A Rapid Method For the Determination of *Trans* Unsaturation in Fats and Derivatives[1]

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Abstract

The *trans* unsaturation present in samples of unsaturated fats and derivatives may be determined from measurement of the infrared absorbance at two wavelengths, one due to *trans* configuration of a double bond and the other due to the ester or acid group. The ratio of the two absorbance values has a linear relationship with the *trans* unsaturation content. Thus, a drop of sample is diluted with CS_2 and the infrared absorbance measured at 10.36 μ and at 8.5–8.6 μ if esters and 10.7 μ if acids. To calculate the per cent isolated *trans* double bonds in the sample, the ratio of the two absorbance values is substituted into the linear equation which was developed from known samples. The method is rapid since the sample need not be weighed nor made up to a known volume and thus may be applied to samples such as those collected from a gas chromatograph or after thin layer chromatography.

Introduction

The measurement of the amount of *trans* double bonds in a sample of fat or fat derivative is an important measurement since in many products, principally partially hydrogenated, the fatty acid that contains a *trans* double bond may be the major fatty acid present. While the G.L.C. method employing a 5 or 6 ft polyester column can be used to determine the fatty acid composition, this method does not reveal the isolated *trans* isomers since they are not separated from the *cis* unsaturated esters. Therefore, the *trans* double bonds must be measured by an independent method to obtain a complete determination of the fatty acid composition.

The infrared absorption at 10.36 μ was shown by Shreve et al. (1) to be a measure of the amount of isolated *trans* unsaturation in a sample. This has become AOCS Standard Method Cd 14–61.

In practice, the sample is weighed into a volumetric flask, made up to volume and the infrared spectral absorbance recorded over the 9.5 to 11 μ region. A base line is drawn and the absorbance due to *trans* double bonds then compared to the absorbance of a known standard sample and the *trans* double bonds content calculated.

In an effort to conserve time of analysis and make possible the analysis of samples that cannot be weighed or made to known volume such as those collected from a gas chromatograph, a new method has been developed.

Experimental Procedures

Theory

The infrared spectra of fats or esters, such as methyl oleate and methyl elaidate, show a large peak at about 8.6 μ due to ester and also a weak absorption at 10.36 μ if no *trans* is present but a peak if *trans* is present. The ratio of the absorbances at these two wavelengths is the basis of this new method.

The per cent *trans* unsaturation (T) in a sample such as methyl esters may be calculated from the equation

$$\% \text{ T} = \frac{\text{100 absorptivity due to } \textit{trans} \text{ unsaturation in sample}}{\text{absorptivity of methyl elaidate}}$$

Since the absorptivity of methyl elaidate is constant, Q, then

$$\% \text{ T} = 100 a_t/Q$$

a_t = absorptivity due to *trans* unsaturation in sample. The absorptivity due to *trans* unsaturation, a_t, of the sample at 10.36 μ is calculated from the absorbance due only to *trans* unsaturation divided by the concentration and cell length term C. However, the total absorbance at 10.36 μ is due to both the *trans* (A_T) and the weak absorption due to the sample (A_S). Thus

$$A_{10.3} = A_T + A_S$$
$$A_T = A_{10.3} - A_S$$
$$\text{and} \quad a_t = A_{10.3} - A_S/C$$

The concentration and cell length term C of the sample may be measured from the absorbance at 8.6 μ which is independent of the *trans* unsaturation in the sample. Thus

$$C = A_{8.6}\,R$$

where R is the slope of the line relating the concentration and absorbance at 8.6 μ. As shown in Figure 1 this is a constant for esters with the same molecular weight. Then

$$a_t = A_{10.3} - A_S/A_{8.6}\,R$$

A_S is also independent of the amount of *trans* unsaturation in the sample but is a fraction of the absorbance at 8.6 μ ($fA_{8.6}$). Thus

$$a_t = A_{10.3} - fA_{8.6}/A_{8.6}\,R$$
$$\text{then} \quad \% \text{ T} = (100\,A_{10.3} - fA_{8.6}/A_{8.6}\,R)/Q$$
$$\% \text{ T} = 100\,A_{10.3} - fA_{8.6}/A_{8.6}\,QR; \quad K = 100/QR.$$

Then

$$\% \text{ T} = K(A_{10.3}/A_{8.6}) - f$$

which is the equation for a straight line. Thus to evaluate K and f the absorbance ratios of several samples that contain known amounts of *trans* isomers are measured and plotted against the *trans* unsaturation content. The slope of the straight line is K and the intercept is f.

Thus, after the value of K and f, the two constants, has been found, all that is necessary to determine the per cent *trans* unsaturation in a sample is the ratio of the total absorbance at the two wavelengths. Since the ratio is independent of the cell length or concentration of the sample, these need not be known.

Practice

The infrared spectrophotometer was adjusted so that the instrument showed zero absorbance at 10.36 μ with both sample and reference cells filled with

[1] Presented at the AOCS Meeting, New York, October 1968.

the solvent, carbon disulfide. Also the zero transmission setting was adjusted so that the instrument covered full 100% span.

The sample was dissolved in carbon disulfide and diluted so that the absorbance at 8.6 μ of esters or 10.7 μ for acids was not over 0.9. Maximum absorbance of peaks at 8.6, 10.36 μ for fats, 8.55 and 10.36 for methyl esters and 10.36 and 10.7 for fatty acids was recorded. The ratio of the absorbance was calculated at 10.36–8.6 for esters and 10.36–10.7 for acids and substituted into the linear equation developed from known samples; the per cent *trans* unsaturation in the sample was then calculated.

Results

Known mixtures were prepared from pure trielaidin:triolein, methyl elaidate:methyl oleate and elaidic acid:oleic acid and the absorbance ratios determined over a wide range of concentrations for each sample. From these data the linear equations were calculated by the least squares method and other statistics measured.

Methyl esters. Mixtures of methyl oleate and methyl elaidate (Hormel Institute, Highly Purified). Samples, per cent methyl elaidate: 0, 37.6, 54.7, 68.0, 77.4, 100. Each sample was analyzed six times over range of absorbance values at 8.55 μ of 0.3 to 0.9.

Calculated equation:

$$\%\ \textit{Trans}\ \text{Unsaturation (methyl elaidate)} = 121.86\ (A_{10.3}/A_{8.55}) - 9.18$$

Index of determination, .9987; Correlation coefficient, .9993; Standard error (35 D.F.), 1.51; F Ratio test statistic, 26923.2 (1, 35 D.F.).

Triglycerides. (Mixtures of trielaidin and triolein, Hormel Institute, Highly Purified.) Samples, per cent trielaidin: 0, 20.5, 37.3, 68.8, 77.8, 100. Forty samples of hydrogenated oil were analyzed by method Cd 14–61. Each known sample was analyzed six times over a range of absorbance values at 8.67 μ of 0.4 to 0.9.

Calculated equation:

$$\%\ \textit{Trans}\ \text{Unsaturation (trielaidin)} = 144.8(A_{10.3}/A_{8.67}) - 16.6$$

Index of determination, 0.995; Correlation coefficient, 0.9976; Standard error (75 D.F.), .974; F Ratio test statistic, 9225.1 (1, 75 D.F.).

Fatty Acids. (Mixtures of elaidic acid and oleic acid, Hormel Institute, Highly Purified.) Samples, per cent elaidic acid: 0, 11.7, 32.3, 56.5, 69.6, 100. Each sample was analyzed five or six times over range of absorbance values at 10.36 μ of 0.9 to 0.2.

Calculation equation:

$$\%\ \text{Elaidic Acid} = 56.78\ (A_{10.36}/A_{10.75}) - 25.8$$

Index of determination, 0.996; Correlation coefficient, 0.998; Standard error (31 D.F.), 4.42; F Ratio test statistic, 8650.5 (1, 31 D.F.).

These data indicate the ratio of the absorbances at the two peaks is correlated with the amount of *trans* double bonds in the sample and thus the equations can be used to calculate the *trans* unsaturation in a sample from the ratio of the absorbances.

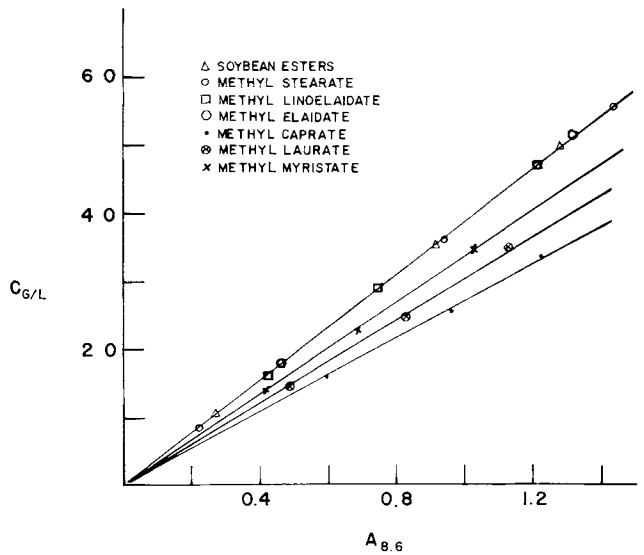

FIG. 1. Absorptivity of methyl esters at 8.6 μ in CS_2 and 0.48 mm path length.

The method is limited to the analysis of samples that do not contain impurities that absorb at other wavelengths than that used to calculate concentration unless the equations were developed from known samples of the same composition. For example, if the equation developed from the analysis of triglycerides was used to calculate the *trans* unsaturation in mixtures of triglycerides and fatty acids, the results would be erroneous since the apparent sample concentration would be based on only the triglyceride part of the sample.

Also, since as shown in Figure 1 the absorptivity at 8.6 μ increases as the molecular weight of the ester decreases, the constants of the equations would be limited to samples of similar molecular weight. Thus, the equations must be developed from known samples that are similar to the unknowns.

The solvent used has an effect on the values of the two constants. For example, the constant K for triglycerides was found to be 144.8 when carbon disulfide was used as a solvent but increased to 181.0 when chloroform was used. This is probably due to the differences in the weak bonding of the solvent with the different groups in the molecules which has an effect on the infrared absorption. Therefore, the equations can be used only when carbon disulfide is used as a solvent.

The analysis of other derivatives of unsaturated fatty acids for *trans* unsaturation should be possible by this method. An absorption band would be selected to use as a measure of the concentration and the ratio of the absorbances due to *trans* unsaturation and to the concentration of the sample measured and an equation developed from samples of known composition.

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REFERENCE

1. Shreve, O. D., M. R. Heether, H. B. Knight and D. Swern, Anal. Chem. *22*, 1261–1264 (1950).

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