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Direct, reagent-free determination of free fatty acid content in olive oil and olives by Fourier transform Raman spectrometry

Barbara Muik [a,b], Bernhard Lendl [b], Antonio Molina-Díaz [a], María José Ayora-Cañada [a,*]

[a] *Department of Physical and Analytical Chemistry, University of Jaén, Paraje las Lagunillas s/n, E-23071 Jaén, Spain*
[b] *Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9/164, A-1060 Wien, Austria*

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Abstract

Fourier transform (FT) Raman spectrometry in combination with partial least squares (PLS) regression was used for direct, reagent-free determination of free fatty acid (FFA) content in olive oils and olives. Oils were directly investigated in a simple flow cell. Milled olives were measured in a dedicated sample cup, which was rotated eccentrically to the horizontal laser beam during spectrum acquisition in order to compensate sample heterogeneity. Both external and internal (leave-one-out) validation were used to assess the predictive ability of the PLS calibration models for FFA content (in terms of oleic acid) in oil and olives in the range 0.20–6.14 and 0.15–3.79%, respectively. The root mean square error of prediction (RMSEP) was 0.29% for oil and 0.28% for olives. The predicted FFA contents were used to classify oils and olives in different categories according to the European Union regulations. Ninety percent of the oil samples and 80% of the olives were correctly classified. These results demonstrate that the proposed procedures can be used for screening of good quality olives before processing, as well as, for the on-line control of the produced oil.

Keywords: Free fatty acids; Fourier transform Raman spectrometry; Olive oil; Olives; Partial least squares (PLS) regression

1. Introduction

Virgin olive oil is a natural product obtained from the olive fruit (*Olea europaea*) using solely mechanical or other physical means. In modern olive oil plants, the oil extraction is carried out by direct continuous centrifugation (tri- or dual-phase decanters) that allow higher throughput than the traditional method based on pressure extraction. These new methods demand sophisticated on-line techniques for process monitoring purposes. Indeed, at the present time different automated systems exist to control the performance of olive oil plants. These allow to regulate and to control operation variables such as water and oil temperature, rate of feeding and amount of obtained oil. In order to minimize oil losses, the on-line control of the oil content in the by-product (olive pomace) has been approached successfully using near-infrared (NIR) spectroscopy [1,2]. Nevertheless, there is still a lack of fast analytical methods to determine the quality of the raw material (olives) and of the produced oil.

When evaluating the quality of virgin olive oil, parameters such as free fatty acid (FFA) content,

* Corresponding author. Tel.: +34-95-3002759; fax: +34-95-3012141.
E-mail addresses: blendl@mail.zserv.tuwien.ac.at (B. Lendl), mjayora@ujaen.es (M.J. Ayora-Cañada).

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peroxide value, spectrophotometric absorption at selected wavelengths (K_{232} and K_{270}) and organoleptic characteristics are considered. Among them, FFA content expressed as percentage of oleic acid (w/w) is one of the main criteria used to establish the different categories of olive oil. According to the European Community, *extra* virgin olive oil, which is the most valuable category and is considered a gourmet oil, may not have a FFA content higher than 1%. The FFA limit in olive oil for direct consumption is 3.3%. Oils with higher FFA content are called *lampante* and must be refined prior to consumption [3]. Under ideal conditions, sound, ripe fruits give *extra* virgin olive oil. However, reasons for decreased olive oil quality can be identified throughout the growth phase, harvest and the oil production process. Anomalies during the process of biosynthesis, microbial activities and environmental conditions are all related to the formation of oil with high content in FFA. Infestation by the olive fly (*Bactrocera oleae*) is a major cause of high FFA content in olives. Falling from the trees causes bruises of the olives, which together with long storage times results in lipolysis increasing FFA content in the produced oil. Finally, a badly conducted extraction process also decreases oil quality [4].

Classification of the olives based on their FFA content prior to processing would be an important first step to improve production of valuable *extra* virgin olive oil. In this way, mixing of high and poor quality oils during the extraction process could be prevented. On-line control of the FFA content of the produced oil could detect anomalies during the extraction process that deteriorate the quality of the oil. Furthermore, this would also allow the storage of oils according to their FFA content in the production plant.

The official method of the European Union for FFA determination in olive oil is based on titration of the oil dissolved in a mixture of ethyl ether and ethanol against an alkali using phenolphthalein as indicator [5]. This method is not suited for process control purposes because it is time-consuming, labor intensive and requires large amounts of solvents. In the last decade, several spectroscopic methods have been proposed for the determination of FFA in oils in order to overcome the drawbacks of the official method. They include automated flow injection spectrophotometric methods [6–8] and determination by Fourier transform infrared (FTIR) spectrometry [9–12]. These methods constitute plausible alternatives to the official method. Nevertheless, for process control purposes it would be desirable to avoid any sample treatment or reaction. Only two of the aforementioned methods [10,11], using attenuated total reflection (ATR)–FTIR measure the FFA content directly in the oil. However, to assure a clean surface of the ATR element a sequence of different rinsing solutions (Triton X-100 solution, distilled water, propanol) needs to be applied prior to measurement of each sample.

Regarding the analysis of olives, the currently employed method is still far less suited for on-line process control. It is also based on a wet chemical titration but requires a further time-consuming extraction step to obtain oil from the olives. Consequently, only few producers measure FFA content in olives prior to processing them. The quality control of the raw material is mostly reduced to visual inspections. To our best knowledge, there is no method reported to determine the FFA content directly in the olives, which would be the desirable approach for quality control of the raw material in the oil production plant.

Raman spectrometry is a very promising tool in process analytical chemistry because many samples can be examined non-destructively in short time without any sample preparation. Moreover, Raman spectra exhibit well-resolved bands of fundamental vibrational transitions thus providing a high content of molecular structure information. In combination with chemometric data evaluation Raman spectrometry is a powerful tool capable of extracting quantitative chemical information also from complex matrices [13,14]. Recent advances in instrument technology have decisively contributed to a rapid increase in industrial applications of Raman spectrometry [15,16]. In the field of edible oil analysis, the technique has already been successfully applied to the determination of the total unsaturation in oils [17,18], oil and fat classification [19], and to the detection of adulterations in virgin olive oil [20,21]. In a previous work, we reported the determination of major compounds, oil and humidity in olives using Raman spectrometry with chemometric evaluation [22]. The aim of the present study is to investigate the capability of FT-Raman spectrometry for the determination of FFA content in olive oil and olives.

2. Experimental

2.1. Instrumentation

A Bruker RFS (FT)-Raman spectrometer fitted with a liquid nitrogen-cooled Ge detector was used to record the Raman spectra. The samples were illuminated by a Nd:YAG laser line at 1064 nm with a power of 500 mW using a focused laser beam. The Raman scattered radiation was collected at a 180° geometry. All spectra were recorded with a resolution of 4 cm^{-1} and were averaged over 150 scans. The recording time was 5 min.

2.2. Reagents

All reagents used were of analytical grade. Oleic acid was purchased from Fluka Chemie GmbH, Buchs, Switzerland. Hexane was obtained from Panreac Quimica SA, Barcelona, Spain.

2.3. Samples

Oils and olives of different Spanish varieties were obtained from olive oil plants in the region of Jaén in a period of 2 months during the harvest 2001–2002. The range of free fatty acid content in the 28 oil samples was 0.20–6.14% expressed as percentage of oleic acid. The oils were used without any pretreatment. In the 43 olive samples, FFA content in terms of percentage of oleic acid ranged between 0.15 and 3.79%. Sample pretreatment of the olives consisted in milling in a hammer mill (strainer size 4 mm).

2.4. Measurement procedures

The oil samples were investigated in a dedicated Raman flow cell, which consisted of a PTFE body and a calcium fluoride window. A palladium mirror was placed at the back of the flow channel, which dimensions at the place of measurement were 2 mm width and 2 mm depth. A peristaltic pump was used to pump the oils through the flow cell, which was cleaned between the measurements with hexane. Spectra were recorded in stopped flow.

The milled olive samples were investigated in a cylindrical cup of 13 mm diameter and 5 mm depth. The sample cup was attached via a magnet to a synchronous motor mounted in the sample compartment. The motor allowed the eccentric rotation of the sample cup around the horizontal axis of the laser beam. The rotation velocity of the motor was 5 rounds/min.

2.5. Reference analysis

Reference analyses were performed at CM Europa SA, Martos (Spain), an accredited laboratory for olive and olive oil analysis. The determination of FFA content in oils involves the following steps: (a) exact weighing of the oil, (b) dissolving of the oil in a 1:1 ethanol diethylether solvent, and (c) titration with a 0.1 M ethanolic KOH solution using phenolphthalein as indicator. For the determination of the FFA content in olives the same method was used after extraction of the oil with the Abencor® method [23]. This method reproduces the industrial process in laboratory scale and consists briefly in milling the olive and stirring the paste slowly and continuously. This is followed by centrifugation to separate the solid from the liquid phase. The final step is the decantation of the oil.

2.6. Chemometrics

Raman spectra in the range 300–3200 cm^{-1} were imported into TQ Analyst 6.1.1 (Thermo Nicolet Corp.). The mean of two recorded spectra per sample was used for chemometric evaluation. Preprocessing procedures, spectral region selection and PLS regression model characteristics are described in detail in the Section 3.

3. Results and discussion

3.1. Spectral features

Fig. 1 shows characteristic FT-Raman spectra of pure oleic acid, olive oil and milled olive, obtained in the above-described measurement conditions for oils and olives. The major bands of the virgin olive oil at 1267 (in plane δ(=C–H) deformation in unconjugated *cis* double bond) 1302 (in-phase methylene twisting motion), 1442 ($\delta(CH_2)$), 1655 (ν(C=C)), 2852 ($\nu_{sym}(CH_2)$) and 2900 cm^{-1} ($\nu_{sym}(CH_3)$) dominate the spectra. The band at 1747 cm^{-1}, which is

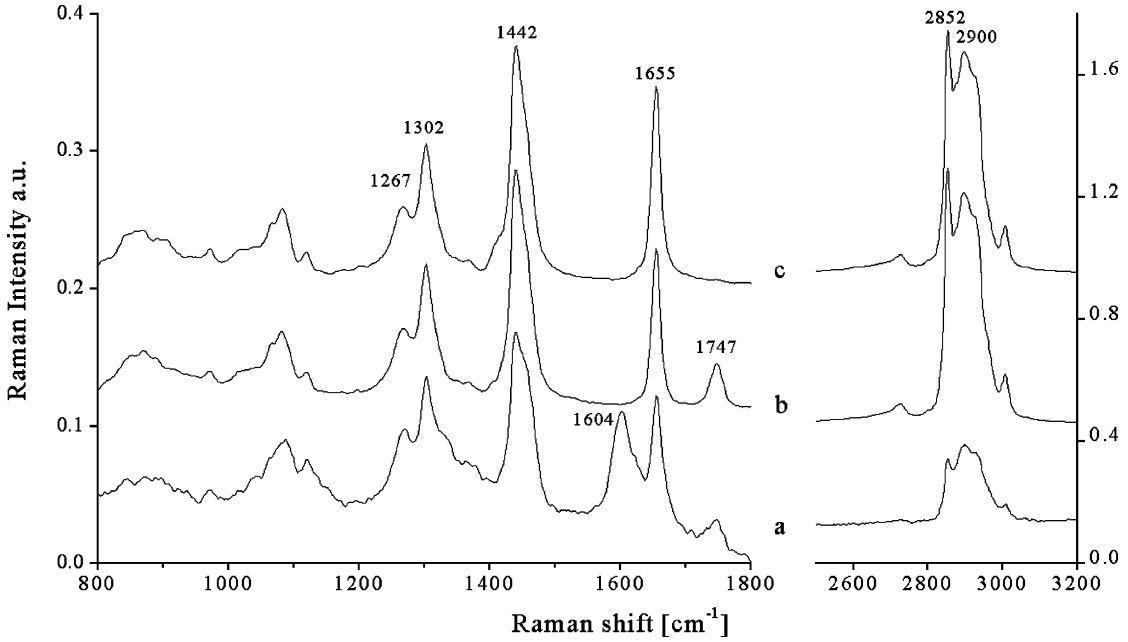

Fig. 1. Raman spectra of (a) milled olives (enhanced by a factor of 5), (b) virgin olive oil and (c) oleic acid.

assigned to the ν(C=O) ester vibration, can be found in both the oil and the olive spectra, but is completely absent in the spectra of the oleic acid. Apart from this band, the spectra of the oil and the oleic acid differ slightly due to the fatty acid composition of the oil and contributions of minor compounds. Comparing the olive with the oil spectra, one major band at 1604 cm^{-1} can be seen. This can be assigned to the aromatic ring stretch of lignin, which is a major compound of the olive kernel. A detailed discussion of the different features and a tentative band assignment can be found elsewhere [22].

3.2. Determination of free fatty acid content in olive oil

Multivariate calibration methods can obtain selective and reliable analyte quantifications from spectral data where the analyte and the matrix composition are varying. Partial least squares (PLS) regression, a flexible full spectra method, was used. In this method, the significant information contained in the Raman spectra is concentrated in a few latent variables that are optimized to produce the best correlation with the concentration information.

The first step in constructing the PLS model was defining a calibration set of 18 and a validation set of 10 samples. Samples of both sets were uniformly distributed across their FFA concentration range, which were 0.20–6.14 and 0.29–5.92% (in terms of oleic acid) for the calibration and the validation set, respectively. Data pretreatment consisted in mean centering the spectra to eliminate common spectral information. To assess the fitting of the model, the root mean square error of calibration (RMSEC) and the correlation coefficient between actual and predicted value for the calibration set (r) were calculated. The optimum number of latent variables for the model was selected based on the root mean square error of cross-validation (RMSECV), which should be minimized. The RMSECV was calculated using the leave-one-out approach. The predictive ability of the model was also taken into account using the root mean square error of prediction (RMSEP) for the validation set.

Region selection can improve the performance of full spectrum models such as PLS regression because models including unnecessary spectral regions tend to over-fit the data, whereas with insufficient regions, valuable information is ignored. The spectral range should include information describing the

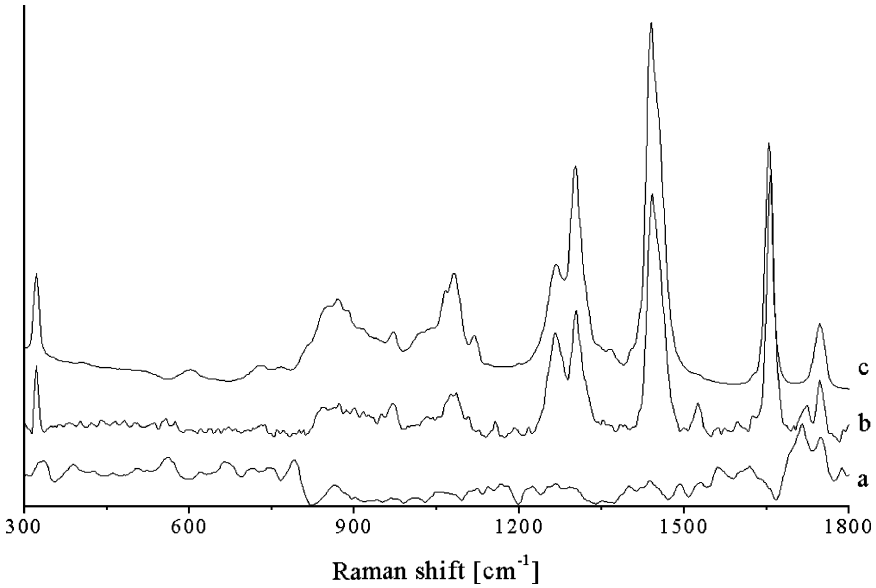

Fig. 2. Correlation (a), variance (b) and mean spectra (c) of the olive oil calibration set.

concentration variation of the analyte and other matrix constituents while excluding regions dominated by noise or other artifacts that might distort the model. Thus, the spectral range between 1800 and 2500 cm^{-1} (not shown) was excluded because it does not contain any bands. Relevant spectral regions can be identified with the used software by computing the correlation spectrum that shows the correlation between the spectral information and component concentrations, and the variance spectrum that shows the spectral variance in the standards. The mean, correlation and variance spectra of the calibration set in the region between 300 and 1800 cm^{-1} are presented in Fig. 2. Variance was found in the main bands in different proportions and additionally at 1720 and 1525 cm^{-1}. Correlation was found in the region between 1680 and 1800 cm^{-1}. The rest of the correlation spectrum shows no characteristic features but noise. Five different spectral regions were examined. The results of the calibration models are summarized in Table 1. Using only the spectral region between 1600 and 1800, which exhibits the highest correlation with free fatty acid content, the calculated model showed poor performance. Only one latent variable was chosen as optimum and both RMSEC (0.72) and RMSEP (0.51) values were very high. This model lacks in spectral information, which is necessary to ensure sufficient modeling. Thus, more spectral variance had to be included. The best results were achieved using the region between 1200 and 1800 cm^{-1}. Broadening the spectral range to 300–1800 cm^{-1} gave a slightly better RMSEC but the predictive ability worsened. This could indicate over-fitting of the model. The inclusion of the 2500–3200 cm^{-1} region also lead to a model with high prediction errors. A reason for this could be that this region showed much higher spectral variance compared with the region between 1200 and 1800 cm^{-1}, but a very low correlation with FFA content (not shown here). Thus, the inclusion of this region does not improve analyte quantification but disturbs the model.

Table 1
PLS regression models for FFA content in olive oil using different spectral regions

Spectral range (cm^{-1})	Latent variables	r	RMSEC (%)	RMSEP (%)
1680–1800	1	0.890	0.72	0.51
1500–1800	5	0.984	0.28	0.48
1200–1800	5	0.985	0.27	0.29
300–1800	6	0.997	0.13	0.42
1200–1800, 2500–3200	4	0.976	0.34	1.03

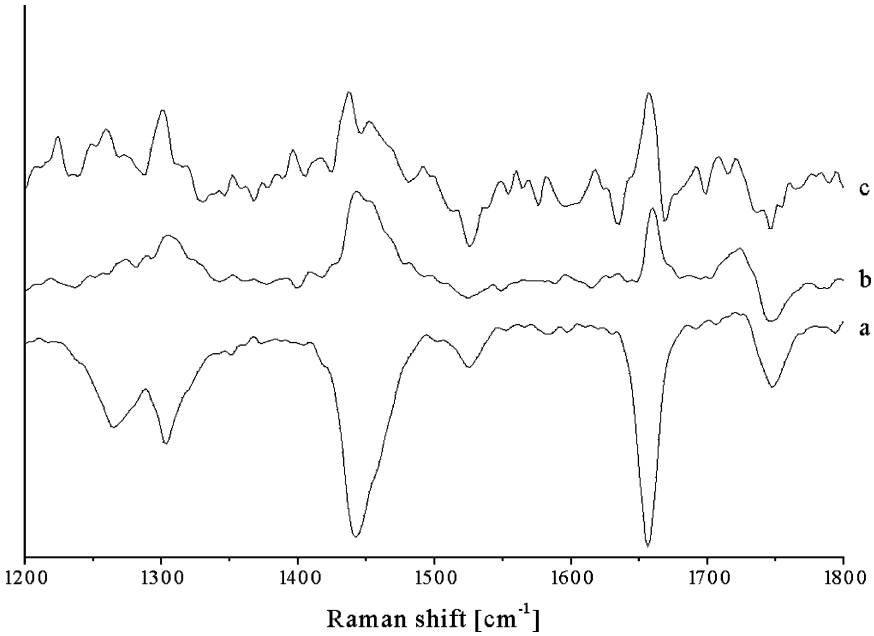

Fig. 3. First three loadings, 1 (a), 2 (b), 3 (c), of the olive oil calibration model in the region 1200–1800 cm^{-1}.

The loading vectors used for the PLS model give qualitative information regarding the spectral features considered to be of importance by the regression. The interpretation of the loadings is generally difficult and only assumptions can be made, because they do not only depend upon the spectral characteristics of the analyte but also of other compounds present in the sample matrix [24]. Nevertheless, they can be examined to ensure that the actual spectral information from the analyte of interest is used. This is especially useful when examining a large spectral range for a small set of calibration samples, as it is the case, because the chance of finding bands that accidentally correlate to the reference measurement can be high. Fig. 3 depicts the computed loadings for the first three latent variables of the model in the range of 1200–1800 cm^{-1}, which altogether account for 89.5% of the spectral and 92.5% of the concentration variances. The first loading shows all the spectral features of the virgin olive oil, thus reflects spectral variations due to compositional differences in the oil matrix. Oils differ in their triacylglycerol composition and consequently also in their free fatty acid composition. Oleic acid is the main fatty acid (up to 80%) but also linoleic, palmitic, and stearic acid are found in significant proportions. These fatty acids differ in their chain length and in the degree of unsaturation. These differences are reflected in the main bands of the first loading located at 1442 and 1655 cm^{-1}, corresponding to $\delta(CH_2)$ and ν(C=C), respectively. The first latent variable explains 72.5% of the spectral variation but only 17.5% of the FFA concentration variation. The major part of the concentration variation (65%) is described by the second latent variable. Its loading shows the decrease at 1747 cm^{-1}, which corresponds to the ν(C=O) ester vibration, and the increase of one band at 1720 cm^{-1}. The latter can be attributed to the ν(C=O) of the carboxylic acid. This band cannot be clearly seen in Fig. 1, where spectra of olive oil and oleic acid are presented, but appears as a tailing of the band at 1655 cm^{-1} and is present in both variance and correlation spectrum (Fig. 2). Bands at 1302, 1440 and 1660 cm^{-1} also appear but their interpretation is rather difficult. They are shifted compared to the bands in the first loading, which could indicate the presence of different FFA. The third loading vector, which accounts for 2% of the spectral and 10% of the concentration variation, contains more noise. The band at 1660 cm^{-1} appears again slightly shifted. The band at 1525 cm^{-1} could be attributed to the ν(C=C) stretching vibration

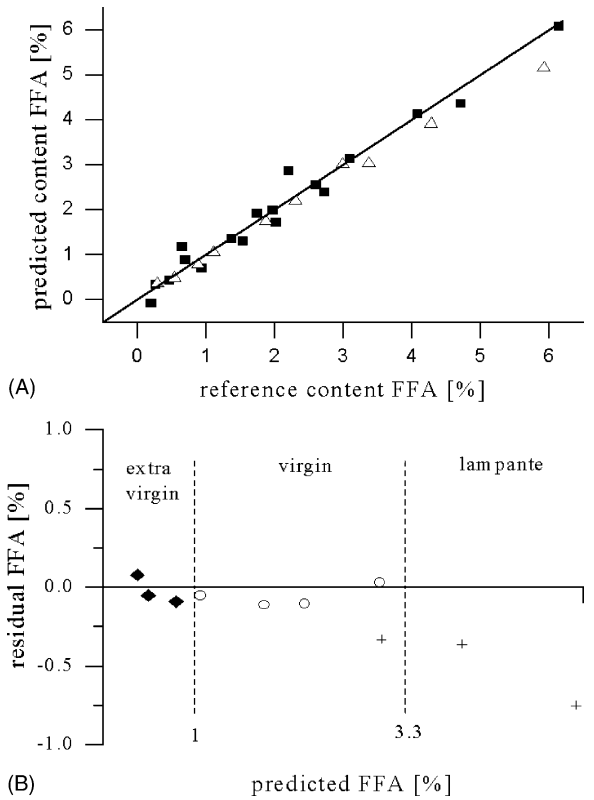

Fig. 4. (A) Predicted vs. reference FFA content for the olive oil calibration: (■) calibration samples; (△) validation samples. (B) Classification of olive oil validation samples according to FFA content: (◆) extra virgin; (○) virgin; (+) lampante olive oil according to the reference analysis.

in conjugated systems, like in carotenoids, which are minor compounds in the oil, but differ significantly in different varieties and give strong Raman signals [25].

The final PLS regression model for determining FFA content in olive oil was built using five latent variables in the region 1200–1800 cm^{-1}. It had a RMSEC of 0.27% and a correlation coefficient (r) of 0.985. Fig. 4A shows the plot of the FFA content predicted by the PLS model based on the Raman data versus the concentrations obtained using the reference analysis. The predictive ability of the model was checked on 10 independent samples providing a RMSEP of 0.29%. An alternative approach to evaluate the predictive ability of the model was performed. All the 28 samples were used to construct the model and a leave-one-out cross-validation procedure was adopted. This model was constructed using five latent variables, had a RMSEC of 0.27% and the RMSECV was 0.41%. The RMSECV is higher than the RMSEP. This can indicate certain instability of the model, which could be avoided if more calibration samples were included in the model.

Fig. 4A also includes the results of the independent validation samples. Samples in the very high concentration range have higher errors than those in the lower range. This could indicate that they do not fit the calibration model, but the inspection of the leverage and residual variance did not reveal that these samples were significantly different from the others. Better predictions could probably be achieved with more calibration samples in this region. Anyway, these errors are of minor importance because content in comestible oils is lower than 3.3% and the method is able to classify these oils as bad quality oils. The qualitative statement derived from these quantitative predictions can be more easily visualized in Fig. 4B. This plot shows the residuals against the predicted FFA content for the validation samples. The dashed lines reflect the concentrations of FFA, which define the three different categories of virgin olive oils according to the European Commission regulations. The different symbols group the samples into the categories as obtained by the reference method. The diamonds are *extra* virgin, the circles virgin and the crosses *lampante* olive oils. Only 1 of the 10 oils was misclassified. Although it is clear that the accuracy of the proposed method is inferior to the official method, results confirm that the method is useful for process control enabling a fast screening of the olive oil categories to store them separately.

3.3. Determination of the free fatty acid content in olives

A method, which is capable of the rapid screening of olives before processing them must work directly on the olive. The time-consuming oil extraction prior to FFA determination is not applicable for process control. The direct spectroscopic investigation of the olive paste is a challenge due to the complexity of the matrix. The main obstacle is the heterogeneity of the sample, which contains kernel, pulp and skin. In a previous work, the quantitative determination of major compounds (oil and humidity) in milled olives by Raman spectrometry was reported. It was found

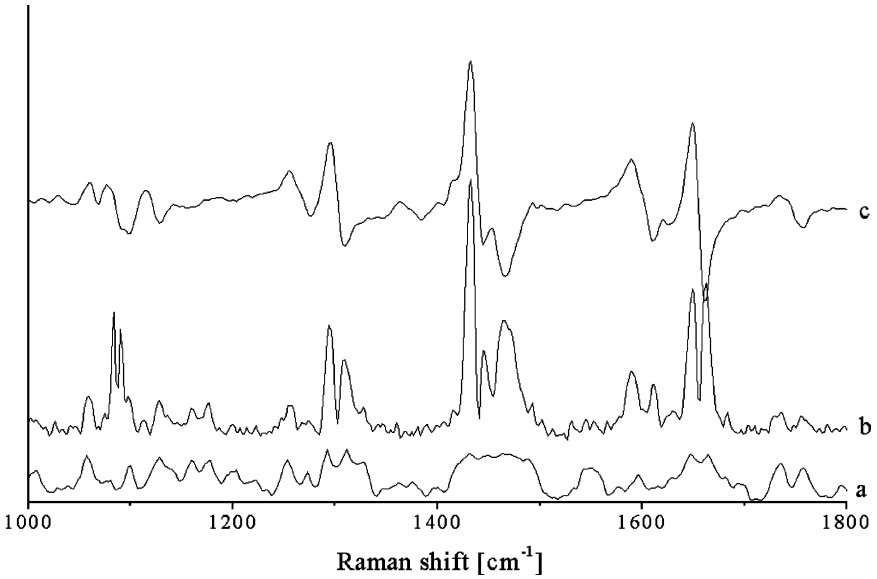

Fig. 5. Correlation (a), variance (b) and mean spectra (c) of the olive calibration set (derivative spectra).

that rotating the sample in the above-described cell enabled reproducible measurements due to the increased illuminated sample volume [22].

For PLS model construction, the 43 olive samples were divided into two sets: a calibration set consisting of 28 samples and a validation set consisting of 15 samples. The samples for the validation set were randomly selected, covering the whole concentration range. Baseline correction was necessary because some of the spectra showed high fluorescence backgrounds. One common and simple method to remove these sorts of baseline effects is calculation of derivative spectra. The derivative spectra were then mean centered. The next step in model development was region selection. For this, the mean, variance and correlation spectra were calculated (see Fig. 5) and the results obtained for the oil were considered, too. Beside the variance in the major oil peaks, there are two additional maxima in the variance spectra. They appear as double peaks due to the use of first derivative spectra. One corresponds to the peak at 1604 cm^{-1} in the original spectrum and reflects the influence of the olive kernel. The other, found at approximately 1085 cm^{-1}, probably derives from changes in the vegetable matter. This region was found to influence the PLS calibration despite its lower correlation with FFA content. Two models were constructed, one using the spectral region between 1200 and 1800 cm^{-1} and the other between 1000 and 1800 cm^{-1}. The results of the calibration for the two models are summarized in Table 2. The model built on the broader spectral range gave better results. Four latent variables were selected as optimum based on the RMSECV. The poorer model was build with three latent variables. The loadings of both models (not shown here) were almost identical, with the only difference of a band at 1085 cm^{-1} in the second, third and fourth latent variable. With the four latent variable model 77.5% of the spectral variance was accounted for and 95.5% of the concentration variance. This means, that more than 20% of the spectral variance was not included in the PLS model. Probably, this spectral variance derives from differences in the vegetable matter of the olive fruit and from scattering effects of the sample surface during spectra acquisition.

Table 2
PLS regression models for FFA content in olives using different spectral regions

Spectral range (cm^{-1})	Latent variables	r	RMSEC (%)	RMSEP (%)
1200–1800	3	0.944	0.37	0.30
1000–1800	4	0.977	0.24	0.28

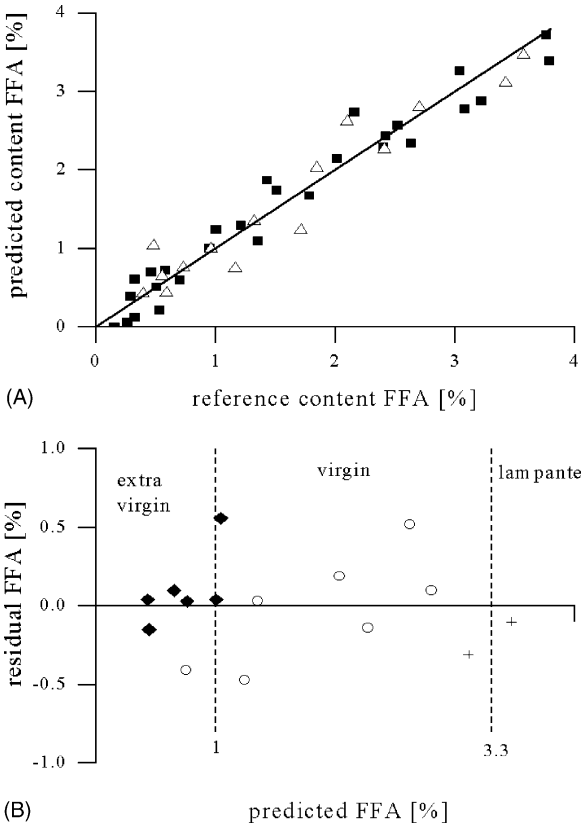

Fig. 6. (A) Predicted vs. reference FFA content for the olive calibration: (■) calibration samples; (△) validation samples. (B) Classification of olive validation samples according to FFA content: (◆) extra virgin; (○) virgin; (+) lampante according to the reference analysis of the extracted oils.

The predictive ability of the model, using the region 1000–1800 cm^{-1} was checked by internal (cross-validation) and external validation. The RMSECV of the leave-one-out cross-validation using the 43 available samples was 0.44%. The external validation was performed splitting the data in two sets. Using a validation set of 15 independent samples the SEP was 0.28%. The results showed a predictive ability similar to the oil method. Fig. 6A depicts the calibration and prediction results for the external validation set.

The ultimate goal of this study was to investigate the possibilities of screening olives, using FT-Raman spectrometry and PLS regression. Therefore, the analytical method should be able to distinguish between high quality olives, from which *extra* virgin olive oil can be extracted, and poor quality olives. For reference analysis, an extraction of the oil from the olives has to be performed. The FFA content in the extracted oil is then used to classify the oil as *extra* virgin (0–1% FFA), virgen (1–3.3% FFA) and *lampante* (>3.3% FFA). Using FT-Raman spectrometry and PLS regression the FFA content of the oil can be predicted directly in the olives. In Fig. 6B, the *x*-axis shows the FFA content, predicted by the PLS model, and the *y*-axis the difference between FFA content obtained by the reference method and predicted by the model. The symbols group the samples into the categories according to results obtained with the reference measurement. Eighty percent of the olives were correctly classified. This result proofs that Raman spectrometry can provide a useful tool to discriminate between different categories of olives, in order to optimize the production of high quality virgin olive oil.

4. Conclusions

We have reported two direct, reagent-free methods to estimate FFA content in olive oil and olives using Fourier transform Raman spectrometry and chemometric evaluation. Although results are less accurate compared to the official method, the here proposed methods are well suited for quality control in process monitoring. Due to its simplicity, the method for olive oil analysis allows the on-line measurement of the produced oil. The method for olive analysis is much faster than the official one, because sample preparation is reduced to a minimum (milling). Using Raman spectrometry, a classification of olives based on their FFA content before processing them to obtain olive oil becomes feasible.

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References

[1] J.A. García Mesa, M. Hermoso Fernández, P. Cáceres Alonso, Grasas y Aceites 47 (1996) 317.
[2] M. Hermoso, M. Uceda, A. García Ortiz, A. Jiménez, G. Beltrán, Acta Hortic 474 (1997) 717 (Proceedings of the Third International Symposium on Olive Growing, vol. 2, 1997).
[3] Commission Regulation (EEC), No. 796/2002, OJ No. L 128/8, 2002.
[4] D. Boskou (Ed.), Olive Oil: Chemistry and Technology, AOCS Press, 1996, p. 114.
[5] Commission Regulation (EEC), No. 2568/91, OJ No. L 248, 1991.
[6] Z. Zhi, A. Rios, M. Valcarcel, Anal. Chim. Acta 318 (1996) 187.
[7] P.G. Nouros, C.A. Georgiou, M.G. Polissiou, Anal. Chim. Acta 351 (1997) 291.
[8] E. Mariotti, M. Mascini, Food Chem. 73 (2001) 235.
[9] A.C. Lanser, G.R. List, R.K. Holloway, T.L. Mounts, J. Am. Oil Chem. Soc. 68 (8) (1991) 448.
[10] A.A. Ismail, F.R. van de Voort, G. Emo, J. Sedman, J. Am. Oil Chem. Soc. 70 (4) (1993) 335.
[11] E. Beltran, M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, I. Montoliu, J. Am. Oil Chem. Soc. 76 (5) (1999) 611.
[12] M.J. Ayora Cañada, A. Ruiz Medina, B. Lendl, Appl. Spectrosc. 55 (3) (2001) 356.
[13] D.S. Himmelsbach, F.E. Barton II, A.M. McClung, E.T. Champagne, Cereal Chem. 78 (4) (2001) 488.
[14] H. Schulz, B. Schrader, R. Quilitzsch, B. Steuer, Appl. Spectrosc. 56 (2002) 117.
[15] J.B. Cooper, Chemom. Intell. Lab. Syst. 46 (1999) 231.
[16] J.M. Chalmers, G. Dent (Eds.), Industrial Analysis with Vibrational Spectroscopy, RSC, UK, 1997.
[17] H. Sadeghi-Jorabchi, P.J. Hendra, R.H. Wilson, P.S. Belton, J. Am. Oil Chem. Soc. 67 (8) (1990) 483.
[18] R.C. Barthus, R.J. Poppi, Vib. Spectrosc. 26 (2001) 99.
[19] V. Baeten, P. Hourant, M.T. Morales, R. Aparicio, J. Agric. Food Chem. 46 (1998) 2638.
[20] V. Baeten, M. Meurens, J. Agric. Food Chem. 44 (1996) 2225.
[21] N.A. Marigheto, E.K. Kemsley, M. Defernez, R.H. Wilson, J. Am. Oil Chem. Soc. 75 (8) (1998) 987.
[22] B. Muik, B. Lendl, A. Molina-Díaz, M.J. Ayora-Cañada, Appl. Spectrosc. 57 (2003) 233.
[23] J.M. Martinez Suarez, E. Muñoz Aranda, J. Alba Mendoza, A. Lanzón Rey, Grasas y Aceites 26 (6) (1975) 379.
[24] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[25] V. Baeten, P. Dardenne, R. Aparicio, J. Agric. Food Chem. 49 (2001) 5098.