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Analysis

Raman spectroscopy in lipid analysis

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Summary

In the last decade, Raman spectroscopy has gained in maturity as a valuable technique for lipid analysis. The technique is non-destructive, does not need sample presentation thereby allowing use in in situ and on-line analysis, and is relatively cost effective. Recent studies have demonstrated the potential of Raman spectroscopy for determining and quantifying different parameters and properties of lipids, and for following oxidative deterioration.

Introduction

The range of techniques at the disposal of the fat and oil industry is continuously expanding. A quick look on the internet shows just a fraction of the analytical solutions proposed in research papers or by private companies dealing with the development and validation of methods. A significant number of the techniques proposed involve titration or separation processes (e.g., iodine value determination by titration, or fatty acid profile determination by gas chromatography) and require increasingly sophisticated equipment and greater expertise. Alongside these techniques, other emerging fast and cost-effective techniques are being proposed. Vibrational spectroscopic techniques including near-infrared (NIR), mid-infrared (IR or MIR) and Raman spectroscopy techniques are among them. With the increasing interest in 'green' methods, more attention is being paid to these techniques as they have the advantage of not requiring the use of reagents except those needed to perform the reference analyses used in the calibration stage of the development and validation of the method.

Features of Raman spectroscopy

NIR and MIR techniques are widely used in food and feed analysis for the simultaneous control of different parameters and properties while Raman use is more restricted. It should be noted that whereas the potential of Raman spectroscopy has been recognized for some time, it is only in the past 15 years that various instrument developments have allowed it to be used more frequently for the control of food products. Several advantages make the vibrational spectroscopic techniques very attractive, especially for the analysis of fats and oils. These techniques are non-destructive, they do not need sample presentation allowing their use in *in situ* and online analysis, and they are relatively cost effective. Today, their costs in terms of instrumentation and implementation are increasingly affordable. The introduction of ready-to-use Raman instruments is also an important step in the extension of the use of this technique. Another interesting feature is that Raman spectroscopy, like other vibrational spectroscopy techniques, allows users to determine simultaneously and rapidly (requiring milliseconds to minutes depending on the instrument and configuration used) a large number of parameters and properties. The fact that the procedure for determining parameters and properties is done in two steps is a considerable advantage. In the first step, the product's spectral information is collected, and in the second step it is transformed by using prediction models into useful information. This allows users to determine several parameters and properties on the basis of a single analysis. This information can then be used to control the product or to follow and adjust a process.

The major limitation of Raman spectroscopy is that it requires, in most of the analytical issues, calibration and validation steps. The calibration step aims to construct predictive models (i.e., mathematical equations) allowing the Raman scattering intensities from different frequencies (also called Raman shift) to be transformed into a figure. The validation step aims to control, in independent samples/batches, the predictive abilities of the constructed models to provide the correct answer for the parameter or property of interest.

Lipid analysis

In the analysis of lipids, Raman spectroscopy, as a fast and reliable vibrational spectroscopy technique, has decisive advantages that should be considered when a solution to analytical issues is being sought. Scattering the radiation phenomenon behind the Raman effect leads to non-polar groups (such as the ethylenic group, C=C) having strong Raman scattering bands. In addition, the fact that during the measurement process, the sample is the source of radiation makes this the technique of choice when the method has to be implemented for the control of a product or a process. It also allows the Raman spectrum of a product to be collected in various types of crystal containers or tubes. No direct contact with the sample is required to collect the spectral information. In order to get the Raman spectrum of a sample, a laser emitting at a defined frequency is used to send a large quantity of photons to the sample. Most of the photons are scattered at the same frequency as the laser source (this is called the Rayleigh phenomenon), but a small fraction interacts with the molecules making up the sample and is scattered at shifted frequencies (this is called Raman phenommenon). Raman instruments are designed to measure the photons scattered at shifted frequen-

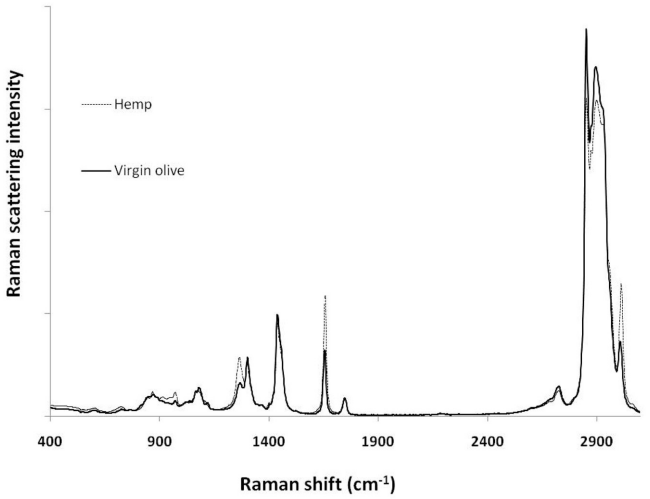

Figure 1. Raman spectra of a virgin olive oil (rich in monounsaturated fatty acids, 79% for the sample analyzed) and a hemp oil (rich un polyunsaturated fatty acids, 73% for the sample analyzed) measured at room temperature (liquid state).

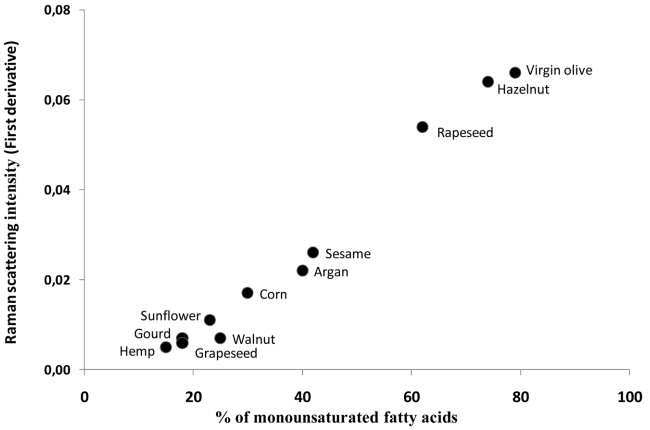

Figure 2. Relationship between the content of monounsaturated acyl groups and the scattering intensities (first derivative value) measured at 1656 cm^{-1} for different oils purchased from a Belgian supermarket.

cies and provide spectra, including the scattering bands observed at the different Raman shifts and characteristics of the sample.

Raman spectroscopy has been shown to be a promising tool for determining and quantifying different parameters and properties of lipids. Thus, it has been proved, for instance, to be of interest in determining the total unsaturation (iodine value) of a sample, the *cis*/*trans* geometrical isomer ratio, the unsaturation of a oil or fat (monounsaturated *vs* polyunsaturated fatty acids), the quantification of a particular triacylglycerol or fatty acid and the study of the crystal structure of specific fat products (Beattie et al. 2004; Muik et al., 2004). These demonstrations have been conducted in various oil and fat matrices of plant or animal origin, such as olive oil or anhydrous milk fats. For example, it is possible to calibrate a Raman spectrometer for the quantification of trilinolein used as an indicator of adulteration in Virgin olive oil (Baeten and Aparicio, 2000) or for the quantification of conjugated linolenic acids (CLA) in milk fat (Meurens et al., 2005). Raman spectroscopy is also valid for analyzing minor compounds of oils and fats. It could be, for example, a valuable technique for detecting carotenoids in oils, or waxes or squalene in the unsaponificable fraction of Virgin olive oil (Baeten et al., 2005).

Figure 1 presents the Raman spectra of a Virgin olive oil (rich in monounsaturated fatty acids, 79% for the sample analysed) and a hemp oil (rich un polyunsaturated fatty acids, 73% for the sample analysed) measured at room temperature (liquid state). These spectra, and the subsequent ones, were acquired with a Vertex 70 – RAM II Bruker Fourier Transform Raman spectrometer. This instrument is equipped with a Nd:YAG laser (yttrium aluminium garnet crystal doped with triply ionised neodymium), which has an output at 1064 nm (9398.5 cm^{-1}), as the source of photons sent to the sample. A liquid-nitrogen cooled Ge detector was used. FT-Raman spectra [3600–100 cm^{-1}] were collected with a resolution of 4 cm^{-1} by co-adding 10 scans for each spectrum. The acquisition time is about 30 seconds per sample. The bands centred in the vicinity of 3005, 2950–2850, 1745, 1670–1650, 1440, 1300 and 1260 cm^{-1} are characteristics of *cis* =C-H, C-H, C=O, *cis* and *trans* C=C, C-H and *cis* =C-H groups, respectively. Figure 2 presents the relationship between the monounsaturated contents and the scattering intensities measured at 1656 cm^{-1} for different oils purchased from a Belgian supermarket.

Raman spectroscopy has been also proposed for the classification, authentication and detection of the adulteration of oils and fats from plant and animal origin (Baeten and Aparicio, 2000; Abbas et al., 2009). As an example, the technique can be used to detect the adulteration of Virgin olive oil with seed oils (e.g., rapeseed or sunflower oil) through the construction of equations for detecting a specific marker, for detecting adulterated samples or for directly quantifying adulterants. For the detection of more sophisticated adulteration, such as the adulteration of olive oil with hazelnut oil, it is worth analyzing the unsaponifiable fraction by Raman spectroscopy (Baeten et al., 2005). In this approach the Raman spectra of minor compounds are used to assess the genuineness of the sample. Whether the authentication by Raman is based on the analysis of the oil or the unsaponifiable fraction, the procedure involves the calibration of the spectrometer for detecting and quantifying a marker or building a large database, including the spectra of genuine oils and adulterated ones.

Raman spectroscopy has also a potential for following oxidation of oils and fats (Muik et al., 2004). The advantage is that in the same analysis, it is possible to follow the disappearance of the fatty acids making up the samples as well as the production of new fatty acid isomers and compounds resulting from the oxidation of the sample. This analysis can be done at the lab level or at the industrial level to follow a process on-line, without interacting with the oxidation process. Indeed, as explained before, the measure is done through the vial or the tube without requirement of direct interaction with the sample.

Conclusion

Raman spectroscopy is now mature enough to be considered as a vibrational technique of value for the analysis of oils and fats. The technique has been proposed for a wide range of applications of interest in the lipid industry. It can be used to set up reliable and fast methods for the lab and process controls for both raw materials and end products or for the follow-up of industrial process.

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