Edible oils and fats authentication by Fourier transform Raman spectrometry

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The European project FAIR-CT96-5053 concerned the application of the FT-Raman and infrared spectroscopy in food chemistry and quality control. Our research mainly concerned the study of the potential of Raman spectroscopy and the comparison with the results achieved in infrared spectroscopy. The discrimination of virgin olive oil from other edible oils, and the detection and quantification of virgin olive oil adulteration have been experimented with this new technique of fast and non-destructive analysis.

Keywords. FT-Raman spectroscopy, virgin olive oil, stepwise linear discriminant analysis, adulteration, authentication.

1. INTRODUCTION

Authentication is of paramount importance in the food industry where incoming batches of raw materials and finished products must be tested for compliance with regulatory and health specifications. The recent dioxin crisis highlighted the importance of checking raw materials for their compliance with these specifications. In the past, other incidents such as the adulteration of olive oils with non-edible oils have shown the potentially serious consequences of such practices. With the emergence of international markets, however, the authentication of food products is receiving increased attention. One of the consequences of trade agreements, such as EU trade agreements and the GATT ratification accord, is the creation of a complex network of excise duties and agricultural subsidies which, in turn, require the establishment of official mechanisms to ensure that a food product is what it is claimed to be (Lees, 1999). There has also been an increase in the number of the products carrying Denomination of Origin (DO) information and/or labels. This trend is a result of efforts by regional authorities and producers to protect and support local production. An additional important aspect in the authentication of fat products is the social and cultural impact of adulteration.

Several techniques for assessing the authenticity of food products have been proposed. The authentication methods applied to oils and fats can be classified as chemical (= separative) or physical (= non-separative) (Figure 1). Separative techniques, such as gas chromatography, focus on the existence or absence of certain chemical compounds in the adulterated sample. Physical techniques, such as infrared or Raman spectrometry, are based on a combination of measurements (e.g., light absorbance at different frequencies or the whole spectrum). The measurements are carried out directly on the samples or after dilution in a suitable solvent. The most widely used and accepted physical technique for oil and fat authentication is ultraviolet (UV) spectrometry. Other promising physical techniques which have been investigated for oil and fat authen-
Oils and fats authentication by Raman spectrometry

Oils and fats authentication techniques include mass spectrometry, pyrolysis mass spectrometry, GC-electron ionisation mass spectrometry, nuclear magnetic resonance and infrared spectrometry. Several papers have discussed the potential of near- and mid-infrared spectrometry in the authentication of food products; some of these papers are listed in Table 1.

Another branch of vibrational spectrometry is Raman spectrometry which, like infrared spectrometry, provides information about the vibrations of the molecules. This information is contained within the wavenumber or frequency (or Raman shift) spectrum of scattered intensity. From a chemical point of view, both Raman and infrared spectroscopy are based on the vibrational transitions occurring in the ground electronic state of the molecules. Raman scattering arises from the changes in the polarisability or shape of the electron distribution in the molecule as it vibrates; in contrast, infrared absorption requires a change of the intrinsic dipole moment with the molecular vibration.

For many years Raman spectroscopy was considered to be of very limited use in food science. Several drawbacks were noted: the fluorescence, the photo-decomposition and the wavenumber calibration problems, and the difficulty of obtaining high resolution spectra. Only recently, with the introduction of instrumental advances such as the interferometer technology and the near-infrared source, has Raman spectroscopy become more widely used. Table 2 summarises the analytical, spectroscopic and instrumental advantages of FT-Raman spectroscopy. Almost all spectroscopic techniques have these features, but some are specific to FT-Raman spectrometry and are indicated.

The European project FAIR-CT96-5053 (Aparicio, Baeten, 1998) was carried out at the Instituto de la Grasa to evaluate the potential of FT-Raman spectrometry for the authentication of oils (with virgin olive oil as the model).

2. MATERIALS AND METHODS

2.1. Sampling

A set of 138 commercial samples of the most representative oils and fats used in the food industry was analysed. The samples were purchased from Belgian and Spanish producers, retailers and laboratories.

2.2. Raman analysis

All the FT-Raman spectra were acquired on a Nicolet 910 FT-Raman spectrometer (Nicolet Analytical Instrument, Madison, WI). FT-Raman spectra were obtained by placing each sample in front of the laser and focusing the Nd: YAG laser beam onto the sample. Spectra were produced over the Raman shift 3250–0 cm\(^{-1}\). Typically, 200 interferograms were co-added at 4 cm\(^{-1}\) resolution with a sampling time of 4 min.

2.3. Near-infrared (NIR) and FT-mid-infrared (FT-MIR)

NIR spectra of fat and oil samples were recorded with a Pacific Scientific Model 6250 spectrometer (PSCO, NIRSystems, Silver Spring, MD, USA) working in the single beam mode. The FT-MIR instrument used for this work was the collegian model spectrometer of Midac (Costa Mesa, CA, USA). The NIR and part of the FT-MIR spectra were recorded at the Unité de Biochimie de la Nutrition at the Université Catholique of Louvain (Louvain-la-Neuve, Belgium).

3. RESULTS

3.1. The Raman spectrum of an edible oil

Figure 2 presents the FT-MIR (inverse scale, top) and FT-Raman (bottom) spectra of the same virgin olive oil. Both techniques present a spectrum with well-resolved bands showing various scattering intensities (Raman spectrum) or absorbances (FT-MIR spectrum). The polar groups (e.g., C=O, O-H) have strong mid-infrared absorption bands, whereas the non-polar groups (e.g., C=C) show intense Raman scattered bands.

Figure 1. Presentation of some of the methods used to authentify oils and fats (Aparicio, Baeten, 1998) — Diagramme présentant une partie des méthodes utilisées pour l’authentification des huiles et graisses.
3.2. Classification of edible oils and fats: “How to spin spectral data into chemical information”

The main analytical operation in spectroscopy is to extract the information in such a way that it can be used in quantitative or qualitative analysis. The Raman spectrum is a rich source of multivariate data (about 1000 data for one spectrum [4000 to 0 cm\(^{-1}\)] collected with a resolution of 4 cm\(^{-1}\)). The challenge facing the analyst is how to distinguish the spectral data set and isolate the variables that can be correlated with the information of interest (in our case, the authentication issue). A wide range of statistical and data analysis software packages is available. The trick in the multivariate data analysis is not the computation, but the definition of the objective and the subsequent choice of the appropriate statistical analysis or chemometrics.

The various chemometric approaches can be classified according to whether they are supervised (e.g., discriminant analysis) or unsupervised (e.g.,
Principal component analysis. In the first stage, the PCA method was applied to the mean values of the fatty acids content (determined by GC) of the all sample sources. Three clusters of samples appeared: those rich in saturated fatty acids (SFA), those rich in monounsaturated fatty acids (MUFA) and those rich in polyunsaturated fatty acids (PUFA). In the second stage, PCA was applied to the FT-Raman spectral data. The oils and fats clustered into the same three groups described for the first stage. The results of the unsupervised method show clearly that FT-Raman spectroscopy can classify samples according to their degree of unsaturation.

Stepwise linear discriminant analysis. The third stage of the study was to design an arborescent structure for the classification of edible oils and fats. The objective was to establish a series of discriminant functions (DFs) (i.e., mathematical equations) able to separate unknown oil and fat samples from different sources and to study the Raman data used to do that. A flow diagram describing the procedure used to build the arborescent structure was established and was applied to the sample set in order to determine the different DFs. The first step of the flow diagram consisted of assigning an arbitrary number to each sample according to its source. The SLDA procedure was then applied to discriminate the sample groups and to construct the pair of DFs (one for each group).

The next stage focused on the validation of the DFs. The squared Mahalanobis distance from the sample group mean observations (i.e., centroid) was calculated for each sample. Each oil or fat was assigned to the nearest group centroid. If the conclusions of the internal and external validation (i.e., samples used and not used to build the equations) were positive, the established DFs were validated DFs for the two studied sample groups. In the case of one or both groups including samples from the same source, the discrimination was considered partially or fully achieved, respectively. When the sample group included samples from two or more sources, the flow diagram was again applied until each group contained only oils from a single source.

Figure 3a presents the results of the construction of the arborescent structure with the established pair of DFs and the defined groups. First, the calibration samples were separated into two groups (DF 1) on the basis of total amount of unsaturated fatty acids; thus, one group contained samples with a high content of saturated fatty acids (SFA), while the other contained samples rich in unsaturated fatty acids (UFA). The UFA group was then divided into two sub-groups (DF 2) on the basis of type of unsaturation; thus, one sub-group contained oils with a high amount of monounsaturated fatty acids (MUFA), while the samples in the other sub-group contained a predominant fraction of polyunsaturated fatty acids (PUFA). In the PUFA branch, the next stage (DF 3) was the discrimination of the corn oils (COR) —richer in MUFA— from the

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Table 2. Analytical, spectroscopic and instrumental advantages of FT-Raman spectroscopy (Aparicio, Baeten, 1998) — Avantages analytiques, spectroscopiques et instrumentaux de la spectroscopie FT-Raman.

<table>
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<th>Advantages</th>
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<tr>
<td>Rapid analysis</td>
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<td>Direct, non-invasive and non-destructive</td>
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<td><strong>In situ</strong> analysis</td>
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<td>Qualitative and quantitative analysis</td>
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<td>Economic method (e.g. labour saving)</td>
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<td>No use of pollutant solvents</td>
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<td>Scattering process</td>
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<td>Intensity proportional to concentration</td>
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<td>High-resolution spectra</td>
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<tr>
<td>Precise spectral frequency measurement</td>
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<td><strong>Entire vibrational spectra</strong></td>
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<td><strong>Non-polar group information</strong></td>
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<tr>
<td>More significant information</td>
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<tr>
<td><strong>Trace element determination</strong></td>
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<tr>
<td><strong>Fluorescence-free spectra</strong></td>
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<tr>
<td><strong>Spectra insensitive to temperature</strong></td>
</tr>
<tr>
<td>Push button instrumentation</td>
</tr>
<tr>
<td>None or reduced sample preparation</td>
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<tr>
<td><strong>Works well with aqueous samples</strong></td>
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<tr>
<td><strong>Suitable for use at-, on- or in-line process</strong></td>
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<td><strong>Compatible with suitable fibre optics</strong></td>
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Figure 2. FT-mid-infrared (inverse scale) and FT-Raman spectra of a virgin olive oil — Spectre FT-moyen-infrarouge (échelle inverse) et FT-Raman d’une huile d’olive vierge.
The sunflower (SUN) and soybean (SOY) oils — richer in PUFA. The SUN group was discriminated in the last step (DF 4) involving the PUFA branch. Turning to the MUFA branch, canola (CAN, DF 5) and peanut (PEA, DF 6) oils — richer in PUFA — were successively discriminated from the group which included the high oleic sunflower (HOS) and olive (VOO) oils. The DF 7 stage involved discriminating between the HOS and VOO samples.

Figure 3b shows the scattering intensities from Raman shifts selected, the source samples included in the two defined groups and the squared Mahalanobis mean distance (SMD) calculated between the two groups. To study the power of the DFs, different factors were considered.

- The squared Mahalanobis distance between the group centroids (i.e., the point defined by the means of the Mahalanobis distance for all variables in the group) was calculated.
- The classification matrix displayed by the SLDA module of the STATISTICA package allowed the number and percentage of samples to be correctly classified for each group involved; a sample is classified in the group to which it is nearest.
- The ellipses of the 95% confidence region were calculated during the calibration step, using the Mahalanobis distance from the group mean. Their centres were calculated from the means of the group coordinates, and their axes represent the values of the confidence regions. These ellipses allow an
interpretation beyond the simple location of the sample and the calculation of the percentage of samples correctly classified.

For each pair of DFs and each subsequent group, figure 3c regroups the percentage of samples correctly classified according to the nearest group (classification procedure I) and the samples included in the 95% confidence region (classification procedure II). Figure 3d shows the results of DF 5.

3.3. Detection of virgin olive oil adulteration: “Which samples are authentic?”

The potential of Raman spectroscopy for detecting the adulteration of virgin olive oil was also assessed. The FT-Raman spectrometer could be calibrated for the quantification of some triglycerides (e.g., LLL) in edible oil. LLL is present in virgin olive oil at trace levels; a high content of these triglycerides therefore indicates that the virgin olive oil has been adulterated with other kinds of vegetable oil.

The potential of Raman spectrometry was demonstrated for adulterant oils rich in LLL (soybean, corn) as well as for oils poor in LLL (pomace). For this experiment, genuine Virgin olive oils were spiked at the range of 1, 5 and 10% with different adulterant types. The Raman data used in the classification of fats and oils were useful in the authentication of virgin olive oils. Using the information contained in the Raman spectra it is possible to discriminate clearly between genuine and 1% spiked samples (Figure 4). This observation attests to the potential of FT-Raman spectroscopy in the detection of low levels of adulteration. The results of discrimination between the three groups (1, 5, 10%) of adulterated samples indicate that the technique could also be useful in quantifying the adulteration (Baeten et al., 1996).

4. CONCLUSION

Advances in knowledge and technology have undoubtedly led to the increased success in the battle against fraud. Unfortunately, however, the same advances have been used by dishonest people to invalidate the usefulness of official standards. Consequently, it is necessary to update and improve food authentication methods continuously, to develop new analytical techniques, to keep pace with changing practices in the food industry and to keep ahead of those indulging in unscrupulous practices.

The results of the European project FAIR-CT96-5053 (Aparicio, Baeten, 1998) have demonstrated that FT-Raman spectrometry could be useful for classifying samples of oils and fats according to their sources and for detecting and quantifying virgin olive oil adulteration. A combination of spectroscopy and chromatography (where the latter could be used to quantify chemical compounds revealing adulterations, and the results could be used in the multivariate calibration of the former) might be an easier, faster and more accurate way of determining virgin olive oil adulteration than currently available methods.

The methodology developed and tested in the project could be applied to the authentication of other edible oils such as peanut, cottonseed or sesame oil, as well as fat products such as butter and chocolate.

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Bibliography


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