

# Visible Raman spectroscopy for the discrimination of olive oils from different vegetable oils and the detection of adulteration

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We have investigated the potential of Raman spectroscopy with excitation in the visible spectral range (VIS Raman) as a tool for the classification of different vegetable oils and the quantification of adulteration of virgin olive oil as an example. For the classification, principal component analysis (PCA) was applied, where 96% of the spectral variation was characterized by the first two components. A significant similarity between sunflower oil and extra-virgin olive oil was found using this approach. Therefore, sunflower oil is a potential candidate for adulteration in most commercially available olive oils. Beside the classification of the different vegetable oils, we have successfully applied Raman spectroscopy in combination with partial least-squares (PLS) regression analysis for very fast monitoring of adulteration of extra-virgin olive oil with sunflower oil. Different mixtures of extra-virgin olive oil with three different sunflower oil types were prepared between 5 and 100% (v/v) in 5% increments of sunflower oil. While in the present context the adulteration usually refers to the addition of reasonable amounts of the adulterant (given the similarity with the basic product), we show that the technique proposed can also be used for trace analysis of the adulterant. Without using techniques like surface-enhanced Raman scattering (SERS), a quantitative detection limit down to 500 ppm (0.05%) could be achieved, a limit irrelevant for adulteration in commercial terms but significant for trace analysis. The qualitative detection limit even was at considerably lower concentration values. Based on PCA, a clear discrimination between pure extra-virgin olive oil and olive oil adulterated with sunflower oil was achieved. The adulterant content was successfully determined using PLS regression with a high correlation coefficient and small root mean-square error for both prediction and validation. Copyright © 2009 John Wiley & Sons, Ltd.

**Keywords:** Raman spectroscopy; chemometrics; vegetable oils; adulteration detection; olive oil

## Introduction

Olive oil has a high nutritional value and significant health benefits.<sup>[1–3]</sup> It is an essential component of the Mediterranean diet. According to the European Union (EU), olive oils are classified into various categories ranging from the high-quality extra-virgin olive oil to the low-quality olive pomace oil.<sup>[4]</sup> Extra-virgin olive oils are produced using only cold pressing techniques without any thermal or chemical treatments, while olive pomace oil, a fully refined olive oil, is obtained by solvent extraction processes from olive pomace.<sup>[5]</sup> Extra-virgin olive oils are the most desirable on account of their high quality and nutritional properties compared to low-quality pomace olive oil<sup>[6]</sup> and are therefore the most expensive grades. For the last reason, as a matter of fact and for commercial purposes, there is a tendency of mislabeling or adulterating extra-virgin olive oil with refined olive oil or cheaper and similar oils, such as sunflower oil and hazelnut oil.<sup>[7–9]</sup> Therefore, the identification of different oils and the detection of oil adulteration are of great importance from both market and health aspects. Classical methods based on wet chemical or chromatographic analysis for the determination of free acid radicals, peroxide value and fatty acid composition are typically used to detect adulterants and classify olive oil categories. Especially, a standard method based on quantification of stigmastadienes, which are derived from refining processes, is used in order to detect adulteration of virgin olive oil with refined

oils.<sup>[10]</sup> Chromatographic methods such as high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC) have been successfully applied to determine triglycerides<sup>[11]</sup> and the fatty acids,<sup>[12]</sup> respectively, in order to check the quality and detect the adulteration in edible oils. In addition, triglycerides of olive oil and some seed oils (corn, cottonseed, palm, peanut, soybean, and sunflower) are used to detect olive oil adulteration by means of capillary gas chromatography.<sup>[13]</sup>

In olive oil industry, these classical methods are not only time consuming and inconvenient for online process control, but they are expensive as well. Meanwhile, rapid and simple techniques are needed, which would offer online detection in order to boost up the production while reducing the costs and satisfying quality needs of the consumer.

Spectroscopy techniques now have the potential to replace or at least complement these classical methods. Nuclear magnetic resonance (NMR) analysis for detecting adulteration of olive oil has been reported.<sup>[14–16]</sup> With respect to vibrational spectroscopy, near infrared (NIR) absorption has been applied for the

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characterization of several edible oils<sup>[17]</sup> and for olive oil analysis.<sup>[18]</sup> Similarly, Fourier transform infrared (FT-IR) spectroscopy has been demonstrated to be successful for the detection of hazelnut oil in olive oil with a minimum of 5% of the former.<sup>[19]</sup> However, also Raman spectroscopy can provide content-relevant information about molecular vibrations with well-defined and resolved spectral features in various sample categories including liquids, solids, and gases. Raman scattering is based on the rate of change of the molecular polarizability or configuration of electron distribution in the molecule as vibrational transitions occurring mainly in the electronic ground state take place within the molecule.<sup>[20]</sup> The major advantages of Raman spectroscopy are that it requires no sample preparation, it is a rapid and nondestructive tool, and, when combined with a fiber-optic sampling station, it can make such a system ideally suited for online industrial processing, since the direct contact with the sample is not necessarily required to obtain spectra; both excitation and collection of signal light can be performed, e.g., through windows of a production vessel. As a matter of fact, in recent research works, extensive effort has been spent on the authentication of virgin olive oil<sup>[21,22]</sup> and edible oils<sup>[23,24]</sup> by FT-Raman spectroscopy with NIR excitation.

Vibrational spectroscopy in combination with multivariate analysis is a promising analytical technique for online application (for control and monitoring purposes), offering an excellent technology for early detection of olive oil adulteration. FT-Raman spectroscopy in combination with principal component analysis (PCA) and multidimensional scaling (MDS) has been employed to classify oils and fats.<sup>[25,26]</sup> Partial least-squares (PLS) regression has been used to build a calibration model for the prediction of adulterants in olive oil using FT-Raman spectroscopy with excitation at 1064 nm,<sup>[27,28]</sup> and Raman scattering with NIR 780-nm excitation (minimum values of 2 and 5% hazelnut oil, respectively).<sup>[29]</sup>

Although Raman spectroscopy has become a widely used tool for analytical purposes both in industry and in scientific research, it is still considered a new emerging technique in the field of food analysis<sup>[30]</sup> where more extensive research is still to be achieved. To our knowledge, none of the previous research works dealt with olive oil characterization using standard Raman spectroscopy with visible excitation (VIS Raman, e.g. 514.5-nm laser excitation); in most cases a Nd:YAG laser was employed with its emission at 1064 nm. The reason for this might be that for several organic samples the application of dispersive VIS Raman spectroscopy results in considerable problems caused by the strong overlapping fluorescence which makes it very difficult to distinguish the vibrational bands from the intense background signal.<sup>[31]</sup> The advantage of an excitation with blue-shifted light is the increased signal intensity in general ( $\omega^4$  dependence) and in some cases (e.g. for carotenoids) the resonance enhancement, which helps detecting also constituents at lower concentrations. Additionally, compared to FT-Raman, the dispersive technique uses simpler instrumentation, which also can result in a rather compact design and portable devices. For example, a combination of a small frequency-doubled Nd:YAG laser and a fiber-fed fixed-grating spectrometer with an array detector could be used optimally for the spectral range and resolution needed.

In our work, we have demonstrated the potential capabilities of dispersive Raman spectroscopy using visible excitation in combination with appropriate chemometric methods. In the following, we present and discuss our results. We start with the investigation and characterization of different vegetable oils. Then, we discuss the quantitative analysis of olive oil adulteration

with sunflower oil, which is a common adulterant for olive oil because of its high oleic content.<sup>[19]</sup>

## Materials and Methods

### Sample preparation

Seventeen different brands of extra-virgin olive oil and 13 different vegetable oils (rape seed oil, sesame oil, garlic oil, bean oil with omega 3, three different types of sunflower oil, walnut oil, two different types of safflower oil, soybean oil, wheat germ oil, and flaxseed oil) were purchased as commercial products from local grocery stores. Additionally, one sample of extra-virgin olive oil produced by cold press was obtained directly from a farmer. All the oil samples were stored at room temperature until the time of analysis in order to provide realistic testing conditions. First of all, all the 18 extra-virgin olive oils (labeled and cold pressed) were tested by titration, which showed that their free fatty acid (FFA) content was distributed between 0.2 and 0.4%. This is below the maximum range of FFA content for extra-virgin olive oil, which is 0.8–1% expressed as oleic acid percentage.<sup>[32]</sup> Then the pure samples of extra-virgin olive and other vegetable oils were Raman spectroscopically analyzed and classified by using multivariate analysis (PCA). Binary mixtures of extra-virgin olive oil and three different brands of sunflower oil were prepared for the investigation of adulteration. The percentage of sunflower oil in olive oil was varied from 0 to 100% (v/v) in 5% increments. A total of three sets were considered, each set containing 21 different concentrations of sunflower oil.

### Raman experiment

Raman measurements were carried out using the 514.5-nm emission of an argon ion laser (10 mW at sample) for excitation. The laser was focused in the sample contained in 1800  $\mu$ l quartz cuvettes (Starna) using an inverted microscope setup equipped with a 10 $\times$  ultralong-working-distance objective (10 $\times$  ULWD, N.A. 0.2; Olympus). The Raman signal was collected in the backscattering geometry and dispersed by a TRIAX 550 single monochromator (Jobin Yvon) using a 1200 grooves/mm diffraction grating and an entrance slit width of 200  $\mu$ m. For signal detection, a liquid-nitrogen-cooled charge-coupled device (CCD) detector was employed with optimal sensitivity in the visible (blue/green) and a chip size of 2048  $\times$  1024 pixels (Symphony 3500, Jobin Yvon). These unique features allowed for an absolute exposure time of only 3 s. Therefore, the complete spectral range of interest (700–3100  $\text{cm}^{-1}$ ) corresponded to two spectral windows and each window was recorded in just 15 s within which five scans were averaged. Within this time, a noise-free spectrum could be obtained. To obtain sufficiently reliable spectra, the measuring time could be reduced without problems down to 1 s. A spike filter was used to remove cosmic ray peaks. Each sample was analyzed in triplicate. Toluene was used to calibrate the Raman setup (wavenumber position and intensity).

### Data processing

Raw Raman spectra were preprocessed as follows. The baseline of each spectrum was approximated by a fourth-order polynomial fit in order to subtract the fluorescence background. Raman spectra were regarded as multidimensional data sets, where each object consisted of  $n$  variables corresponding to the Raman intensities

(counts) recorded at the different Raman shifts ( $\text{cm}^{-1}$ ). These data sets were then analyzed by a multivariate analysis method<sup>[33]</sup> as described below.

### Statistical analysis

PCA and PLS regression were performed using the robust commercial software package Unscrambler (v 9.7; CAMO A/S). In order to compare the different bean oils with the 18 extra virgin olive oils as well as to discriminate extra-virgin olive oil from olive oil adulterated with the three sunflower oil types, a qualitative classification was performed by PCA. PCA is one of the most common multivariate analysis methods used to reduce the dimensionality of large data sets by finding combinations of variables that describe the major trends in the data.<sup>[34]</sup> In addition to this, the PLS regression method was applied in order to (quantitatively) determine the sunflower oil content in adulterated olive oil.<sup>[35]</sup> Usually, for the PLS analysis the samples are divided into a calibration set and a validation set. However, in our work the validation step was performed using full cross validation. In this process one sample is left out from the calibration process, followed by the construction of a calibration model using the remaining samples, which is then tested on the sample left out; this procedure is repeated until each sample has been left out once. The validation procedure consisted of two looping steps: First, the model is built with full cross validation. Second, outlying samples (spike samples) are detected and removed. Then, the model is rebuilt from scratch without the outliers so that the information used to produce them in the previous step is removed from the calibration set. This procedure is repeated until there are no more outliers, provided that the calibration set still contains enough samples to validate the model with good estimates of the root mean-square error (RMSE). In our case, a good validation was obtained already in the first loop without the existence of any spike samples. Finally, this yielded a model with good RMSEs both for calibration and prediction. The model accuracy was assessed by calculating the RMSE, which measures the average difference between predicted and measured response values – it can be interpreted as the average modeling error, as expressed in Eqn (1).

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^i (y_{\text{ical}} - y_{\text{iref}})^2}{N}} \quad (1)$$

Here,  $y_{\text{ical}}$  is the calculated (predicted) value using the PLS model,  $y_{\text{iref}}$  the reference value and  $N$  the total number of samples used for calibration.

To evaluate the degree of linearity of the model, correlation coefficients ( $R^2$ ) between the actual and predicted values were computed.<sup>[36]</sup>

## Results and Discussion

Raman spectra of 13 different bean oils were measured under green excitation at 514.5 nm. The Raman spectra were recorded in the spectral region  $700\text{--}3100\text{ cm}^{-1}$ , as shown in Fig. 1. While the different oil spectra exhibit changes in the Raman intensities of many lines, there are no noticeable changes in the positions (Raman shift) of these bands. However, three bands around  $1008\text{ cm}^{-1}$  ( $\text{C--CH}_3$  bending),  $1150\text{ cm}^{-1}$  ( $\text{C--C}$  stretching), and  $1525\text{ cm}^{-1}$  ( $\text{C=C}$

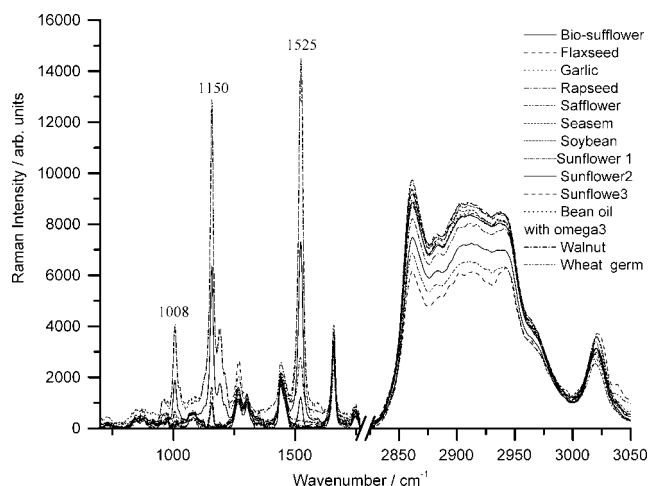


Figure 1. Raman spectra of 13 different bean oils.

stretching) attributed to carotenoides<sup>[37]</sup> are missing in some oil samples (garlic oil, bean oil with omega 3, sunflower oil, soybean oil, and rape seed oil). These bands are not seen in practically all oil spectra obtained with red and NIR excitation, which can be explained by the resonance enhancement when using the green laser for Raman spectroscopy. Qualitatively, the similarity of these spectra reflects the similar chemical composition between the selected oils. The spectra of extra-virgin olive oil and the ones adulterated with sunflower oil are shown in Fig. 2. It is noticeable that the carotenoid bands at  $1008$ ,  $1150$ , and  $1525\text{ cm}^{-1}$  decrease with increasing concentration of sunflower oil, while the other remaining bands only show slight asymmetric changes of their shapes. The Raman spectra of 18 extra-virgin olive oils were compared with those of 13 different bean oils and with those of 21 samples of extra-virgin olive oil adulterated with sunflower oil in order to reveal characteristic spectral variations. Raman scattering of edible oils are extensively described in the literature.<sup>[38,39]</sup> Based on previous reports,<sup>[27,40]</sup> the assignment of major peaks is given in Table 1. The main differences between olive oil and sunflower oil were observed in the  $800\text{--}1800$  and  $2850\text{--}3020\text{ cm}^{-1}$  regions; these regions were used to establish the PLS model for quantitative analysis.

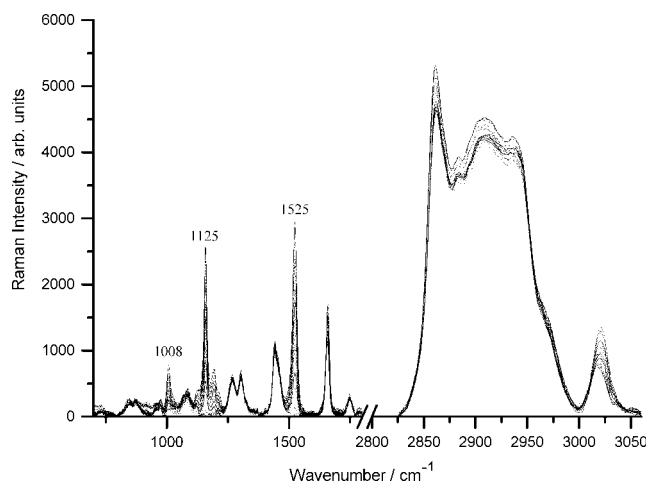


Figure 2. Raman spectra of pure extra-virgin olive oil and the one adulterated with sunflower oil.

**Table 1.** Assignment of major Raman peaks in edible oils

| Wavenumber (cm <sup>-1</sup> ) | Molecule/group                     | Vibrational mode          |
|--------------------------------|------------------------------------|---------------------------|
| 3005                           | <i>cis</i> RHC=CHR                 | =C–H symmetric stretching |
| 2924                           | –CH <sub>2</sub>                   | C–H asymmetric stretching |
| 2897                           | –CH <sub>3</sub>                   | C–H symmetric stretching  |
| 2850                           | –CH <sub>2</sub>                   | C–H symmetric stretching  |
| 1750                           | RC=OOR                             | C=O stretching            |
| 1650                           | <i>cis</i> RHC=CHR                 | C=C stretching            |
| 1525                           | RHC=CHR                            | C=C stretching            |
| 1440                           | –CH <sub>2</sub>                   | C–H bending (scissoring)  |
| 1300                           | –CH <sub>2</sub>                   | C–H bending (twisting)    |
| 1265                           | <i>cis</i> RHC=CHR                 | =C–H bending (scissoring) |
| 1150                           | –(CH <sub>2</sub> ) <sub>n</sub> – | C–C stretching            |
| 1008                           | HC–CH <sub>3</sub>                 | CH <sub>3</sub> bending   |
| 968                            | <i>trans</i> RHC=CHR               | C=C bending               |
| 868                            | –(CH <sub>2</sub> ) <sub>n</sub> – | C–C stretching            |

### Classification using PCA

To gain insight into (1) the similarity between the extra virgin olive oil and bean oils, and (2) the characteristic differences between pure extra-virgin olive oil and those adulterated with sunflower oil, statistical analysis was performed using PCA classification. In the first case (1), two separate PCA models were built with a limited number of samples from each group (extra-virgin olive vs other vegetable oils); then, the remaining samples of each group were added to the classification in order to check how they are distributed between the two groups. The results are shown in the Cooman's plot<sup>[19]</sup> (Fig. 3), which represents the best way to visualize the similarity between the newly classified samples and the two groups. In fact, this plot displays the samples in the two models (groups) by checking how well the spectra of unknown samples match the spectra of the calibration set, which allows us to visualize how far the groups are from each other.<sup>[41]</sup> The newly classified samples are displayed as light gray triangle symbols, while the samples in the calibration set for the two models are displayed as black circles and squares. It is noticeable that the olive oils appear to be separated from the other oils with an explained spectral variation of 96% for the first two components. Among all vegetable oils, sunflower oil was the closest to olive oil and walnut oil was the farthest; these findings are in good agreement with previous research work.<sup>[25,42]</sup>

Since the Raman spectra are determined by the chemical composition of the oils, this result reflects the high chemical similarity of sunflower oil to olive oil. For this reason, sunflower oil was selected as the best candidate to adulterate olive oil. Figure 4 shows the clear separation between pure extra-virgin olive oil and the adulterated samples. Using only two principal components, 98% of the desired information within the Raman spectra can be described. Moreover, PCA successfully distinguished the adulterated samples even with the smallest addition of sunflower oil, as low as  $\leq 5\%$ .

### Quantification using PLS

The estimation of the sunflower oil content in extra-virgin olive oil was performed using a PLS regression. The PLS regression was calibrated with the calibration sample set and the validation was achieved by full cross validation. The optimum number of

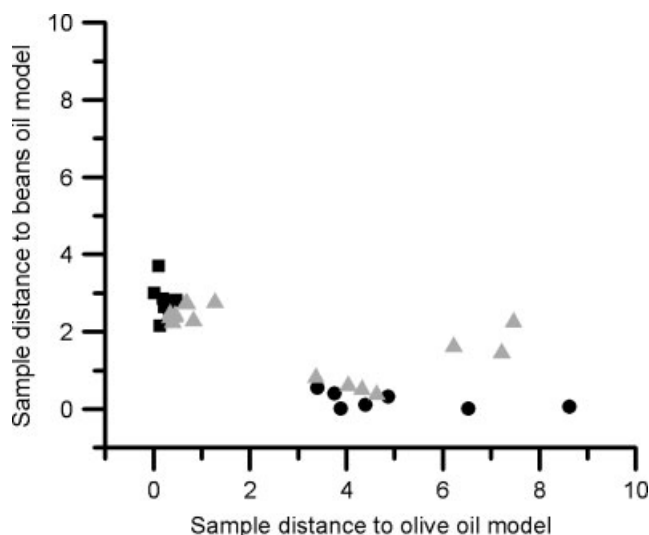


**Figure 3.** Cooman's plot for the classification of 18 extra-virgin olive oils and 13 different bean oils.

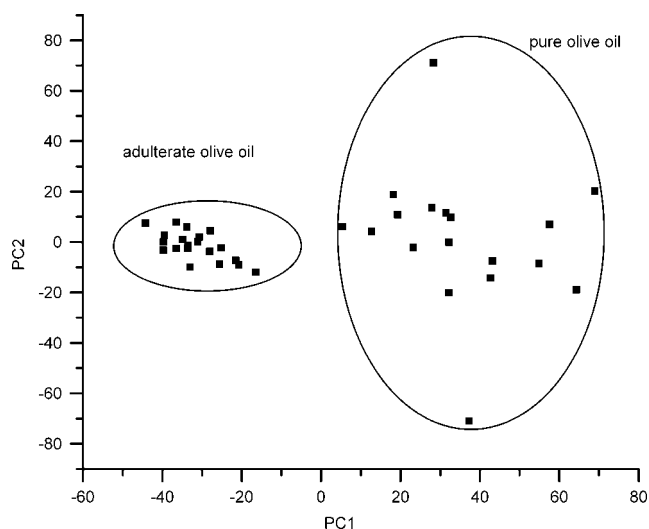


**Figure 4.** Principal component for the classification of 18 pure olive oil samples and 21 olive oil samples adulterated with sunflower oil.

principal components (PCs) used in this calibration model was checked by a residual variance plot against the PCs, which gives an indication of how much of the variation in the data is described by the different components. Models in which the residual variance tends to zero with as few components as possible are best suited to explain the variation in the data (Fig. 5). The PLS score plot in Fig. 6 helps in the detection outliers among samples and in the present case indicates that only negligible deviations occur. To find out which variables (Raman shifts) are important for the prediction, the regression coefficients of the model were plotted against the Raman shift (Fig. 7). This plot shows the relationship between the regression coefficients and the independent variables (Raman shifts); large positive and negative variables are statistically significant in predicting unknown values. Figure 7 clearly shows that the PLS method uses practically the whole Raman spectra to determine the content of sunflower oil adulterant; this is due to the previously mentioned considerable similarity between virgin olive oil and sunflower oil.

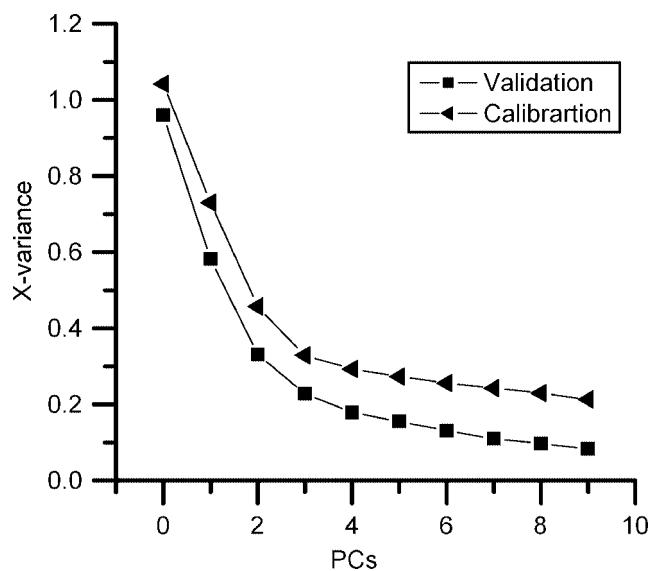


Figure 5. Residual variance for PLS model.

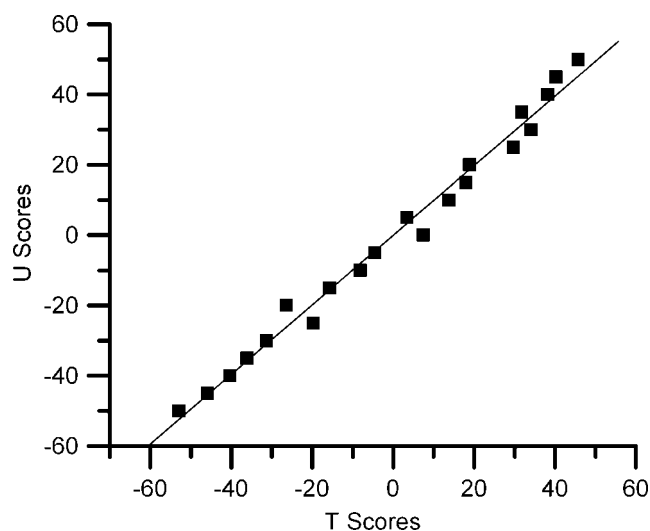


Figure 6. Scores of PLS model for outlier detection.

Figure 8 shows the comparison between the measured (sample preparation) values of sunflower oil content in extra-virgin olive oil against the values determined from the Raman spectra. To assess the fitting of the model, the prediction error (RMSE) and the correlation coefficients were calculated. The estimated values of the RMSE and the correlations for the models are summarized in Table 2 for adulterant concentration ranging from 5 to 100%. It shows that the sunflower oil labeled 'sunflower2', which is organic oil, offers the optimum calibration model among all other sunflower oils, with lowest RMSE and highest  $R$ . That could be due to the higher content of free fatty acid in organic oil since this oil showed the lowest intensity of the band at  $1750\text{ cm}^{-1}$ , which is reported to decrease with the increase of FFA in edible oils.<sup>[43,44]</sup> This high content of FFA in organic oil can be attributed to the absence of refining, whereas conventional oils (the two remaining ones in the table) had lower FFA content.<sup>[45]</sup>

In order to further assess the capability and also the limitation of the PLS model, we have extended the calibration range by

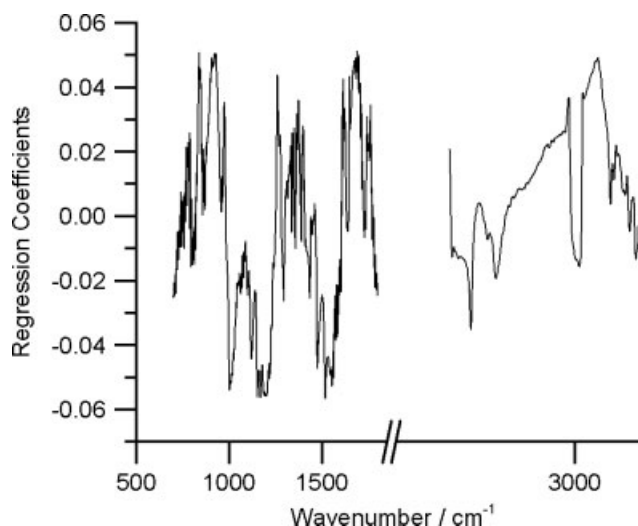


Figure 7. Regression coefficients of Raman shift.

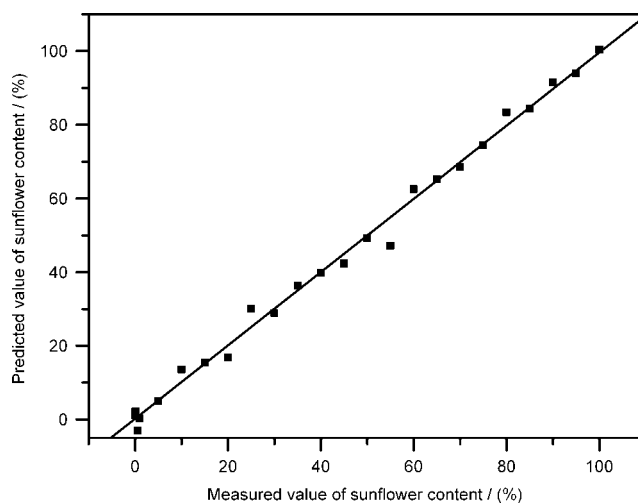


Figure 8. Calibration curve for sunflower oil-adulterated extra-virgin olive oil of PLS model.

minimizing the sunflower oil content in extra virgin olive oil from 3, 2, 1, 0.5 down to 0.05%, corresponding to a detection limit of 500 ppm. This value would not be of commercial interest in connection with adulteration of olive oil. However, in some cases it might be important to also detect traces of other oils, like hazelnut oil, which could cause allergic reactions. Although the detection of the adulteration could still be noticed at even lower concentrations (qualitative analysis), reliable and precise models were obtained only down to 0.05% sunflower oil as the minimum concentration with a low error ( $\text{RMSECV} = 0.036$ ) and high correlation coefficients ( $R^2 = 0.971$ ).

## Conclusions

In this work, we have demonstrated that dispersive Raman spectroscopy with excitation in the visible spectral region (VIS Raman) in combination with appropriate chemometrical methods can be used as a fast and nondestructive method for the identification of plant oils. Particularly, the adulteration of olive

**Table 2.** Calibration and validation statistics for sunflower ranged between 5 and 100% detection in olive oil

| Type of sunflower oil | RMSEC<br>(RMSE calibration) | RMSECV<br>(RMSE cross validation) | $R^2$<br>(calibration) | $R^2$<br>(cross validation) |
|-----------------------|-----------------------------|-----------------------------------|------------------------|-----------------------------|
| Sunflower 1           | 2.56                        | 3.59                              | 0.992                  | 0.981                       |
| Sunflower 2           | 0.92                        | 1.33                              | 0.993                  | 0.988                       |
| Sunflower 3           | 2.32                        | 3.05                              | 0.987                  | 0.971                       |

oil with similar oils, such as sunflower oil here, can easily be detected for a low concentration of adulterant (e.g. 1%) with very short measuring times of few seconds. For a reliable quantitative analysis, a lower detection limit of approximately 500 ppm (0.05%) was determined, which demonstrates the usability of VIS Raman in trace detection (the qualitative detection limit was even considerably lower). The amount of sunflower oil in adulterated extra-virgin olive oil could be quantitatively determined by applying the PLS regression method to the Raman spectra. Owing to the simple setup (no Fourier transform technique required) and the intense signals resulting from the visible laser excitation, VIS Raman spectroscopy is a powerful tool for the online detection important for quality control applications.

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