

Measuring Unsaturation in Milkfat and Other Oils by Differential Infrared Spectroscopy

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Abstract

Infrared absorbance at 3.3μ was directly related to the iodine value of milkfat samples with unusual unsaturation when a totally saturated analog was a reference. Unsaturation was determined from differential infrared absorbance using a calibration curve. This technique was suitable for seven natural vegetable oils but not castor oil or cod-liver oil. The calibration curve was not applicable to hydrogenated oils because of the decrease in intensity due to isomerization (*cis-trans*) of the olefinic bond.

Introduction

Concern with saturated dietary fats has promoted efforts to modify the natural fat composition of foods such as milk and beef. Such research would be aided by development of rapid techniques for determining unsaturation since present methods are time consuming (2).

Sinclair et al. (8) first reported a relationship between infrared absorbance bands at 3.3 and 3.4μ and the number of double bonds with *cis* configuration in unsaturated fatty acids. Arnold and Hartung (1) used a procedure based on a similar relationship to determine the extent of unsaturation in various food fats and oils. For speed and simplicity we investigated differential infrared spectroscopy for the analysis of unsaturation. The differential technique directly measures intensity at an isolated absorption band and precludes the need for establishing empirical relationships involving several bands. This paper presents the results of our investigation and describes a method for determining unsaturation in natural fats.

Materials and Methods¹

Butter prepared from churned cream and washed with water was converted to butteroil

by warming to 39 C and centrifuging to remove the residual butter serum. Butteroils with elevated concentrations of linoleic acid were also examined. These butteroils, furnished by Dr. L. F. Edmondson of this laboratory, were prepared from the milk of cows with increased unsaturated fatty acids in their milkfat (6).

Cod-liver oil and the nonprocessed vegetable oils (almond, corn, castor, olive, safflower, sesame seed, soybean, and sunflower seed) were purchased from local retail stores. Tristearin, stearic acid, and the pure '*cis*' fatty acids, i.e., oleic, vaccenic, linoleic, and linolenic, were from the Hormel Institute (Austin, Minn.). Hydrogenated samples of butteroil and olive oil, prepared by Yoncoskie et al. (10), were furnished by R. A. Yoncoskie of this laboratory.

The differential infrared spectra were obtained with a Perkin-Elmer 421 grating spectrophotometer which was operated at a scan speed of $100\text{ cm}^{-1}/\text{min}$. Matched $.50\text{ mm}$ sodium chloride cells were the sample and reference. Spectral grade carbon tetrachloride was the solvent.

A solution (2% by weight) of each natural oil was scanned against a 2% solution of tristearin. Fatty acids were studied as .5% solutions with stearic acid solution as the reference. Only the C-H stretching region from 3.0 to 4.0μ was scanned for the natural oils and fatty acids. The hydrogenated samples were studied as 4% solutions with a tristearin or a completely saturated butteroil solution as reference. Hydrogenated samples were scanned from 3.0 to 4.0μ and from 10.0 to 11.0μ . All optical densities were measured in triplicate. Iodine values were determined by the Hanus method (2).

Results

Differential infrared spectra, in wavelengths between 3.2 to 3.4μ , for 2% solutions of butteroils with iodine numbers of 33, 58, and 79 (Fig. 1) revealed that as the degree of unsaturation increased, the intensity of the peak

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¹Mention of brand or firm name does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

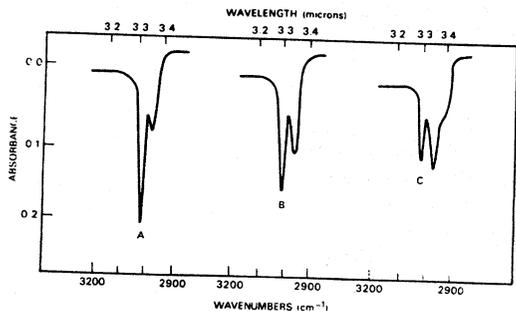


FIG. 1. Differential infrared absorbance scans in the 3.1 to 3.5 μ region for 2% solutions of butteroils of decreasing unsaturated fatty acids. A, B, C had iodine values of 79, 58, 33.

at 3.3 μ increased. A linear relationship existed between differential infrared absorption maxima at 3.3 μ and the iodine values for 2% solutions of butteroils containing unusual amounts of unsaturated fatty acids (Fig. 2).

Data from nine nonprocessed vegetable oils, cod-liver oil, and eight butteroil samples also gave linear relationships between intensity and unsaturation (Fig. 3).

An equation (Eq. 1) for the line in Fig. 2 is:

$$A = .0024 I + .0023 \text{ where } A = \text{absorbance and } I = \text{iodine values.}$$

The correlation coefficient, r , relating absorbance and iodine values for all the data except for castor oil and cod-liver oil, was .999.

The relationship between differential infrared absorbance at 3.3 μ and the number of double bonds in some *cis*-fatty acids is in Fig. 4.

Curves generated from linear regression

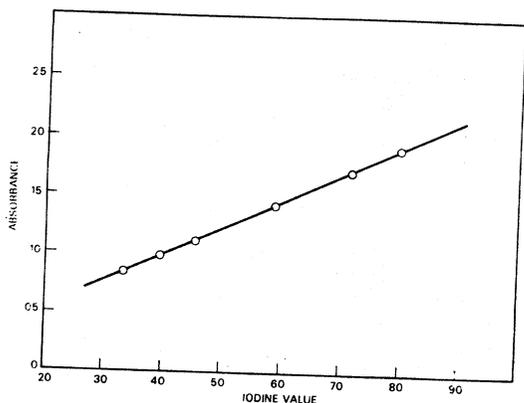


FIG. 2. Plot of differential infrared absorbance at 3.3 μ vs. iodine value for 2% solutions of a series of butteroils with increasing amounts of unsaturated fatty acids.

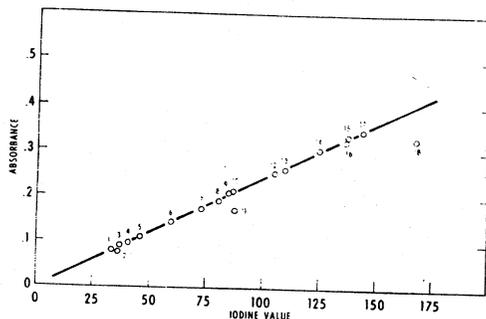


FIG. 3. Plot of differential infrared absorbance at 3.3 μ vs. iodine value for 2% solutions of cod-liver oil and a series of vegetable oils and butteroils (1 to 8—series of butteroils, 9 to 10—olive oils, 11—castor, 12—almond, 13—sesame, 14—corn, 15—sunflower, 16—soy, 17—safflower, 18—cod-liver).

analysis of data from the differential infrared absorbance versus iodine value for 4% concentrations of the natural oils, the hydrogenated milkfat and olive oil samples, and from both natural and hydrogenated samples are compared (Fig. 5). Data for the hydrogenated oils were plotted to show where these points fall.

The equations for the lines in Fig. 5 are:

$$\text{For the natural oils:} \\ A = .0048 I + .0046 \\ r = .999 \quad [2]$$

$$\text{For the hydrogenated samples:} \\ A = .0044 I - .0209 \\ r = .992 \quad [3]$$

$$\text{For all samples, hydrogenated and natural:} \\ A = .0050 I - .0237 \\ r = .995 \quad [4]$$

Comparison of patterns from the differential infrared absorption and the standard infrared absorption in Fig. 6 showed that resolution of the peaks was more definite from the differential scan.

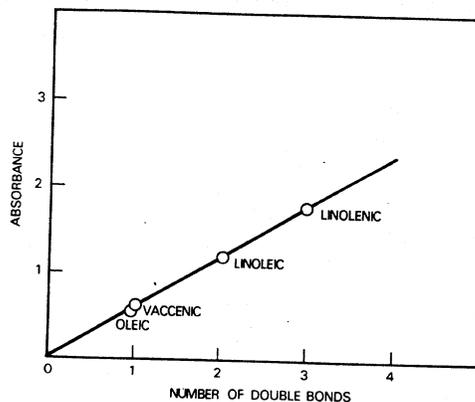


FIG. 4. Effect of unsaturation in some *cis* fatty acids on differential infrared absorbance at 3.3 μ .

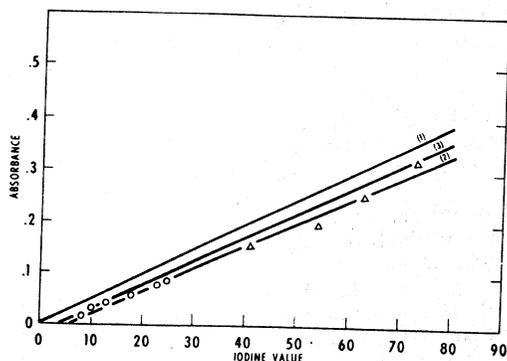


FIG. 5. Comparison of curves from linear regression analysis of data from native butteroils (1), hydrogenated butteroils and olive oils (2), combined data from native butteroils and hydrogenated oils (3). Plotted points are \circ —hydrogenated butteroils, Δ —hydrogenated olive oils.

Discussion

Differential infrared absorbance at 3.3μ was directly related to the number of double bonds for *cis*-unsaturated fatty acids. Because most naturally occurring fatty acids are *cis*-isomers (9), we applied the differential infrared technique to the series of butteroils made from the milk of cows fed protected feed to increase unsaturation in the milkfat

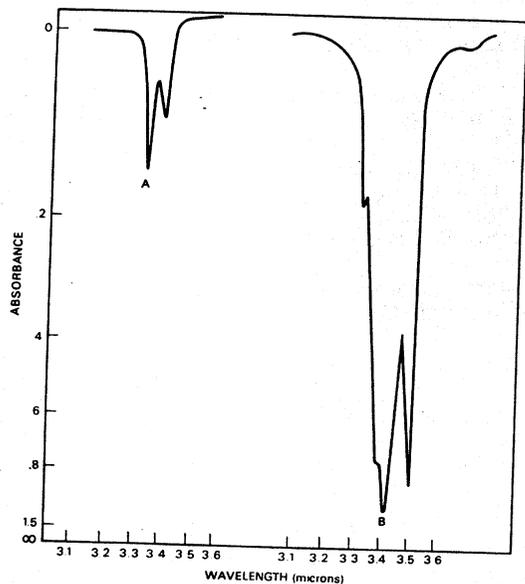


FIG. 6. Comparison of differential infrared scan with conventional infrared scan. A—butteroil solution vs. tristearin solution, B—butteroil solution vs. carbon tetrachloride.

and showed the method worked extremely well for these samples which had iodine values ranging from 33 to 79.

The differential infrared method is applicable to a number of unprocessed vegetable oils. All of the points representing differential infrared absorbance versus iodine value fell on a straight line with the exception of castor oil and cod-liver oil. These two points are real as the absorbance and iodine values for each of those samples were measured twice as many times as the other samples. The low absorbance of the castor oil could be explained by its fatty acid composition. Since ricinoleic acid comprises 85 to 95% of the fatty acids in castor oil (9), the inductive effects of the hydroxyl group in this acid could reduce the intensity of the peak at 3.3μ . The reason for the low value for the cod-liver oil is unexplained but might have been caused by hydrogenation during processing.

Absorbance at 3.3μ was lower for hydrogenated samples of butteroil and olive oil than for the natural unprocessed oils, which agrees with Arnold and Hartung (1). When the absorbance versus iodine number was plotted for the hydrogenated oils, their points did not fall on the curve with the other oils nor was the relationship as linear. The results are not necessarily unexpected since there is a difference in absorbance at 3.3μ depending on the conformation of the double bond in the fatty acids, *trans* bonds having lower absorbance than *cis* bonds (8). *Cis-trans* isomerization occurs during hydrogenation with the *trans* acids reaching a maximum of $\frac{1}{2}$ to $\frac{3}{4}$ the total olefinic bonds in hydrogenated fats and oils (5, 7). Therefore, since the ratio of *cis-trans* bonds changes during hydrogenation, peak intensity versus unsaturation would not be expected to be linear.

Standard deviations from the appropriate regression equations, by propagation of error techniques (11), showed there was greater error in iodine values for the hydrogenated samples. Standard deviations of the iodine values for the natural unprocessed oils were 1.8% and for the hydrogenated oils 4.8%.

If the equation for all the oils (Eq. 4) is used to predict iodine values for hydrogenated and nonhydrogenated samples, the standard deviation would be 3.2%. Therefore, Eq. 4 could be used for studying treated and untreated oils, but accuracy would be decreased. Thus, this differential infrared technique using Eq. 1 or 2, depending on the concentration, is recommended primarily for native butteroils and vegetable oils.

Absorbance at 10.3μ was measured for the hydrogenated samples because others have used differential infrared spectroscopy at this wavelength to detect adulteration of butteroil by hydrogenated fats (3, 4). Absorbance at this wavelength is due to *trans* unsaturation. We found a slight increase in absorbance on partial hydrogenation of the butteroil and olive oil indicating the formation of *trans*-olefinic isomers.

Tristearin was the reference throughout this investigation because most of the triglycerides in the oils contain C_{18} fatty acids. Tristearin blocks out most of the aliphatic C-H absorbance in the 3.4 to 3.5μ range for the samples, and since it is a pure saturated triglyceride, it does not absorb at 3.3μ , the olefinic stretching band. A completely saturated butteroil sample ($I = 1.8$) was the reference in one study and gave the same results as tristearin.

A concentration of 2% was used for the native oils because it gave absorbance in a range useful for all the samples. A 4% solution was needed for the hydrogenated oils due to the low iodine values of some of the samples. No special instrumental settings were needed and a gain of 4.2 gave good results with little noise problems. Because of differences among instruments, however, a working standard curve should be developed for each instrument.

Differential infrared spectroscopy to determine unsaturation offers several advantages over other methods. A sharp isolated peak is produced (Fig. 6) when a saturated sample is used in the reference beam. Once a standard curve is established, the method is simple and rapid. The sample and reference are dissolved in the solvent, the absorbance maximum at 3.3μ is measured, and the iodine value is determined from the standard curve. Scanning time is short because only one absorbance maximum is used and a small area of the spectrum is scanned. Since a 2% solution gives satisfactory results, little sample is needed for the

determination, and, if necessary, the sample could be recovered afterwards.

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