

RAMAN SPECTROSCOPIC DETECTION OF OLIVE OIL ADULTERATION

APPLICATION NOTE RAMAN-001 (A4)

Author: A.J.R. Bauer, Ph.D.

Abstract

Olive oil is among the most commonly adulterated food products. Raman spectroscopy has the potential to be utilized in measurements of olive oil to establish purity from adulteration with other, less expensive, oils. This application note reviews some of the most compelling data present in recent scientific literature to support this claim.

Background and Motivation

Extra-virgin olive oil is in high demand, and as a result commands high prices. Due to this fact, adulterated olive oil is reported to be the biggest source of agricultural fraud in Europe. It has been estimated that while less than 10% of the world olive oil production meets the criteria for being labeled as extra-virgin, about 50% is thusly labeled.¹ This is a problem, both from the perspective of label fraud, but also in terms of the health impacts of the product. Olive oil has recently been found to have significant health benefits, benefits that will not be received as expected if the oil has been adulterated or mixed with other oils. Further, some fraudulent oil has been discovered to be poisonous.²

The majority of fatty acids found in olive oil are monounsaturated. According to nutritionists, this type of fatty acids may help lower your risk of heart disease by improving related risk factors; monounsaturated fatty acids have been found to lower total cholesterol and low-density lipoprotein cholesterol levels. They may also help normalize blood clotting. Recent research shows that monounsaturated fatty acids may also improve insulin levels and blood sugar regulation.³

Quality control of edible oils is currently performed with instruments inside the analytical laboratory. Among the most commonly used techniques to perform QC and adulteration detection of oils are gas chromatography (GC), gas chromatography mass spectrometry (GCMS) and high-performance liquid chromatography (HPLC)⁴⁻⁷. In order for oil analysis to be performed more readily, a small and robust device is needed that can be carried into the field.

The fatty acids in olive oil are present largely as triacylglycerols (three fatty acid moieties with a glycerol backbone). Olive oil contains more oleic acid (monounsaturated) and less linoleic and linolenic acids (both polyunsaturated) than other vegetable and seed oils. The fatty acid profile of olive oil has the following unsaturated components: oleic acid (65-85%), linoleic acid (4-15%), palmitic acid (7-16%) and linolenic acid (<1.5%).⁸ This high concentration of oleic acid represents the highest percentage of monounsaturated fats of any commonly used oil.

The fatty acid composition profiles are naturally different for other oils, providing the possibility of differentiating olive oil from them with vibrational spectroscopy. For example, the fatty acid profile of soy bean oil has the following unsaturated components: oleic acid (19-30%), linoleic acid (48-58%), palmitic acid (7-12%) and linolenic acid (5-9%).⁸ Since these fatty acids have different structures, they will also have different intramolecular vibrations, suggesting that Raman spectroscopy could be used to determine oil quality and reveal the presence of adulterants.

Oleic acid, α -linolenic acid and linoleic acid are all 18-carbon carboxylic acids. Oleic is monounsaturated, having only one *cis* double bond. Linoleic acid has two and α -linolenic acid has three *cis* double bonds and are; therefore, polyunsaturated. Raman spectroscopy was first applied to measurements regarding these products in 1972, when used to determine the *cis/trans* isomer content of edible oils using the features at 1656 cm^{-1} (*cis*) and 1670 cm^{-1} (*trans*).⁹

The application of Raman spectroscopy to adulteration detection has been investigated in a variety of different studies which follow the changes in the vibrational spectrum as olive oil is doped with other oils, hazelnut and soy bean oil in particular.

The study of olive oil adulterated with hazelnut oil was performed by López-Díez and colleagues at the University of Manchester Institute of Science and Technology.¹⁰ They acquired Raman spectra of three sample sets, one set of Italian extra virgin olive oils (known to be authentic) from a variety of origins, one set of hazelnut oils having a variety of origins and a set of samples generated by mixing one of the olive oils with hazelnut oil and sunflower oil. Hazelnut oil was chosen because of its chemical and physical similarity to the olive oil. The spectra were acquired with 20 mW of excitation light at 780 nm, over a spectral range of 1000 to 3000 cm^{-1} with a resolution of approximately 6 cm^{-1} . Spectra were collected in quadruplicates, and the mixtures of olive oil with sunflower and hazelnut oil were prepared and measured over the range 0 to 100% in increments of 5%. Raw data were exported to Grams and then to MatLab for data analysis.

The Raman spectra are shown in figure 1. This figure shows 5 brands of extra virgin olive oil with sunflower and hazelnut oil spectra. The chief features of interest in these spectra are 1075 cm^{-1} (C-C stretch, group- CH_2)_n, 1263 cm^{-1} (=C-H bending, group *cis*-RHC=CHR), 1298 cm^{-1} (bending, group- CH_2), 1440 cm^{-1} (C-H scissoring group - CH_2), 1652 cm^{-1} (C=C stretching, group *cis*-RHC=CHR), 1741 cm^{-1} (C=O stretching, group RC=OOR) 2851 cm^{-1} (C-H symmetrical stretch, group CH_2).

The data analysis involved in this study started with a de-noising step and normalization to the peak at 1427 cm^{-1} , assigned to the scissoring-bending mode of $-\text{CH}_2$. The data were readily discriminated by composition and region of origin by applying principal component analysis (PCA), resulting in natural clusters that separated oils produced in Sardinia, for example, from those produced on the Italian peninsula. Hazelnut oils were likewise readily segregated by production region. PCA also clearly separates the olive and hazelnut oils from one another. A PCA plot for the extra virgin olive oil and hazelnut mixtures places them in order of composition.

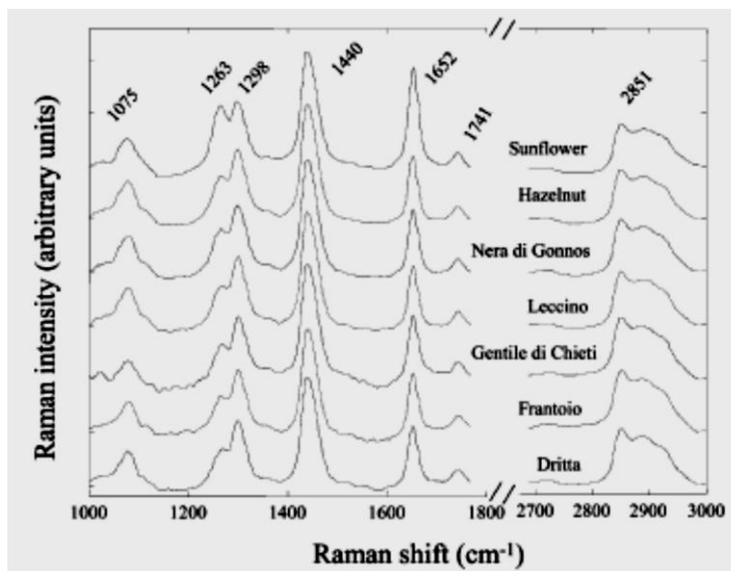


Figure 1. Raman spectra of extra virgin olive oil, hazelnut and sunflower oils (from reference 10).

A partial least squares (PLS) approach was used effectively to create a model that produces an excellent match between expected and predicted concentrations. Although pleased with the output, the authors criticize PLS because this method does not explicitly yield information in terms of the input variables. The authors therefore made use of genetic programming, which are evolutionary computational techniques used to optimize a desired mathematical expression to produce explanatory rules. Gmax-Bio (Aber Genomic Computing, Aberystwyth, U.K.) was utilized in this investigation. Results from both PLS and GP indicated that instead of one or two features leveraging the models, that the entire spectrum was used to create the model used to calculate the hazelnut contamination from the raw data.

This study demonstrates that Raman spectroscopy can be used to identify the presence of hazelnut oil in samples of extra virgin olive oil. Additionally, given only the two components, multivariate analysis can be used to determine the concentration of the adulterant in the olive oil.

Another study reporting successful use of Raman spectroscopy to identify adulterated olive oil was performed by the Chinese Academy of Inspection and Quarantine who did a quantitative study on soybean, corn and sunflower seed oil adulteration of olive oil.¹¹ This study documents Raman spectroscopic measurements and data analysis on large sets of extra virgin olive oil mixed with all three adulterants from 0 to 98%. The spectrometer had an excitation laser with a wavelength of 785 nm, delivering 200 mW to the samples. Raman data were collected from 200 to 2000 cm^{-1} , at a resolution of 8 cm^{-1} . Scanning time was about 30 s. These authors focused on the data acquired from 1000-1800 cm^{-1} , the location of most of the signal variability between these oils. The features were assigned in the same way as those in the previous example. Band ratios between standards and samples were calculated after the raw data were preprocessed with intensity calibration, background correction and normalization to the 1441 cm^{-1} band. The bands at 1265 and 1654 cm^{-1} were the primary focus, as they correspond to the *cis*-($=\text{C-H}$) vibration and the *cis*-($\text{C}=\text{C}$) vibration, and are correlated to high unsaturated fatty acid content in the oils. A linear relationship between the intensity at 1265 cm^{-1} and the mass percentage of adulterant oil was found to be present, and the calibration curve applied to calibration verification samples. The authors report relative errors between 7 to 28% with olive oil samples spiked with 20% of the other oils, and

17 to 54% at the 5% adulterant level. The results were best when the standards' origin and brand matched those of the sample.

These authors also performed quantification by the support vector machine method (SVM). SVM is well described in the paper. In brief, it maps data into higher dimensional feature space and uses either linear or nonlinear processes to transform the data. Subsequent processing minimizes the errors between true data and modeling data, which helps to minimize the prediction errors. Using Raman feature intensities at 1082, 1265, 1300, 1441, 1654 and 1742 cm^{-1} , ChemSVM was used to build a calibration model that returned values better matched to the real spiked concentrations, most of which were reported to possess relative errors of less than 10%. This paper demonstrates again that this important measurement can be performed with Raman spectroscopy, and that multivariate analysis is likely necessary to achieve good measurement goals.

Studies toward the discovery of counterfeit or adulterated extra virgin olive oil have also been performed with a TSI Raman spectrometer (EZRaman-A), using a 785 nm excitation laser with 300 mW of input power. Raman shift signals were collected from 250 to 2350 cm^{-1} , as shown in figure 2. Using these conditions, an acquisition time of 8 s and proprietary software to perform baseline subtraction and normalization to the feature at 1438 cm^{-1} , the S/N in this data is sufficient to resolve a new peak at 1525 cm^{-1} , not identified in previous publications. This band is recognized as one of three bands of carotenoid (1525 cm^{-1} is a C=C stretch). Materny and coworkers have reported that this band can be used to identify the authenticity of extra virgin olive oil, because these natural color centers are missing in nearly all other types of oils.¹²

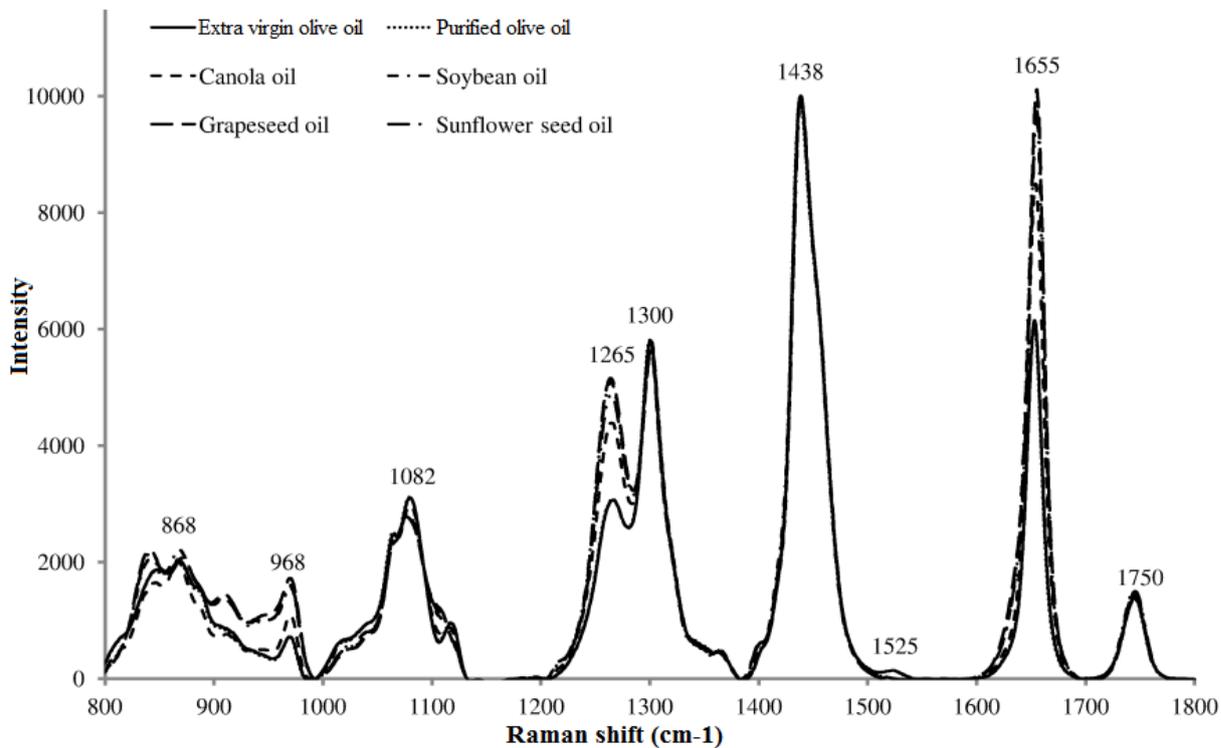


Figure 2. Edible oils analyzed with TSI EZRaman-A. Not the presence of an extra peak at 1525 cm⁻¹.

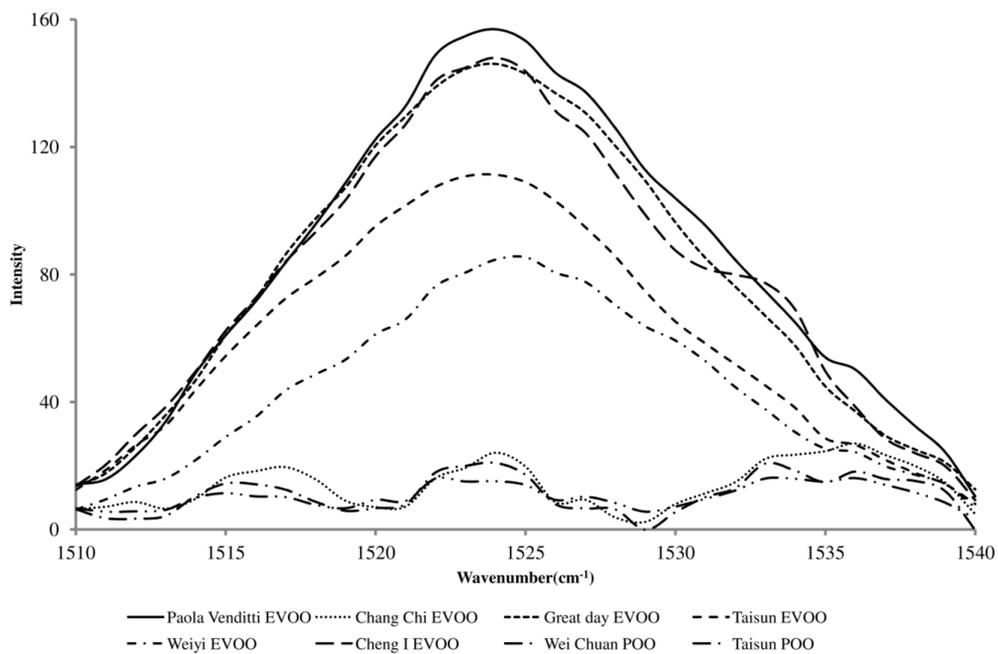


Figure 3. Feature at 1525 cm⁻¹, as a function of olive oil grade. This graphic needs some help.

Summary

Preliminary work using Raman spectroscopy indicates that this approach may be useful the discovery of fraudulent olive oil labeling and production and packaging processes. If proven to live up to the potential present in this work, Raman-based monitoring of olive oil quality could permit consumers to be more confident of product quality, and additionally to usher in a new era of tighter regulation in this important and world-wide market.

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USA	Tel: +1 800 874 2811	India	Tel: +91 80 67877200
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