The use of Raman spectroscopy and methods of quantum chemistry for assessing the relative concentration of triglycerides of oleic and linoleic acids in a mixture of olive oil and sunflower seed oil

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Abstract. The Raman spectra of five samples of sunflower seed oil and five samples of cold-pressed olive oil of various brands are recorded in the range of 500–2000 cm\(^{-1}\). Within the framework of the B3LYP/6-31G(d)/6-31+G(d,p)/6-311G(d) methods, the structural models of eight fatty acids (oleic, linoleic, palmitic, stearic, \(\alpha\)-linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic) are constructed, and also within the framework of the B3LYP/6-31G(d) method, the structural models of triglycerides of the first four of the above acids are obtained. The vibrational wavenumbers and intensities in the IR and Raman spectra are calculated. The Raman spectra of olive oil and sunflower seed oil were simulated by using the supermolecular approach. We investigated the dependence of the relative intensity of the vibrational bands \(\nu_{\text{exp}}=1660\) and 1445 cm\(^{-1}\) on the concentration of triglycerides in oils of oleic and linoleic acids and the dependence of the intensity of these bands on the degree of saturation of fatty acids.

1. Introduction

The great interest in determining the fatty acid composition of vegetable oils is associated, on the one hand, with the crucial role of fats in a correct diet and, on the other hand, the detection of changes in the typical fatty acid composition of vegetable oils makes it possible to distinguish the natural product from its counterfeit. This is especially true for olive oil, which has excellent organoleptic properties and beneficial effects on the human body and is the most often forged. A large number of counterfeits is because the world production of olive oil is relatively small, and its price is rather high compared to many other vegetable oils. In connection with this, quite a lot of works are devoted to the application of Raman spectroscopy for studying the fatty acid composition of various oils of plant and animal origin [1–9]. In particular, Raman spectroscopy and subsequent theoretical analysis by the methods of partial least squares and principal components were used to determine the falsification of olive oil when it was diluted with sunflower oil and hazelnut oil [1,2]. Baeten and Aparicio [3] showed that the Raman spectrometer could be calibrated for determining the percentage of linoleic acid triglyceride in olive oil in revealing the adulteration of olive oil by dilution with vegetable oils. Zhang et al. [4] proved that there is a correlation between the intensities of the experimental Raman bands with
wavenumbers 1265 and 1655 cm$^{-1}$ and the percentage of triglycerides of oleic and linoleic acids in oils, which can be used to determine the adulteration of olive oil.

According to the chromatographic analysis of various oils [10], it is known that the fatty acid base of olive oil and sunflower seed oil is oleic (omega-9) and linoleic (omega-6) acids, of which the latter is essential, that is, not synthesized by the body from other acids but enters it only with food. That's why when planning a proper diet, it is very important to balance both between essential polyunsaturated acids omega-3 and omega-6 and between polyunsaturated and monounsaturated, of which oleic acid is the most common.

In the present work, we used Raman spectroscopy in conjunction with supermolecular simulation [11] of the spectral properties of some olive oils and sunflower seed oils to estimate the relative concentration of triglycerides of oleic and linoleic acids in their mixture.

2. Experimental and calculations
Experimental studies were carried out using a QEPRO-RAMAN Raman spectrometer (Ocean Optics, United States) in the range of 500–2000 cm$^{-1}$. The wavelength of the excitation laser was 785 nm. The resolution of the instrument was 8 cm$^{-1}$. Five samples of sunflower seed oils and five samples of olive oil were selected as objects of research, for which the fatty acid composition was determined experimentally [12]. To obtain the experimental dependence and to test of the applicability of the empirical dependences of the intensity of Raman bands on the relative concentration of triglycerides of oleic and linoleic fatty acids in the mixture, obtained in the present study, we also recorded the Raman spectra of four mixtures (20, 40, 60, and 80%) of olive oil and sunflower seed oil, respectively.

The calculations for triglycerides of oleic, linoleic, stearic, and palmitic acids were performed by the B3LYP/6-31G(d) method, and the calculations for individual fatty acids were carried out by the B3LYP/6-31G(d)/6-31G(d,p)/6-31+G(d,p)/6-311G(d,p)/6-311G(d)/6-31+G(d,p) method using the program [13]. And also the structures and Raman spectra of four fatty acids belonging to the omega-3 series, namely, α-linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids, were also calculated.

Molecular models of olive oil and sunflower seed oil were built on the basis of four models of fatty acids (oleic, linoleic, palmitic, and stearic). The concentration of these acids in the models was set in such a way that it corresponded to the average value for the five oils tested. Then the values were normalized so that their sum was 100% because the concentration of other types of fatty acids in oils was not taken into account. Thus, in the olive oil model, the concentration of oleic acid was 73.9%, linoleic acid 9.4%, palmitic acid 13.5%, and stearic acid 3.2%, while in the sunflower seed oil model, these values were 64.1, 25.9, 6.6, and 3.4%, respectively. The process of adulteration of olive oil with sunflower seed oil was simulated, by changing the ratio of oleic and linoleic acids, taking into account the overall change in the saturated acid concentration in the transition from the olive oil model to the sunflower seed oil model. The theoretical Raman spectra of the models of the investigated oils were constructed using the author's program in units of the Raman cross section [14]. The half-width of all the Lorentz contours was set to 10 cm$^{-1}$. For better agreement with experiment, the calculated values of the vibrational wavenumbers were corrected by linear frequency scaling [15]. The integrated intensity of the experimental bands was determined by counting the number of pixels in the contour bounded from below when the resolution of the spectrogram was 600 dpi.

3. Results and discussion
The experimental Raman spectra of five samples of olive oil and five sunflower seed oil are shown in Figure 1. In the Raman spectrum of the oils under consideration, several intense bands are observed in the region of 500–2000 cm$^{-1}$ (Figure 1). According to the calculation, the first two of them, $v_{exp}=1660$ and $v_{exp}=1445$ cm$^{-1}$, are formed by the vibrations of only unsaturated parts of fatty acid chains (in-phase stretching vibrations of C=C bonds), and the latter is caused only by vibrations of saturated parts (deformation scissor oscillations of the CH2 groups and the terminal CH3 group). The next partially resolved band has two maxima: the first at $v_{exp}=1306$ cm$^{-1}$, according to the calculation, is formed by
deformation torsional vibrations of the CH\textsubscript{2} groups, and the second at \(v_{\text{exp}}=1270\ \text{cm}^{-1}\) is caused predominantly by deformation in-phase vibrations of the C–H bonds of unsaturated fragments. Thus, a change in the length of fatty acid chains or the degree of their unsaturation naturally affects the intensity of these bands, which enables them to be used to estimate the relative concentration of unsaturated acids in vegetable oils. For our research, we select the first two bands as analytical ones because they do not overlap in the Raman spectrum and do not require the separation of the contours to determine the integrated intensities. The remaining Raman bands in this frequency region are formed, according to calculations, by various deformational vibrations of the C–H bonds and CH\textsubscript{2} groups and also by stretching vibrations of the C–C bonds of the saturated sections of the chains. A weak band at \(v_{\text{exp}}=1750\ \text{cm}^{-1}\) is also observed in the spectrum, formed by stretching vibrations of the C=O bonds of carbonyl groups.

![Figure 1](image1)

**Figure 1.** Experimental Raman spectra of (a) sunflower seed oils (samples 1–5) and (b) olive oils (samples 6–10) in the range of 500–2000 cm\textsuperscript{-1}.

The theoretical Raman spectra of oleic and palmitic acids constructed with three times the intensity values and their triglycerides, as well as the experimental Raman spectra of two types of vegetable oils with the spectra of the corresponding theoretical models, are compared in Figure 2.

![Figure 2](image2)

**Figure 2.** (a) Theoretical Raman spectra of (1) oleic acid and (2) palmitic acid constructed with three times the intensity values (dashed curve), and their triglycerides (solid curve). (b) Experimental spectra of the samples of (4) olive oil and (6) sunflower seed oil and (3, 5) spectra of the theoretical models of these oils, calculated by the B3LYP/6-31G(d) method.

It is seen in Figure 2a that glycerol cross linking has a weak effect on the Raman spectra of fatty acids, in contrast to, for example, their IR spectra. In the given spectral region, there are practically no significant frequency shifts and the intensity of the main Raman bands is actually directly proportional to the number of chains. This enables using the structural models of only individual fatty acids for the theoretical simulation of the Raman spectra of vegetable oils, which saves considerable time for theoretical calculations.

In order to determine how the wavenumbers and the relative intensity of the investigated Raman bands depend on the selection of the theoretical level, we calculated the models of the fatty acids...
under consideration using six standard basis sets. The results of these calculations are presented in Table 1.

**Table 1.** Wavenumbers (cm⁻¹) and relative intensities (values in parentheses) of two Raman bands in the spectra of unsaturated fatty acids and the models of olive oil and sunflower seed oil calculated within the framework of the B3LYP method with different basis sets

| Fatty acids and models of oils | Basis set |
|-------------------------------|-----------|
|                               | 6-31G(d)  | 6-31G(d,p) | 6-31+G(d,p) | 6-311G(d) | 6-311G(d,p) | 6-311+G(d,p) |
| Oleic acid                    | 1517/1743 | 1502/1740 | 1493/1722 | 1502/1726 | 1491/1721 | 1489/1714 |
|                               | (3.38)    | (3.26)    | (1.71)    | (2.48)    | (2.32)    | (1.59)    |
| Linoleic acid                 | 1517/1746 | 1502/1744 | 1493/1725 | 1502/1730 | 1491/1725 | 1489/1716 |
|                               | (1.28)    | (1.24)    | (0.68)    | (0.94)    | (0.89)    | (0.64)    |
| Model of olive oil            | 1517/1744 | 1502/1741 | 1493/1722 | 1502/1727 | 1491/1722 | 1489/1714 |
|                               | (3.47)    | (3.35)    | (1.78)    | (2.39)    | (1.65)    | (2.56)    |
| Model of sunflower seed oil   | 1517/1746 | 1502/1744 | 1493/1725 | 1502/1730 | 1491/1725 | 1489/1717 |
|                               | (1.77)    | (1.71)    | (0.94)    | (1.24)    | (0.88)    | (1.31)    |

The relative intensity of the Raman bands under consideration, calculated in the framework of the theoretical B3LYP method depends quite strongly on the type of the basis set (Table 1). The expansion of the basis set and, especially, the addition of diffuse functions to the basis leads to a significant decrease in the relative intensity value for these Raman bands. Comparison of these values with the mean experimental values (4.11 for olive oils and 2.69 for sunflower seed oils) shows that a better agreement is obtained when using the 6-31G(d) basis set. A rather large discrepancy in the relative intensities of Raman bands between the theoretical and experimental values indicates that it is not possible to use only the theoretical data obtained in this paper to construct an analytical dependence that could be used for practical purposes. It should be noted that, in spite of significant discrepancies in the values of relative intensities, their functional dependence on the concentration of oleic and linoleic acids in oils is described in good agreement with the experiment. The functional dependence has a nonlinear character since the intensity of the Raman bands under consideration depends in different ways on the number of vibrations that form them.

The theoretical dependences of the intensity of the Raman bands under consideration on the number of deformation vibrations of the CH2 and CH3 groups and the forming stretching vibrations of the C=C bonds are presented in Figure 3.

**Figure 3.** Theoretical dependence of the intensity of the Raman bands at (a) \( \nu_{\text{exp}} = 1445 \text{ cm}^{-1} \) on the number of deformation vibrations of CH2 and CH3 groups and (b) \( \nu_{\text{exp}} = 1660 \text{ cm}^{-1} \) on the number of stretching vibrations of the C=C bonds in the spectra of (1) palmitic, (2) stearic, (3) oleic, (4) linoleic, (5) \( \alpha \)-linolenic, (6) arachidonic, (7) eicosapentaenoic, and (8) docosahexaenoic acids calculated by the B3LYP/6-31G(d) method.
With an increase in the degree of unsaturation of the fatty acid chain, the intensity of the Raman band formed by the stretching C=C vibrations rapidly increases linearly (Figure 3). When the length of the fatty acid chains is the same, the number of CH2 groups decreases proportionally as the degree of saturation increases, which leads to a decrease in the intensity of the second Raman band, but the intensity decrease is much slower than its growth in the former case. In the case of theoretical simulation of the dependence of the relative intensity of Raman bands on the concentration of oleic and linoleic acids in mixtures of the two types of oil, the fact that the average concentration of saturated acids in them is different is also taken into account. For example, in olive oil, this value is 16.3%, and it is 9.8% in sunflower seed oil. This allowance improves the agreement with the experiment, because the deformation vibrations of both unsaturated and saturated acids contribute to the intensity of the Raman band at $\nu_{\text{exp}}=1445$ cm$^{-1}$. Figure 4a shows a relationship that takes into account this change. It has a logarithmic character and is described by the equation $y = 0.09\ln(x) + 0.83$, where $x$ is the ratio of oleic and linoleic acids and $y$ is the difference between the experimental and theoretical values of the relative intensity of the Raman bands of the mixtures of the oils under consideration.

![Figure 4a](image)

**Figure 4.** (a) Difference between the experimental values of the relative intensity of Raman bands and the Raman spectra of ten oil samples obtained by simulation: (1–5) sunflower seed oils and (6–10) olive oils; the difference between the experimental and theoretical values in percent is given in parentheses. (squares) Average values for the samples of sunflower seed oil and olive oil; (dotted line) approximation of the difference between the experimental and theoretical values and the ratio of oleic and linoleic acids in the samples. (b) Dependences of the relative intensity of Raman bands on the concentration ratios of oleic and linoleic acids, constructed with different parametrization: (1) model constructed on the basis of only theoretical calculations, (2) model constructed on the basis of theoretical calculations taking into account the empirical shear coefficient of 0.78, and (3) model constructed on the basis of theoretical calculations with allowance for the functional dependence presented in Figure 4a. (4) Experimental values of the relative intensities of Raman bands for the samples of olive oil (a group of points on the right) and sunflower seed oil (on the left) and (plus) their average values. (5) Experimental results for the mixture of olive oil and sunflower seed oil; the ratio of the concentrations of these oils in the mixture is given below the data point.

The theoretical dependences of the relative intensity of the two Raman bands on the concentration ratios of oleic and linoleic acids (Figure 4b) are constructed with different parametrization. In the first case, the dependence is constructed only from theoretical calculations using the B3LYP/6-31G(d) method. In the second case, the shear coefficient (0.78) was used as the empirical parameter, which was calculated as the average value of the difference between the experimental values of the relative intensity of the Raman bands and the Raman spectra of ten oil samples, obtained by simulation (Figure 4a). This dependence is described by the equation $y = 0.59\ln(x) + 3.01$ and is most suitable for
determining the percentage concentration of oleic and linoleic acids in a mixture of olive oil and sunflower seed oil close to 1:1.

In the third case, the functional dependence of the difference between the experimental and theoretical values of the relative intensity of the Raman bands on the concentration ratios of oleic and linoleic acid in the test oil samples, also shown in Figure 4a, was selected as the correction parameter. This dependence is described by the equation \( y = 0.50\ln(x) + 3.06 \). It is universal and can be used in estimating both the percentage concentration of oleic and linoleic acids in pure oils and in their mixtures and, accordingly, for determining the adulteration of cold-pressed olive oil with sunflower seed oil. Using this relationship, the average error in determining the proportion of olive oil in a mixture of two oils was 5%.

4. Conclusions
The Raman spectra of cold-pressed olive oil and sunflower seed oil were simulated by using the supermolecular approach. A functional relationship between the relative intensity of the two experimentally observed Raman bands at \( \nu_{\text{exp}} = 1660 \) and 1445 cm\(^{-1} \) on the concentration of triglycerides of oleic and linoleic acids in oils is found, which is well approximated by the logarithmic dependence. Experimental and theoretical simulation of the dilution of cold-pressed olive oil with sunflower seed oil is carried out, resulting in the production of functional relationships that can be used in practice both for estimating the relative concentration of oleic and linoleic acids in olive oils and sunflower seed oils and for assessing the fraction of sunflower seed oil in the mixture when cold-pressed olive oil is adulterated.

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