Detection of Virgin Olive Oil Adulteration by Fourier Transform Raman Spectroscopy

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The authenticity of products labeled as virgin olive oil is of great importance from commercial and health aspects. The combination of Fourier transform-Raman (FT-Raman) spectroscopy with multivariate procedures has been used for predicting the level of adulteration in a set of virgin olive oil samples that were adulterated with soybean, corn, and raw olive residue (olive pomace) oils at 1, 5, and 10%, respectively. Six genuine virgin olive oil samples, differing in their chemical composition, were selected from a set of 1428 European samples. The best result in prediction of adulteration was an adjusted R^2 value of 0.964, determined by regression on principal components (PCR), giving 100% correct discrimination between genuine and adulterated samples and 91.3% correct classifications at different adulteration levels.

Keywords: *Olive oil; adulteration; FT-raman spectroscopy; chemometrics; authentication*

INTRODUCTION

Virgin olive oil, a gourmet oil highly prized for its delicious flavor (Morales et al., 1995; Overton and Manura, 1995), is sometimes adulterated with cheaper oils by dishonest traders. Adulterants may include olive residue and esterified virgin olive oils (IOOC, 1985; Firestone et al., 1985) or seed oils that are locally available and less expensive (Firestone et al., 1988; Salivaras and McCurdy, 1992). This procedure is harmful for emergent virgin olive oil markets whose local consumers buy virgin olive oil for its potential health benefits (Viola and Audisio 1987) and are surprised to receive an oil that does not have these benefits (Kafatos and Comas, 1991).

Advances in knowledge and technology have undoubtedly led to greater success in the fight against adulteration over the years, but it is equally true that the same advances have also been used by dishonest people to invalidate the usefulness of official standards. Currently there is a proliferation of proposals attempting to demonstrate that adulterants in virgin olive oil can be easily detected (Li-Chan, 1994), though most of them do not show any advantage over official methods (EU, 1995).

Numerous methods have been proposed, including pyrolysis-mass spectrometry (Goodacre et al., 1992), gas chromatography (GC)-electron ionization mass spectrometry (Brumley et al., 1985), ^{1}H NMR and ^{13}C NMR (Zamora et al., 1994), ultraviolet (UV) spectrophotometry (Passaloglou, 1990) and infrared (IR) spectroscopy (van de Voort et al., 1992, 1994a,b; Ismail et al., 1993; Safar et al., 1994; Sato, 1994; Lai et al., 1994; Wesley et al., 1995). The alternative is still liquid and gas chromatography (Gracian, 1968; Flor et al., 1993; Antoniosi et al., 1993; Firestone, 1993), a time-consuming technique that requires several steps to complete quantification, that is the current basis of olive oil adulteration standards (EU, 1995). It is accepted that chromatography (HRGC-HPLC) is the most accurate technique (Lanzón et al., 1989), though it is destructive and laborious, uses pollutant solvents, and is inappropriate for on-line control, a demand that is heard more and more from farmers and cooperative societies.

The alternative to chromatography should be techniques that do not use pollutant solvents, work with intact samples, give rapid results, can detect lower adulteration and are suitable for on-line controls. Raman spectroscopy (Gerrard and Birnie, 1990; Vickers and Mann, 1991) meets these requirements, but has only recently, and infrequently, been applied to the quantitative analysis of oils (Sadeghi-Jorachi et al., 1990; Lerner et al., 1992) and authentication of certain edible oils by their *cis/trans* isomer composition (Bailey and Horvart, 1972). Dispersive raman spectroscopy had been considered of very limited use in this field because of fluorescence interference, photodecomposition, wavelength calibration, lack of a precise frequency base from scan to scan, and difficulty in attaining high-resolution spectra (Chase, 1987).

These problems have been overcome in the Fourier transform (FT) raman spectrometers with the use of Michelson interferometers and microcomputers for data processing. The most important advantage of FT-raman for oil samples is that the spectra are fluorescence-free because the operating frequency of the Nd:YAG laser, (1.064 μm) is well below the threshold for fluorescence processes caused by carotenes, which are widely present in many virgin olive oils (Kiritsakis, 1991). These instruments have also dramatically improved the signal-to-noise ratio by the averaging of hundreds of scans per sample (Wilson, 1990), higher light energy throughput and speed of analysis (Baranska et al., 1987), superior spectral resolution, and better wavelength calibration (Grasseli and Bulkin, 1991), etc.

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S0021-8561(96)00011-8 CCC: $12.00

Based on these advantages, a few relevant studies have recently been published, such as those concerning unsaturation in oils and margarines (Sadeghi-Jorachi et al., 1990), authentication of certain edible oils by their *cis*/*trans* isomer composition (Bailey and Horvart, 1972), characterization of polyunsaturation in cooking oils (Lerner et al., 1992), and studies on the double bond position in linseed oil during its isomerization process (Chmielarz et al., 1995). These studies clearly state that FT-Raman could be useful in detection of virgin olive oil adulterations because although there are no *trans*-unsaturated fatty acids in virgin olive oil, there are *trans*-acids in any refined vegetable oils added in adulteration processes.

In this paper, we describe the first application of FT-Raman spectroscopy in the detection of virgin olive oil adulteration. FT-Raman was calibrated with different percentages of trilinolein added to a sample of virgin olive oil; other genuine virgin olive oil samples were adulterated with olive pomace, soybean, and corn oils at percentages 1, 5, and 10%, respectively.

MATERIALS AND METHODS

Sampling. The International Olive Oil Council (IOOC, 1985) has defined and classified the different olive oil products. Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil. The oil has not undergone any treatment other than washing, decantation, centrifugation, and filtration. Refined olive oil is obtained by refining virgin olive oil under conditions that do not lead to alteration of the initial glyceride structure. Olive pomace oil (olive residue oil) is the oil obtained by extracting with solvents the olive residue remaining after mechanical extraction of the virgin olive oil and made edible by refining methods that do not lead to alteration of the glyceride structure.

The largest virgin olive oil database (Aparicio and Alonso, 1994) was used to select those samples of virgin olive oil, from a set of 1428, with differing composition in chemical compounds (fatty acids, triglycerides, alcohols, sterols, hydrocarbons, etc.). Samples were selected by the statistical multivariate procedure of principal components and later the set was refined taking into account their composition in triglycerides and sterols.

FT-Raman calibration was carried out with virgin olive oil of the Coratina variety at a normal level of ripeness. Six samples, adulterated with trilinolein 99% (Sigma Chemical Company, Saint Louis, MO) in the range 1−10% (w/w), plus an unadulterated control were used in this process. Six virgin olive oils, obtained from varieties Hojiblanca (3) and Picual (3) at a medium level of ripeness, were the genuine samples selected. Each of these samples was adulterated with soybean, corn, or olive pomace oils at 1, 5, or 10% (w/w), respectively. The set consisted of 24 samples [six samples at three levels of adulteration (1, 5, and 10%) plus six genuine samples]. The adulterant oils, 100% purity, were selected as being characteristic of low, medium and high proportion of problems in detection of virgin olive oil adulteration.

Instruments. All the FT-Raman spectra were acquired on a Nicolet 910 FT-Raman spectrometer (Nicolet Analytical Instrument, Madison, WI). The nitrogen-cooled Ge detector of the spectrometer provided a superior signal-to-noise performance, allowing for reduce scan time. The 900 series houses a continuous wave Nd:YAG laser with a TEM_{∞} output at 1.064 μm. The laser power is variable from zero to 1.200 mW at the focus after filtering. In this work, however, the operating power of 500−540 mW provided the near-IR excitation.

Prior to calibration or analysis, the high quality 5-mm NMR tubes were cleaned with 1% Triton X-100 solution (Sigma Chemical) and then by hexane and then dried. A reference background emittance spectrum was taken of each of the clean tubes and stored on disk, even thought their absorbance was near zero. Approximately 1 g of each sample was introduced into the NMR tubes. FT-Raman spectra were obtained by placing each sample in front of the laser and focusing the Nd:YAG laser beam into the oil. All spectra were taken in the 180° backscattering refractive geometry and passed into the interferometer. Spectra were produced over the Raman shift 3250−100 cm^{-1}. Typically, 100 interferograms were co-added at 4 cm^{-1} resolution with a 4-min sampling time.

Individual sample emittance spectra were then recorded and ratioed, against the reference background emittance spectrum of the tube used, to obtain the absorbance spectra of each sample. The tubes were cleaned after each sample by aspirating the oil from the glass, washing twice with hexane, and wiping dry. The cleaned glass was checked spectrally to ensure no residue of the previous sample remained on the glass walls.

The spectrometer was coupled to a Nicolet 680D workstation under Nicolet software, version 3.20. Binary files of the spectral data were accessible to personal computers through the NIC2DOS program. Spectra Calc program, version 2.2 (Galactic Industries Corp., St. Louis, MO) was used for spectra manipulations and transformations.

Statistical Analysis. Classical multivariate procedures were applied; they are, regression on principal components (PCR; Martens and Næs, 1989), stepwise linear regression analysis (SLRA) and stepwise linear discriminant analysis (SLDA; Tabachnick and Fidell, 1983), and cluster analysis (CA; Massart and Kaufman, 1983).

The SLDA was applied to show the wavelengths, and hence structural groups. Adulteration is thus detected because SLDA works with the ratio between the inter-to-intra dissimilarities among samples belonging to each level of adulteration (0, 1, 5, 10%). Hierarchical CA was used to show basic groups of adulterated samples during calibration of samples spiked with trilinolein (LLL). The agglomerative procedure used was Ward's method because its criterion allows grouping the samples so that the increase in heterogeneity is least. City-block (Manhattan) was the distance selected as it is conceptually attractive for measurements where there are two or more different vibrations for the same molecule.

The two regression procedures were SLRA and PCR. The SLRA procedure yielded information about those bands explaining the percentages of adulteration or LLL added to a genuine virgin olive oil. Bands were selected taking into account the values of *F*-to-Enter and *F*-to-Remove of *F*-distribution at $p = 0.05$. The latter procedure, PCR, was applied on the principal components selected after the cross-validation process had been repeated 10 times.

Statistica (Statistica, 1992) and BMDP (BMDP, 1994) were the statistical libraries selected. The former was run on a 486 DX4 computer and the latter on a DEC VAX 3200.

RESULTS AND DISCUSSION

A first-derivative mathematical treatment was applied over 15 convolution points, and the Savitsky-Golay 8-point smoothing function over the range 1250−3030 cm^{-1}. The dataset was normalized by Z-scores (autoscaling). Later, information concerning the baseline was removed from the datasets as it is not relevant to this study. Zones of the spectra retained were 3030−2811, 2757−2709, 1766−1722, 1679−1631, 1482−1360, 1338−1250 cm^{-1}. The raw (a) and derivative (b) spectra of a genuine virgin olive oil are shown in Figure 1. Using the ratio CHISQ/DF (ratio of χ^2 to number of degree of freedom), one multivariate outlier was detected and removed.

Trilinolein Addition Model. Calibration was carried out with samples of genuine virgin olive oil spiked with 99% (LLL) in the range 1−10% (w/w). The LLL is present in virgin olive oil at trace levels; hence, the addition of LLL emulates the adulteration of virgin olive oil with other kinds of vegetable oil. The addition of

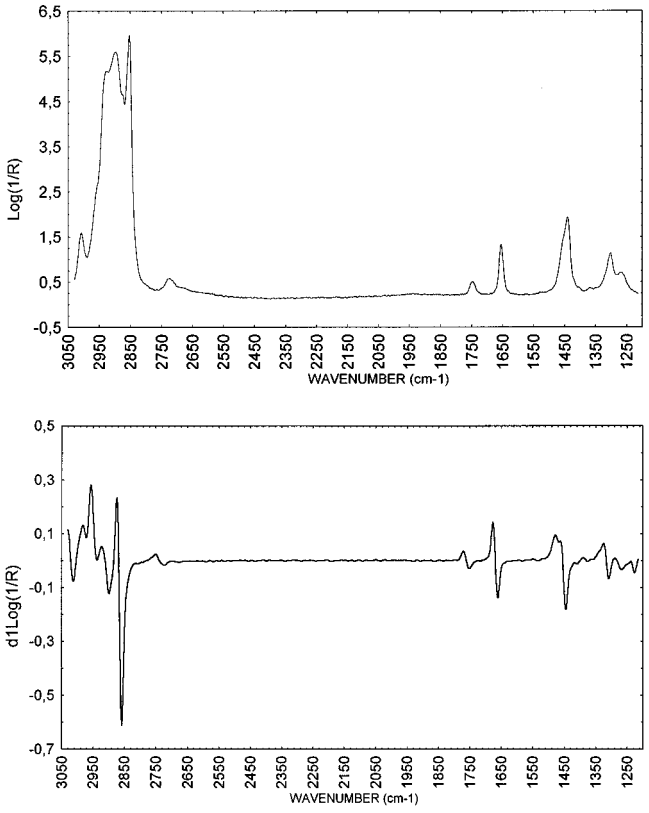

Figure 1. Virgin olive oil spectrum: (a, top) FT-Raman raw spectrum; (b, bottom) FT-Raman first-derivative spectrum.

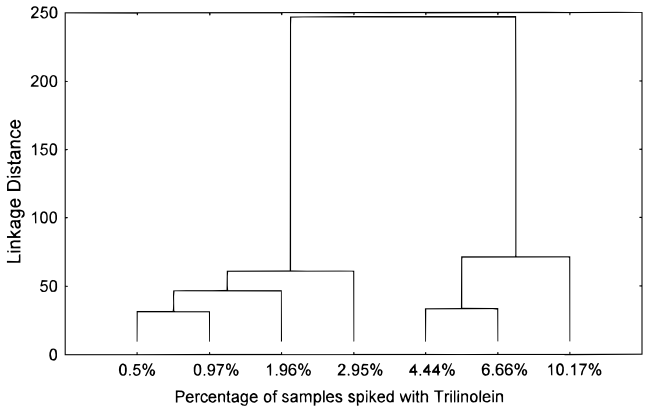

Figure 2. Cluster analysis of genuine samples (LLL = 0.5%) and samples spiked with trilinolein.

LLL to a genuine virgin olive oil sample will be expressed as adulteration percent.

Cluster analysis of the retained spectral data (Figure 2) shows two clear groups, the upper and lower 5% of LLL addition. Beyond this distinction, the dendrogram displays progressive differences between the spiked samples, indicating that calibration could be linear.

Many regression procedures, from partial-least squares to PCR, can be applied in the calibration models. In this study, we opted for SLRA because a great part of each spectrum does not add relevant information but, on the contrary, the results can be disturbed with noise information. Furthermore, there are not enough samples to ensure that results are not attained by chance with other statistical procedures (Tabachnick and Fidell, 1983).

The restrictive conditions imposed on the procedure, through F-to-Enter and F-to-Remove, makes the results sufficiently reliable, whereas the multiple regression

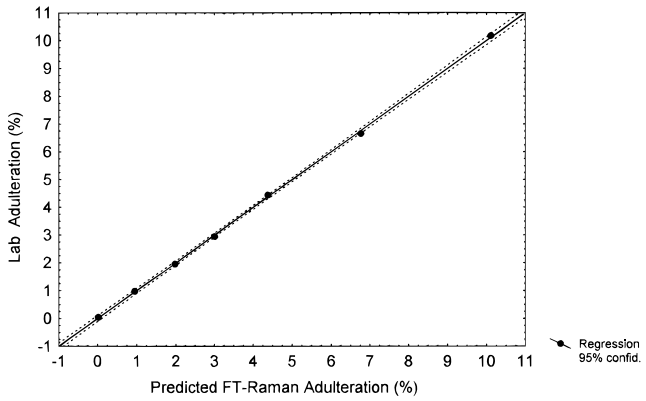

Figure 3. Plot of lab adulteration against predicted FT-Raman adulteration.

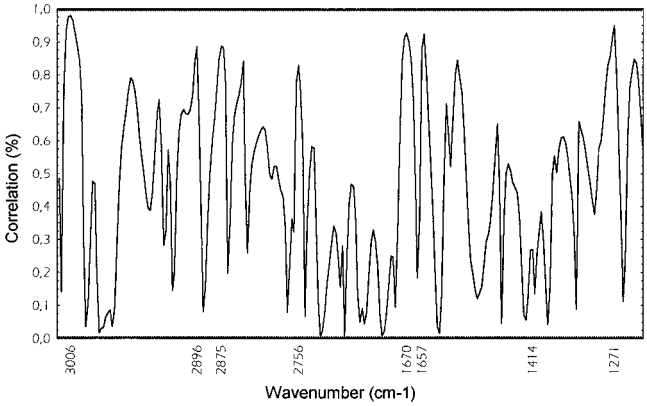

Figure 4. Correlations between wavenumbers. The wavenumbers of zones of correlation above 0.9 represent the same information from a mathematical viewpoint.

coefficient R^2 change (Tabachnick and Fidell, 1983) reveals the relative importance of each wavenumber selected by difference between the adjusted R^2 before and after wavenumber selection.

The graph obtained when the laboratory values are plotted against predicted adulteration are shown in Figure 3. Four wavenumbers (3006, 2873, 1657, 1270 cm^{-1}) were enough to reach an adjusted R^2 of 0.998 (R^2 = 0.999). However, the selection of these wavenumbers is a mathematical compromise with respect to the other wavenumbers inside a region. A plot of the retained wavenumbers of the spectra against the correlation between their intensities and the percentage of olive oil spiked with LLL is shown in Figure 4. There are regions whose correlation with adulteration is >0.9. The wavenumbers selected by SLRA are inside these regions, but their selection is due to mathematical criteria, as every wavenumber inside the region is, more or less, equally significant.

The assignments of Raman wavenumbers (Baranska et al., 1987; Sadeghi et al., 1990; Grasseli and Bulkin, 1991; Bailey and Horvart, 1972), selected by the statistical procedure, are displayed in Table 1. The characteristic 3006 cm^{-1} wavenumber (outside the range 3010−3020 cm^{-1}) of unsaturated fatty acids, visible in the spectrum of linoleic acid, is the result of symmetrical stretching vibration in $-CH{=}CH-$ (*cis* olefin; Safar et al., 1994). There is high correlation (0.89−0.98) with the percentage of LLL in this region (from 3002 to 3010 cm^{-1}), as shown in Figure 4, and the values (*Z*-scored) of wavenumber 3006 cm^{-1} against the percentage of LLL added to the genuine sample are shown in Figure 5.

Table 1. Characteristics of Raman Scattered Wavenumbers Useful in the Trilinolein Adulteration Model

molecule	group	wave-numbers[a] (cm^{-1})	wave-number[b] (cm^{-1})	correlation[c]
$-CH_3$	ν sym (C−H)	2860−2880	2873	0.89
cis RCH=CHR	ν sym (=C−H)	3007	3006	0.98
cis RCH=CHR	ν (C=C)	1650−1660	1657	0.88
cis RCH=CHR	δ (=C−H)	1270	1270	—

[a] δ = deformation; ν = stretching; sym = symmetric. [b] Wavenumbers described by references. [c] Wavenumbers selected by mathematical procedures. [d] Minimum correlation between the selected wavenumbers and described by references.

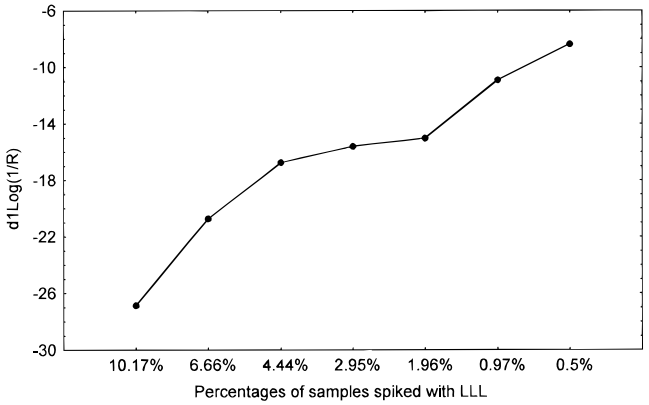

Figure 5. Values of wavenumber 3006 cm^{-1}, ν sym (=C−H), in the samples spiked with LLL.

The selected wavenumbers agree with the chemical structure of trilinolein (C18:2, [*cis*,*cis*]-9,12). The Raman technique should thus be able to detect adulteration of virgin olive oil with those vegetable oils (safflower, soybean, sunflower, rapeseed, grapeseed, linseed, etc.) containing high amounts of LLL, because the detection level of LLL can be low enough with the selected wavenumbers (Figure 3). However, the adulteration of olive oil is more complex than a simple addition of LLL because olive oil can be adulterated with vegetable oils LLL contents that are very low or similar to that of the genuine product. Thus, other chemical compounds (*trans*-fatty acids, hydrocarbons, sterols, etc.) have been used in fraud detection (E.U., 1995) with relative success. Most of these compounds are produced during refining (Lanzón, 1990) by processes that must not be used to produce virgin olive oil (E.U., 1991).

Virgin Olive Oil Adulteration with Pomace, Soybean, and Corn Oils. The previous results show the ability of the Raman technique to detect the percentages of LLL in spiked virgin olive oil. However, adulterations can be carried out with vegetable oils whose amounts of LLL are very low, and the detection of fraud by chromatography can be difficult (Proto, 1992). We selected three oils with different levels of LLL quantified by HPLC (Dieffenbacher and Pocklington, 1992) to verify the wavenumbers selected in the previous calibration and the advantages of using supplementary wavenumbers in detecting adulteration.

The same process just described was followed, except the real percentage of LLL in the samples was taken into account. The genuine samples (LLL = tr., 0.08, 0.27, 0.48, and 0.49%) selected four wavenumbers (3010, 2878, 1666, 1270 cm^{-1}), which are inside the groups described in Figure 4 for the LLL model, reached an adjusted R^2 of 0.961. We checked the model by successively adding the samples adulterated with soybean, which needed only three wavenumbers (3010, 2878, and 1270 cm^{-1}) to reach an adjusted R^2 of 0.92, and later with pomace oil, with which the model reached an adjusted R^2 of only 0.86. However, values of adjusted regression coefficient dropped abruptly (adjusted R^2 = 0.63) when the samples adulterated with corn oil were added to the dataset. No reason has been found for the observation that the wavenumbers became useless with this adulteration.

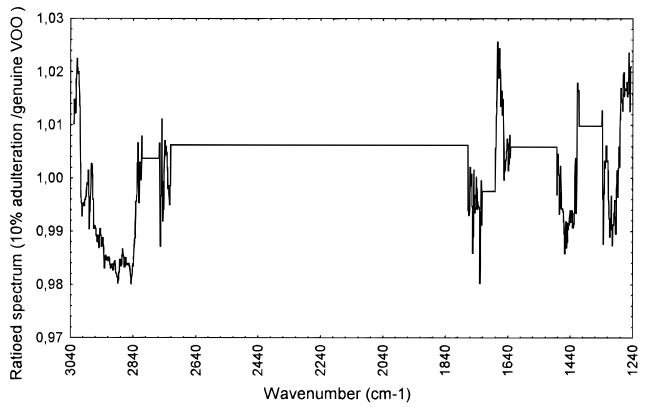

Figure 6. Ratioed spectrum. The *X*-axis represents the wavenumber and the *Y*-axis the values of the ratioed spectrum.

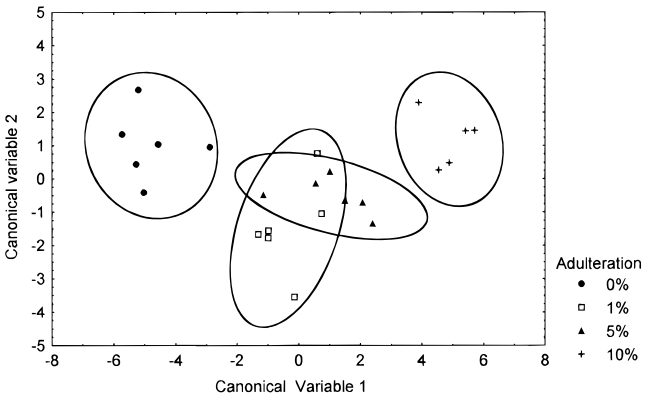

Figure 7. Results of stepwise linear discriminant analysis with the first two canonical variables.

This loss of calibration can be compensated for by using other wavenumbers that indicate adulteration but are related with chemical compounds other than LLL. A ratioed spectrum is shown in Figure 6. The *X*-axis represents the wavenumber and the *Y*-axis the results obtained by dividing the mean spectrum of genuine samples by the mean spectrum of samples spiked with vegetable oils at 10%. The highest peaks are located at wavenumbers where the differences between genuine samples and samples spiked with other vegetable oils at 10% are the greatest.

Following the process suggested by Martens and Næs (1989), though using regression on principal components, 20 factors were calculated by PCA (explained variance: 96%) and regression on the first four factors, were selected by crossvalidation, and reached an adjusted R^2 of 0.964 (p = 0.032). However, these factors represent almost all wavenumbers, because only a few of them are significative for detecting adulteration. SLDA (Figure 7) was applied on the wavenumbers selected by the first factor, which explains 71% of variance. The wavenumbers selected (3007, 2989, 2980, 2954, 1758, 1745, 1630, 1440,1414, 1297 cm^{-1}; Table 2) allowed 91.3% of the samples to be correctly classified (a sample of each one of the groups adulterated at 1−5% was interchanged each other). Mathematical criteria

Table 2. Characteristics of Raman Scattered Wavenumbers

molecule	group[a]	wave-number[a] (cm^{-1})	wave-number[b] (cm^{-1})	correlation[c]
−RCH=CHR	ν sym (=C−H)	3007	3007 2989[d]	—
−CH	ν (C−H)	2980	2980[d]	—
−CH3	ν as (C−H)	2950	2954	0.97
−CO−O−	ν (C=O)	1755	1758[d]	0.96
RCOOR	ν (C=O)	1745	1745	—
cis RCH=CHR	ν (C=C)	1630	1630[d]	—
$(CH_2)_n$	δ (C−H)	1440	1440	—
cis RCH=CHR	δ (C−H)	1410	1414[d]	0.93
trans RCH=CHR	δ (C−H)	1290	1297[d]	0.92

[a] δ = deformation; ν = stretching; sym = symmetric; as = asymetric. [b] Wavenumbers described by references. [c] Wavenumbers selected by mathematical procedures. [d] Minimum correlation between the selected wavenumbers and described by references. [e] Characteristic FT-IR and FT-MidIR scattered bands.

were used to select these wavenumbers from zones of the spectra, but other wavenumbers of these zones could be used with similar success.

The ellipses of Figure 7 show the 95% confidence regions for each adulterated group (0, 1, 5, 10%; Shiffman et al., 1981). Their centers have been calculated from the means of the group coordinates, and their axes represent the values of the confidence regions for each dimension calculated by multiple regression. These ellipses allow an interpretation beyond the simple location of samples; that is, (i) genuine samples constitute a clear group because they are too different from the adulterated samples. This group shows considerable individual variation among its samples that can be explained by the botanical characteristics of the varieties selected, Picual and Hojiblanca; (ii) the virgin olive oil samples adulterated at 10% also show clear differences from the other groups. These samples are placed on left side of the figure, and the genuine samples on the right side; (iii) there is a clear overlapping region between samples spiked at 1−5% and some samples appear in the fuzzy region of their ellipses. The position of these sets of samples, between the genuine and 10% adulterated samples, is logical enough.

SLRA was also applied on these selected wavenumbers, the independent variable being the percentage of adulteration [i.e., 0 (genuine samples), 1, 5, and 10]. Using all spectra data (standard method and intercept included in the model; in fact, a multiple regression analysis) the adjusted R^2 reached 0.92, whereas this value dropped to 0.78 ($R^2 = 0.90$) when SLRA used only the wavenumbers 3007, 2989, 2980, and 1414 cm^{-1} that were selected by the value of F-to-Enter at $p = 0.05$.

Conclusions. The results of this study show that FT-Raman spectroscopy could be useful in detecting adulteration beyond the limits achieved by techniques currently suggested by the standards (E.U., 1995). However, more work is needed to reach the repeatability and reproducibility of wet techniques and to ensure the selection of only those wavenumbers that have chemical meaning.

A combination of spectroscopy and chromatography (where the latter could be used to quantify chemical compounds revealing adulterations, and the results could be used in the multivariate calibration of the former) could be able to detect virgin olive oil adulteration more accurately, easily, and rapidly than current standards.

ACKNOWLEDGMENT

We acknowledge our indebtedness to Drs. Ángel Justo and Miguel-Ángel Avilés of Instituto de Ciencias de los Materiales (CSIC, Sevilla, Spain) who helped us in solving many experimental problems.

LITERATURE CITED

Antoniosi, N. R.; Carrilho, E.; Lanças, F. M. Fast quantitative analysis of soybean oil in olive oil by high-temperature capillary GC. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1051−1053.

Aparicio, R.; Alonso, V. Characterization of virgin olive oils by SEXIA expert system. *Prog. Lipid Res.* **1994**, *33*, 29−38.

Bailey, G. F.; Horvat, R. J. Raman spectroscopic analysis of the *cis/trans* isomer composition of edible vegetable oils. *J. Am. Oil Chem. Soc.* **1972**, *49*, 494−498.

Baranska, H.; Labudzinska, A.; Terpinski, J. *Laser Raman Spectrometry: Analytical Applications*; Ellis Horwood: Chichester, U.K., 1987.

BMDP. *BMDP Statistical Software*, release 7.0; University of California: Los Angeles, CA, 1994.

Brumley, W.; Sheppard, A. J.; Rudolf, T. S.; Shen, C-S. J.; Yasaei, P.; Spon, J. A. Mass spectrometry and identification of sterols in vegetable oils as butyryl esters and relative quantification by gas chromatography with flame ionization detection. *J. Assoc. Off. Anal.Chem.* **1985**, *68*, 701−709.

Chase, B. Fourier transform raman spectroscopy. *Anal. Chem.* **1987**, *59*, 881A−889A.

Chmielarz, B.; Bajdor, K.; Labudzinska, A.; Klukowska-Majewska, Z. Studies on the double bond positional isomerization process in linseed oil by UV, IR, and Raman spectroscopy. *J. Molec. Struct.* **1995**, *348*, 313−316.

Dieffenbacher, A.; Pocklington, W. D. Determinations of the composition of triglycerides in vegetable oils in terms of their partition by high performance liquid chromatography (HPLC). In *Standard Methods of the IUPAC*, revised 7th ed.; Paquot, C., Hautfenne, A., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1992; pp 323−400.

E. U. *Off. J. Eur. Union*. 1991, July 11th, Regulation 2568/91.

E. U. *Off. J. Eur. Union*, 1995, March 29th, Regulation L 69/3−69/4.

Firestone, D.; Summers, J. L.; Reina, R. J.; Adams, W. S. Detection of adulterated and misbranded olive oil products. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1558−1562.

Firestone, D.; Carson, K. L.; Reina, R. J. Update on control of olive oil adulteration and misbranding in the United States. *J. Am. Oil Chem. Soc.* **1988**, *65*, 788−792.

Firestone, D. Fats and Oils. *J. AOAC Int.* **1993**, *76*, 133−136.

Flor, R. V.; Hecking, L. T.; Martin, B. D. Development of high-performance liquid chromatography criteria for determination of grades of commercial olive oils. Part I. The normal ranges for the triacylglycerols. *J. Am. Oil Chem. Soc.* **1993**, *70*, 199−203.

Gerrard, D. L.; Birnie, J. Raman spectroscopy. *Anal. Chem.* **1990**, *62*, 140R−150R.

Gracian, J. The chemistry and analysis of olive oil. In *Analysis and Characterization of Oils, Fats and Fat Products*; Boekenoogen, H. A., Ed.; Wiley: (Interscience): London, U.K., 1968; Vol. 2, Chapter 6.

Goodacre, R.; Kell, D. B.; Bianchi, G. Neural networks and olive oil. *Nature* **1992**, *359*, 594.

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

IOOC, International Trade Standard Applying to Olive Oil and Olive-Residue Oils. COI/T.15, 1; International Olive Oil Council: Madrid, Spain, 1985.

Ismail, A. A.; van de Voort, F. R.; Emo, G.; Sedman, J. Rapid quantitative determination of free fatty acids in fats and oils by Fourier transform infrared spectroscopy. *J. Am. Oil Chem. Soc.* **1993**, *70*, 335−341.

Kafatos, A.; Comas, G. Efectos biológicos del aceite de oliva sobre la salud de las personas. In *El Aceite de Oliva*; Kiritsaki, A. K., Ed.; A. Madrid Vicente: Madrid, Spain, 1991; Chapter 17 .

Kiritsakis, A. K. *Olive Oil*; American Oil Chemists' Society: Champaign, IL, 1991.

Lai, Y. W.; Kemsley, E. K.; Wilson, R. H. Potential of FT-IR spectroscopy for the authentication of vegetable oils. J. *Agric. Food Chem*. **1994**, *42*, 1154−1159.

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

Lanzón, A. Alteraciones producidas en los componentes del insaponificable de los aceites de oliva como consecuencia de los procesos de refinación. Ph.D Dissertation, Universidad de Sevilla, Spain, 1990.

Lerner, B.; Garry, M.; Walder, F. Characterization of polyunsaturation in cooking oils by the 910 FT-Raman. *Abstract of Papers*, 2nd International Workshop on FT-IR, Antwerp, Belgium; Vansant, E. F., Ed.; University of Antwerp: Wilrijk, Belgium, 1992; pp 75−82.

Li-Chan, E. Developments in the detection of adulteration of olive oil. *Trend Food Sci. Tech*. **1994**, *5*, 3−11.

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

Massart, D. L.; Kaufman, L. *The Interpretation of Analytical Chemical Data by the Use of Cluster Analysis;* Wiley Interscence: Chichester, U.K.,1983.

Morales, M. T.; Alonso, M. V.; Rios, J. J.; Aparicio, R. Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. *J. Agric. Food Chem*. **1995**, *43*, 2925−2931.

Overton, S. V.; Manura, J. J. Analysis of volatile organic in cooking oils by thermal desorption gas chromatography mass spectrometry. *J. Agric. Food Chem*. **1995**, *43*, 1314−1320.

Passaloglou, S. A comparative study of UV spectrophotometric methods for detection of olive oil adulteration by refined oils. *Z. Lebensm. Unters. Forsch*. **1990**, *191*, 132−134.

Proto, M. Linoleic acid and trilinolein contents of some seed oils and olive oil. *Ind. Aliment*. **1992**, *31*, 36−38.

Sadeghi-Jorachi, H.; Hendr, P. J.; Wilson, R. H.; Belton, P. S. Determination of the total unsaturation in oils and margarines by FT-Raman spectroscopy. *J. Am. Oil Chem. Soc.* **1990**, *67*, 483−486.

Safar, M.; Bertrand, D.; Robert, P.; Devaux, M. F.; Genot, C. Characterization of edible oils, butters and margarines by Fourier transform infrared spectroscopy with attenuated total reflectance. *J. Am. Oil Chem. Soc*. **1994**, *71*, 371−377.

Salivaras, E.; McCurdy, A. R. Detection of olive oil adulteration with canola oil from triacylglycerol analysis by reversed-phase high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **1992**, *69*, 935−938.

Sato, T. Application of principal-component analysis on near-infrared spectroscopic data of vegetable oils for their classification. *J. Am. Oil Chem. Soc.* **1994**, *71*, 293−298.

Shiffman, S. S.; Reynolds, M. L; Young, F. W. *Introduction to MultiDimensional Scaling. Theory, Methods and Applications*; Academic: Orlando, FL, 1981; Chapter 10.

Statistica. *User's Guide*, version 3.1; StatSoft: Tulsa, OK, 1992.

Tabachnick, B. G.; Fidell, L. S. *Using Multivariate Statistics*; Harper & Row: New York, 1983.

van de Voort, F. R.; Sedman, J.; Emo, G.; Ismail, A. A. Rapid and direct iodine value and saponification number determination of fats and oils by attenuated total reflectance/Fourier transform infrared spectroscopy. *J. Am. Oil Chem. Soc.* **1992**, *69*, 1118−1123.

van de Voort, F. R.; Ismail, A. A.; Sedman, J.; Dubois, J.; Nicodemo, T. The determination of peroxide value by Fourier transform infrared spectroscopy. *J. Am. Oil Chem. Soc.* **1994a**, *71*, 921−926.

van de Voort, F. R.; Ismail, A. A.; Sedman, J.; Emo, G. Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy. *J. Am. Oil Chem. Soc.* **1994b**, *71*, 243−253.

Vickers, T. J.; Mann, C. K. Quantitative analysis by Raman spectroscopy. In *Analytical Raman Spectroscopy;* Grasselli, J. G., Bulkin, B. J., Eds.; Wiley: Chichester, U.K., 1991; Chemical Analysis Series, Vol. 114, Chapter 5.

Viola, P.; Audisio, M. *Olive Oil and Health*; International Olive Oil Council: Madrid, Spain, 1987.

Wesley, I. J.; Barnes, R. J; McGill, A. E. J. Measurements of adulteration of olive oils by near-infrared spectroscopy. *J. Am. Oil Chem. Soc.* **1995**, *72*, 289−292.

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

Zamora, R.; Navarro, J. L.; Hidalgo, F. J. Identification and classification of olive oil by high-resolution ^{13}C nuclear magnetic resonance. *J. Am. Oil Chem. Soc.* **1994**, *71*, 361−364.

Received for review January 3, 1996. Accepted May 31, 1996.[⊗] This work was supported by Junta de Andalucia (Spain) and project COMMET 94/15.

JF9600115

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.