Authors:

Ian Robertson

PerkinElmer, Inc.
Shelton, CT

Advantages of Adulterant Screen for Detection of Olive Oil Adulteration by Attenuated Total Reflectance (ATR) FT-IR

Introduction

Olive oil is an increasingly popular food product worldwide, with consumption in the U.S. alone having increased by about 50% in the last 10 years. Over three million tons annually of olive oil are produced worldwide, with approximately 75% of this being produced in Spain, Italy, and Greece. The U.S. now imports over 300,000 tons of olive oil annually.

Olive oil is considered to be healthy edible oil and is linked to the low incidence of heart disease associated with a Mediterranean diet. It is low in Saturated Fatty Acid (SFA) and Polyunsaturated fats (PUFA) but high in the healthier Monounsaturated fats (MUFA), known to lower cholesterol.

Extra Virgin Olive Oil (EVOO) is a premium product that can command a higher price than "standard" olive oils. This makes it highly susceptible to fraudulent activity. A report by the E.U. Committee on the Environment, Public Health, and Food Safety says olive oil is among the products most prone to food fraud. There were 267 oil adulteration incidents reported to the U.S. Pharmaceutical Food Fraud Database, with the vast majority occurring over the past three years.

Adulteration of EVOO with lower quality olive oils, or other lower cost edible oils, is frequently reported in the media. The most common adulterants include: hazelnut oil, sunflower oil, soybean oil, corn oil, rapeseed oil, and olive pomace oil. Fraudulent activities, such as dilution or even substitution with other lower cost oils containing additional chemicals, that enable the oil to appear to be of higher quality oil and pass routine screening tests are on the rise.

This application note describes a fast, simple, low-cost solution to screen olive oils for adulteration.

Materials and Methods

Mid-infrared spectroscopy is a well-established technique for the analysis of edible oil samples. The PerkinElmer Spectrum Two™ FT-IR, a high-performance compact FT-IR instrument utilizing the modern ATR sampling technique, offers fast and easy measurements of samples within the food industry. Diamond™ ATR accessories, such as the PerkinElmer Universal ATR (UATR), are extremely robust and allow the instrument to be used in the harshest of laboratories or even in remote environments. The Diamond ATR crystal requires only a very small volume of the sample to be tested and can easily be cleaned between samples, in situ, using laboratory tissue and a small amount of a suitable solvent, such as hexane for edible oils.

In this study the PerkinElmer Spectrum Two, equipped with a UATR sampling accessory, has been used to analyze a series of pure and adulterated olive oils and common adulterant spectra. A typical olive oil spectrum is shown in Figure 2. Spectra were recorded at 4 cm^{-1} resolution with a scan time of one minute per sample.

The prominent features in the spectrum are the bands in the region of 2930 cm^{-1} due to the –CH- stretch of the hydrocarbon chains and in the region of 1740 cm^{-1} due to the carbonyl groups in the triglyceride.

Discriminating Olive Oil from Other Edible Oil Types

The infrared spectra of different edible oils will be similar, only varying by the constituent chains on the triglyceride backbone, since their molecules contain the same chemical groups. However, there are small, observable differences between the different oil types. Figure 3 shows the ATR spectra of three different oil types: olive oil, sunflower oil, and rapeseed (canola) oil.

These spectral differences are significant enough to be able to develop a classification method for these different oils. There are a variety of ways to classify materials based on their infrared spectra. For this type of problem Soft Independent Modeling of Class Analogy (SIMCA), a Principal Components Analysis (PCA) based method, is a good approach to take. Building a SIMCA method requires the measurement of a variety of samples for each type of material you wish to classify. The calibration set of samples should cover all sources of variation normally encountered for that particular material, such as different sources, different batches, or different manufacturing processes. The method will build individual models to completely characterize each of the materials. Each material, in this case the individual oil types, generates its own cluster in this model that should be separated from the other clusters calculated for the other materials being classified. A SIMCA model has been generated for the three types of edible oils in this study. Figure 4 shows the SIMCA model with each oil having its individual cluster, clearly separated from those of the other materials.

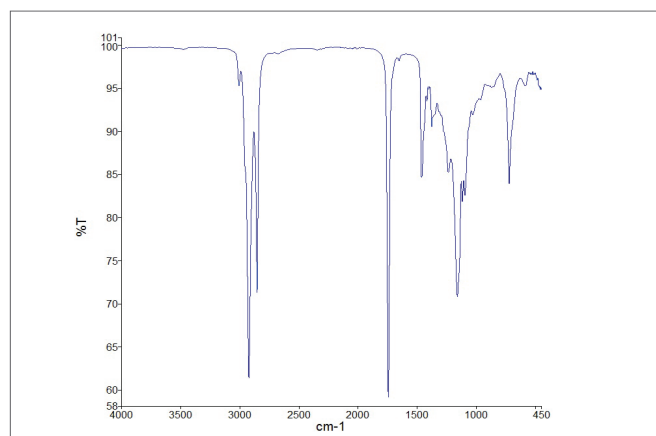Figure 1. The PerkinElmer Spectrum Two and UATR.
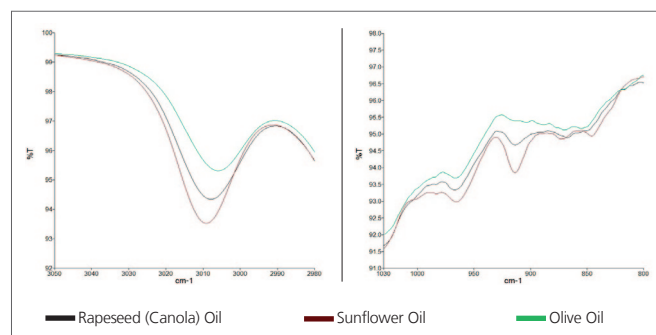

Figure 2. Diamond ATR spectrum of olive oil.

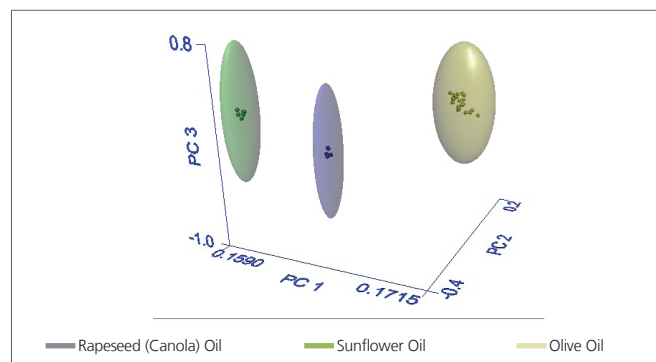Figure 3. Spectral differences between olive oil, rapeseed oil and sunflower oil.

Figure 4. SIMCA model for three edible oil types. Olive oil, rapeseed oil, and sunflower oil.

Classifying a material consists of measuring the IR spectrum and using the SIMCA model to predict to which cluster the spectrum belongs. If the spectrum does not fall into one of the three classes of materials then it is likely to be a different material or contaminated/adulterated oil. Further data investigation would be required to determine the reason that the sample has failed the test.

Quantifying Levels of Known Adulterants in Olive Oil

If the identity of the adulterant is known then it is possible to quantify the amount of adulterant present. This involves the preparation and measurement of the IR spectra of standard mixtures of the olive oil with the adulterant oil. The IR spectra for a series of standards are shown in Figure 5.
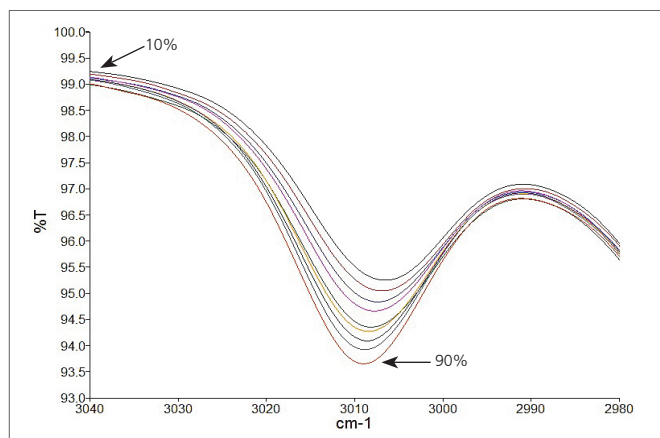

Figure 5. Standards from 10% - 90% Sunflower Oil.

Partial Least Squares (PLS1) Calibrations have been generated for mixtures of olive/sunflower oils and olive/rapeseed oils ranging from 0 to 100% olive oil. The calibrations are shown in Figure 6.
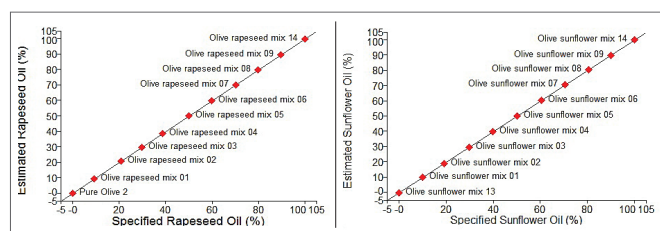

Figure 6. PLS1 Calibrations for Olive/Rapeseed and Olive/Sunflower oils.

An independent validation set of three samples were used to test the calibration model. The validation plot is shown in Figure 7.
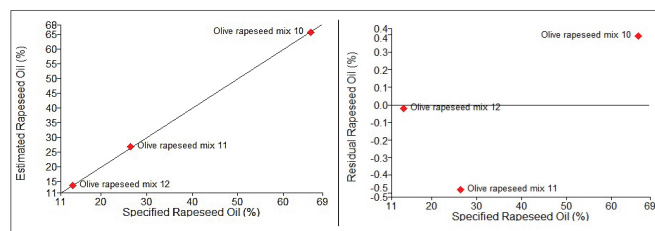

Figure 7. Independent validation samples for olive/rapeseed mixtures.

Adulterant Screen™ Algorithm for Detecting "Known" and "New" Adulterants in Olive Oil

The two statistical approaches taken so far would allow for:
a.) checking that the material is the correct material (SIMCA) and b.) quantifying the amount of a single, known adulterant (PLS).

An alternative approach is available using an Adulterant Screen Algorithm. The approach is simple:

1. Generate a library of unadulterated material samples spectra exactly as for SIMCA. This library should span as much as possible the natural variation of the material, due to differences between batches, suppliers or processing parameters, etc.

2. Generate spectra of adulterants of concern. These spectra should be of the pure adulterant material, not mixtures. (As new adulterant materials emerge these can easily be added to the adulterant library in the future.)

These two sets of spectra are registered in the software, and the method is ready to use.

In this study, a series of 24 olive oil spectra were measured from commercially purchased oils. These 24 spectra were used to generate a library of the unadulterated material. The objective of this study was to specifically look for adulteration with either sunflower or rapeseed oils. Single spectra of the two adulterants were measured and stored with the method. The Adulterant Screen method was tested using samples adulterated with known concentrations of the other oil types and also with pure olive oil. The results are shown in Table 1.

Table 1. Adulterant Screen results for a series of method validation standards.

Sample Name	Adulterant	Level	Unidentified Components	Adulterant Screen Pass/Fail
Sunflower 18.66% Std	Sunflower Oil	0.19208	Probable	Fail
Sunflower 68.80% Std	Sunflower Oil	0.69011	Probable	Fail
Sunflower 38.10% Std	Sunflower Oil	0.38183	Probable	Fail
Sunflower 100.0% Std	Sunflower Oil	1.00328	Probable	Fail
Rapeseed 66.02% Std	Rapeseed Oil	0.64944	Probable	Fail
Rapeseed 26.41% Std	Rapeseed Oil	0.26367	Probable	Fail
Rapeseed 13.79% Std	Rapeseed Oil	0.14083	Probable	Fail
Rapeseed 100.0% Std	Rapeseed Oil	0.99191	Probable	Fail
Pure Olive Oil	No Adulterants	-	Unlikely	Pass

In all cases, except the pure olive oil, the adulterated samples generated a "Fail" result indicating the presence of an adulterant. Not only does the Adulterant Screen algorithm correctly identify the adulterant, but it also gives an estimated level of that contaminant without the requirement for running quantitative calibration standards. The level of the contaminant is reported as the proportion of the total spectrum contribution arising from that component. The results table demonstrates the ability of this algorithm to classify like SIMCA and additionally provide approximate estimates of concentration of the adulterants without the need to generate extensive quantitative models.

When a sample spectrum is scanned, the algorithm first compares it to a PCA model generated from the reference materials. This model is then augmented with each of the adulterant spectra in turn. If including a given adulterant in the model greatly increases the fit of the sample spectrum, it is likely that the adulterant is actually present in the sample.

Figure 8 shows the residuals observed from the analysis of 13.79% rapeseed validation standard.

Note: the spectral region from 2450-1850 cm^{-1} (the region where the diamond absorptions due to the Diamond ATR are intense) was excluded from the method.
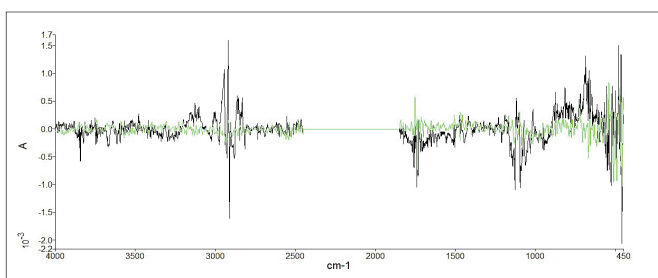

Figure 8. Spectral residuals before (black) and after (green) fitting adulterants.

In this case the residuals are significantly decreased by fitting the spectrum of the pure rapeseed oil indicating the presence of that adulterant in the sample.

Summary

ATR-FT-IR on the Spectrum Two allows for a fast, easy, and low-cost method for screening olive oil samples for adulterants. The information required from the analysis will determine which will be the most appropriate data analysis method to use. Data has been demonstrated using three different approaches – SIMCA , PLS, and Adulterant Screen. These are summarized below:

SIMCA – Is the product what it says it is and does it fall within the expected variation within that class of material? If not, further data analysis will be required.

PLS - For known adulterants it is possible to generate complete quantitative calibrations by preparing suitable standard mixtures. This will give accurate quantitative results.

Adulterant Screen algorithm – Is the product what it says it is and has it been adulterated? If adulteration is likely then try to identify the adulterant from known adulterants and give a semi-quantitative measure of how much of the adulterant is present.

The Adulterant Screen algorithm offers significant benefits over the other two approaches:

Faster method development
• The Adulterant Screen algorithm simply requires the collection of the spectra of the unadulterated material and the known adulterants.

Simple upgrade of methods
• When new potential adulterants are identified they can simply be added to the library of adulterant spectra.

Greater sensitivity than SIMCA
• Achieved by utilizing a library of spectra of potential adulterants.

Whichever statistical approach is utilized it can be deployed using a Spectrum Touch™ method, employing a simple user interface for the routine operator. Figure 9 is an example of the results screen for an adulterated sample.
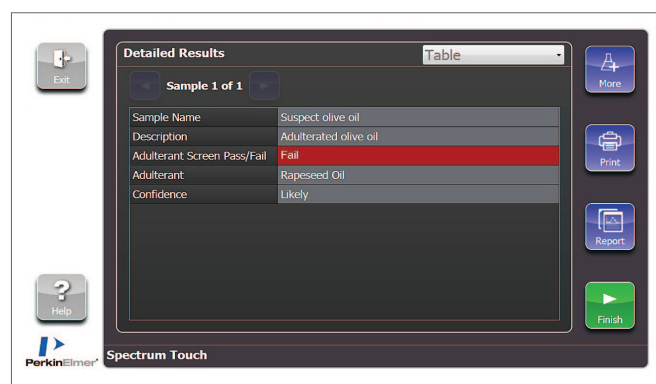

Figure 9. Spectrum Touch software showing result from Adulterant Screen.