, T. Differences in Hydrocarbons of Virgin Olive Oils Obtained from Several Olive Varieties. *J. Agric. Food Chem*. **1996**, *44*, 1723−1726.

(32) Bastic, M.; Basrçtic, J. L.; Jovanovic, J. A.; Spiteller, G. Hydrocarbons and other weakly polar unsaponifiables in some vegetable oils. *J. Am. Oil Chem. Soc*. **1978**, *55*, 886−891.

(33) Lanzón, A.; Cert, A.; Albi, T. Detección de la presencia de aceite de oliva refinado en el aceite de oliva virgen. *Grasas Aceites* **1989**, *40*, 385−388.

(34) Gandul-Rojas, B.; Mínguez-Mosquera, M. Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. *J. Sci. Food Agric.* **1996**, *72*, 31−39.
(35) Ozaki, Y.; Cho, R.; Ikegaya, K.; Muraishi, S.; Kawauchi, K. Potential of near-infrared Fourier transform Raman spectroscopy in food analysis. *Appl. Spectrosc.* **1992**, *46*, 1503−1507.
(36) Keller, S.; Löchte, T.; Dippel, B.; Schrader, B. Quality control of food with near-infrared excited Raman spectroscopy. *Fresenius' J. Anal. Chem.* **1993**, *346*, 863−867.
(37) Bulkin, B. J. The Raman effect: An Introduction. In *Analytical Raman Spectroscopy*; Grasselli, J. G., Bulkin, B. J., Eds.; Miller and Stace: New York, 1991; p 1−43.
(38) Aparicio, R.; Ferreiro, L.; Rodríguez-Morales, J. L. *Characterization of Andalusian Virgin Olive Oils: Expert System for Identifying Oils* (in Spanish); Junta de Andalucía (Dirección General de Investigación Agraria): Sevilla, Spain, 1991; pp 39−54.
(39) Luchetti, F. Introduction. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J. L., Aparicio, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 1−16.
(40) Baeten, V.; Aparicio, R.; Marigheto, N. A.; Wilson, R. H. Olive oil analysis by infrared and Raman spectroscopy: methodologies and applications. In *Handbook of Olive Oil: Analysis and Properties*; Harwood: J. L., Aparicio, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 209−245.
(41) The Commission of the European Communities. Development and assessment of methods for the detection of adulteration of olive oil with hazelnut oil. Research project Growth G6RD-CT2000-00440.

Received for review February 5, 2001. Revised manuscript received July 26, 2001. Accepted August 6, 2001.

JF010146X