Determination of the Total Unsaturation in Oils and Margarines by Fourier Transform Raman Spectroscopy

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An improved Raman spectroscopic procedure for the determination of the total unsaturation in oils and fats using Fourier Transform Raman (FT-Raman) spectroscopy is described. An important advantage of FT-Raman for these samples is that the spectra are fluorescence-free unlike dispersive Raman which often uses visible excitation. Samples can be analyzed without any pre-treatment thus eliminating the need for dissolution in toxic solvents. The short acquisition time of FT-Raman and the ease of application allowed for a rapid sample turnover.

KEY WORDS: Dispersive Raman, fluorescence, FT-IR, FT-Raman, iodine value, unsaturation.

In the food industry, the levels of unsaturation of fats and oils are of importance in terms of the nutritional values and fat processing. These levels are often measured by a standard iodine value (IV) determination. (1-3).

Infrared (IR) spectroscopy has been used as an analytical tool in the fat industry for many years (4-8), and it has often been employed to measure the concentrations of *trans* isomers (9-11) in fats and oils. However, an independent determination of the *cis* isomer content (12,13) is needed when the total unsaturation is required. Determination of the total degree of unsaturation in margarines based on measurement of the weak IR absorption band at 1660 cm^{-1} (C=C stretching band, ν(C=C)) (14) also fails since margarines often contain water which attacks alkali halide windows and it has a strong IR absorption band at 1660 cm^{-1} (0-H bending vibration).

Raman spectroscopy using visible excitation (15-17), on the other hand, has only recently been applied to quantitative analysis of oils (18). Although water has no interference and is readily available, cheap glass cells can often be employed (this is not the case in infrared). Raman spectroscopy using visible excitation suffers from fluorescence from the sample or the impurities present in it. In oils and margarines, fluorescence is often caused by the added carotenes and coloring ingredients. However, recently with the use of Nd/YAG lasers, Michelson interferometers and near-infrared detectors (19-21), it has been possible to overcome this problem because the operating frequency of the laser is well below the threshold for most fluorescence processes. FT-Raman has many of the advantages of FT-IR, frequency calibration can be very reproducible, an extensive range of software (subtraction, deconvolution etc.) is readily available, and existing FTIR spectrometers can be converted easily and cheaply to FT-Raman operation (22).

In this report, we describe the first application of FT-Raman spectroscopy in the determination of the total degree of unsaturation of intact samples, hence eliminating the need for extraction, filtration and dissolution, thus giving a simpler, quicker and more efficient method than the IR and IV techniques.

EXPERIMENTAL PROCEDURES

Five oils (linseed, sunflower, corn, peanut and olive) and seven margarines from different manufacturers were purchased from a local supplier. An aliquot (5mL) of each oil was poured into five identical glass bottles, deaerated by N_2 and sealed. The remaining portions were used in FTIR analysis and IV determinations. The IV measurements were carried out for all the oils and margarines by the standard Wijs technique (1).

A Digilab FTS 60 FTIR spectrometer equipped with a deuterated triglycine sulphate detector was employed to acquire the IR transmittance spectrum of a thin film of each oil sandwiched between two KBr plates. Each spectrum was the result of coaddition of 100 interferograms at a resolution of 12 cm^{-1} over the frequency range of 700-3250 cm^{-1}.

A Spex 1401 double monochromator equipped with a Ga-As photomultiplier and a spectra-physics Krypton ion gas laser operating at 647 nm and 130 mW were employed to obtain the dispersive Raman spectra of margarines at 12 cm^{-1} resolution. Margarine samples were inserted into glass capillary tubes and placed in the laser beam.

All the FT-Raman spectra were acquired on a Perkin-Elmer 1710 FT-Raman spectrometer which was equipped with a quartz beamsplitter and an InGaAs detector running at room temperature. A Spectrom c.w Nd:YAG laser with a TEM_{00} output at 1.064 μm and operating power of 400 mW provided the near-infrared excitation. Typically, 100 interferograms were co-added at 12 cm^{-1} resolution with a 4-min sampling time. The instrument has been described in detail elsewhere (23).

The FT-Raman spectra of five oil samples were obtained by placing each sample bottle in front of the laser and focusing the beam into the oil. Back-scattered light was collected with a lens system and passed into the interferometer. Margarine samples were analyzed by smearing them into a solid sample holder (5mm depth) and focusing the Nd:YAG laser beam onto the surface of each sample. FT-Raman spectra were produced over the Raman shift of 700-3250 cm^{-1}.

The quantitative program measured the ν(C=C) intensity and ratioed it to the intensity of the CH_2 scissoring $\delta(CH_2)$. This was achieved by measuring the height of the peak at about 1661 cm^{-1} from a base line (1628-1694 cm^{-1}), and ratioing it to that at 1444 cm^{-1} (base line 1382-1512 cm^{-1}).

In the margarine sample, a second quantitative program was employed to ratio the ν(C=C) band area to the area of the $\delta(CH_2)$ scissoring band. The total band areas for ν(C=C) and $\delta(CH_2)$ were measured above base lines drawn from 1628-1694 cm^{-1} and 1382-1512^{-1}, respectively.

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TABLE 1

Characteristic Infrared Absorption and Raman Scattered Bands of Sunflower oil*

Peak number	Wavenumber	FT-Raman	FTIR	Remarks
1	3014	Y	—	asymmetric olefinic C-H stretch ν_{asym} (=C-H)
2	3007	—	Y	symmetric olefinic C-H stretch ν_{sym} (=C-H)
3	2926	Y^a	Y	asymmetric aliphatic C-H stretch ν_{asym} (−C-H)
4	2855	Y	Y	symmetric aliphatic C-H stretch ν_{sym} (−C-H)
5	1745	Y(w)	Y	C=O stretching in an ester ν(C=O)
6	1661	Y	Y(v.w)	C=C stretching ν(C=C)
7	1444	Y	—	>CH_2 scissoring deformation $\delta(CH_2)$
8	1306	Y	—	in phase methylene twisting motion
9	1272	Y	—	in plane =C-H deformation in an unconjugated *cis* double bond
10	1163	—	Y	C-O stretch of an ester ν(C-O)
11	724	—	Y	=C-H planar bending

*Y = indicates a Raman scattering or IR absorption band; w = weak band; v.w = very weak band; and ^{a} = This band is shifted to 2905 cm^{-1}.

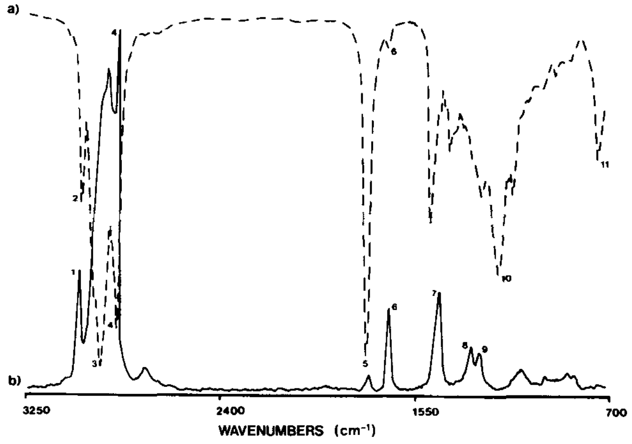

FIG. 1. The infrared transmittance (a) and Raman scattered (b) spectra of a sunflower oil recorded at 12 cm^{-1} resolution.

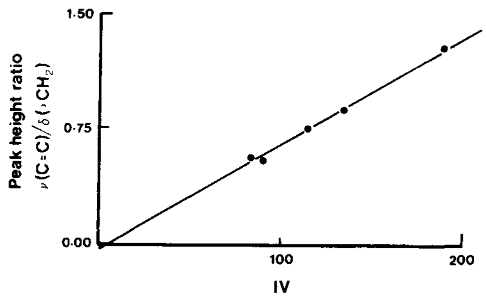

FIG. 2. The peak height ratio $\nu(C{=}C)/\delta(CH_2)$ against the iodine values (IV) of five commercial oils.

RESULTS AND DISCUSSIONS

The transmittance IR spectrum of one of the oils (sunflower) over the frequency range of 700-3250 cm^{-1} is shown in Figure 1(a). This is an example of a very good quality IR spectrum with a very high signal-to-noise ratio (S/N). The FT-Raman spectrum of the same oil over the Raman shift of 700-3250 cm^{-1} is presented in Figure 1(b). In this spectrum the vibrational bands can be easily identified and the S/N is more than sufficient for quantitative analysis. Although both spectra were recorded in a reasonably short time (4 min) and represent active vibrational modes of an oil, the IR and Raman techniques do not give rise to identical information. Table 1 illustrates some of the characteristics of IR absorption and Raman scattered bands of sunflower oil. This table indicates that IR and Raman techniques are complimentary for structural assignment in sunflower and other oils.

The plot of $\nu(C{=}C)/\delta(CH_2)$ band intensities against the IV of the five oils is shown in Figure 2. This plot gave rise to a linear calibration with an equation of $(\nu(C{=}C)/\delta(CH_2)) = (6.845 \times 10^{-3})IV - (2.489 \times 10^{-2})$ and a correlation coefficient of 0.9987.

Although the dispersive Raman spectra of pure oils have recently been published (18), margarines often produce a great deal of fluorescence due to added ingredients. The dispersive Raman spectrum of a margarine (based on hydrogenated sunflower oil) over the Raman shift of 995-1895 cm^{-1} is shown in Figure 3(a). This spectrum is extremely noisy and suffers from a great deal of fluorescence. Vibrational bands hardly could be identified, and quantitative analysis was an impossibility. The acquisition time was also very long (20 min), and fat samples often melt when struck by high energy laser powers over a long period. The FT-Raman spectrum of the same margarine over the same Raman shift is presented in Figure 3(b). FT-Raman produced an exceptionally

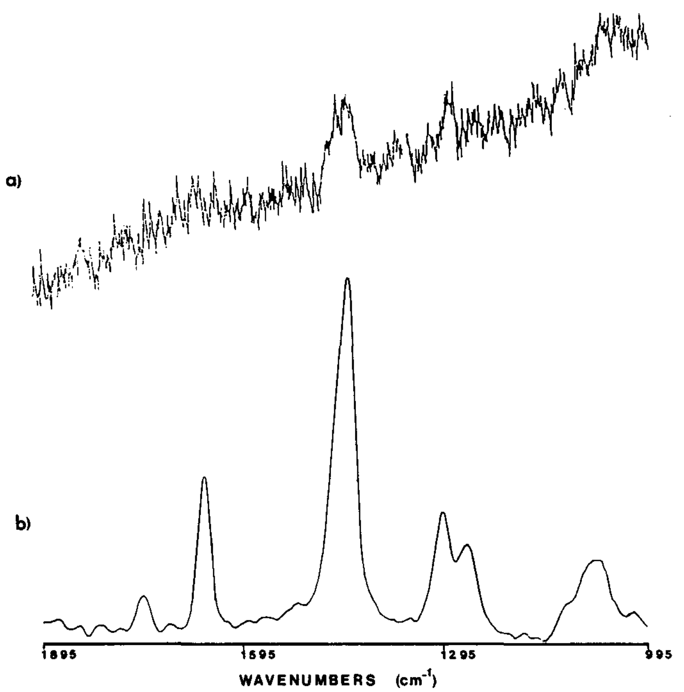

FIG. 3. Comparison of a) dispersive Raman, 647 nm excitation and b) FT-Raman, 1.064 μm excitation spectra for a sunflower margarine. Both spectra were recorded at 12 cm^{-1} resolution. Excitation powers were 130 mW for dispersive Raman and 400 mW for FT-Raman measurements.

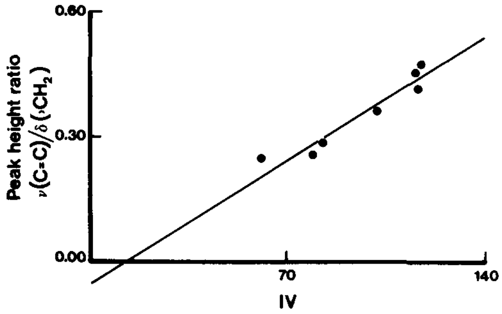

FIG. 4. The peak height ratio $\nu(C=C)/\delta(CH_2)$ against the iodine values (IV) of seven commercial margarines.

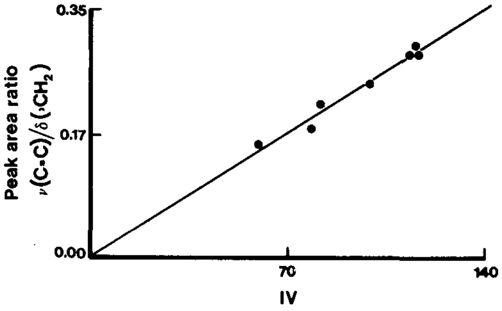

FIG. 5. The band area ratio $\nu(C=C)/\delta(CH_2)$ against the iodine values (IV) of seven commercial margarines.

improved spectrum with very good S/N in one-fifth of the acquisition time than that of dispersive. The vibrational bands could easily be assigned and the band intensities may readily be measured from a base line. Figure 4 illustrates the peak height ratio ($\nu(C=C)/\delta(CH_2)$) against the IVs of seven different margarines. A straight line with an equation of ($\nu(C=C)/\delta(CH_2)$) = (4.205×10^{-3})IV − (5.433×10^{-2}) and a poor correlation coefficient of 0.9495 was obtained. A much better calibration line (Fig. 5) with a higher correlation coefficient of 0.9899 and an intercept much closer to the origin was produced when the total area of the $\nu(C=C)$ was ratioed to that of $\delta(CH_2)$ and plotted against the IV. This calibration line gave rise to an equation of ($\nu(C=C)/\delta(CH_2)$) = (2.488×10^{-3})IV − (2.390×10^{-3}). In margarines, the total unsaturation determination based on peak area measurements produced more accurate results since margarines often contain appreciable amounts of *trans* isomers. *Trans* isomers give rise to $\nu(C=C)$ Raman scattered bands at around 1671 cm^{-1} (18) which is about 10 cm^{-1} higher than the *cis* $\nu(C=C)$ band. This, in turn, causes an increase in the width of the total $\nu(C=C)$ band, due to the low instrumental resolution used.

FT-Raman instrumentation is developing rapidly especially in improvement of detectors (24,25). These developments should make spatial acquisition faster, and hence very rapid analysis of IVs of oils and fats in quality control should be possible. Remote on-line measurements (i.e. during hydrogenation) are also feasible since the coupling FT-Raman spectrometers to experiments through optical fibers have been achieved (26).

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