A Rapid Method for the Estimation of *trans* Unsaturation in Hydrogenated Oils and Fats

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Abstract

Glycerides and methyl esters of fatty acids containing *trans* unsaturation both show peaks at 10.38 μ. The absorptivities of the glycerides ($a_{10.38}$ μ) have a straight line relationship with the concentration of the *trans* unsaturation, calculated as methyl elaidate. This relationship has been utilized as a quick method for estimating the *trans* unsaturation in hydrogenated fats such as Vanaspati. The infrared spectrum of a given hydrogenated fat is taken in the region of 9–11 μ, the absorptivity at the *trans*-peak is calculated, and the corresponding methyl elaidate content is read from a graph.

Introduction

HYDROGENATION OF OILS AND FATS with metal catalysts results in the formation of *trans* isomers (1–7). Edible products such as shortenings, margarines (8,9), and Vanaspati (10,11) have been shown to contain *trans* isomers (mostly monoenoic) to the extent of 20–50%. Since isolated *trans* double bonds have a specific absorption band at about 10.36 μ, infrared spectrophotometry (12–18) is the method of choice to estimate these. Since such primary standards as oleic acid or methyl oleate and elaidic acid or methyl elaidate are easily prepared, it is generally recommended (15–17) that the oils and fats be converted to their corresponding acids or methyl esters and the infrared determination be made on these. If the estimation is done on the glycerides themselves, triolein and trielaidin have been recommended (12,17–18) as standards. Kaufmann *et al.* (18) have observed that infrared estimation of *trans* on the glycerides generally give higher values and have recommended that, for accurate analysis, the determinations must be made on the methyl esters. In a recent study (19) of *trans* isomers estimation by the baseline technique, it has been shown that a positive correction is needed with the methyl esters as the infrared spectrum of pure *cis* esters at 10.36 μ goes above the chosen baseline (10.02–10.59 μ) while for glycerides a negative correction is required as the corresponding baseline (10.05–10.67 μ) lies below that for a pure *cis* triglyceride. For accurate determinations with glycerides the primary standards required are too numerous and consequently the calculations will be too complicated. In commercial hydrogenation, estimations directly on the glycerides from the autoclave will be of great help in following the course of hydrogenation. This paper describes such a method.

Experimental

Hydrogenated Samples

Five samples were drawn from the plant during the hydrogenation of a batch of cottonseed oil. Their analyses are given in Table I. Gas chromatographic analysis showed that they contained 70–75% unsaturated acids, and this was used for correction for *"cis"* unsaturation in the calculation of the methyl elaidate content of the respective methyl esters.

Vanaspati Samples

Two samples from two lots manufactured on different days were tested. These were blends of hydrogenated peanut, cottonseed, and soybean oils with about 5% sesame oil added as per statutory requirements.

Methyl Oleate

Commercial oleic acid was converted into methyl esters by refluxing with methanol in the presence of about 1% sulphuric acid. A 0.5% solution of methyl esters in methanol was cooled to −15C, and the saturated esters were removed by filtration. The filtrate was cooled to −70C, and the resulting crystals were recrystallized three times at about −65C and −60C. Three preparations were combined and distilled through a packed column at 0.4-mm pressure with the major fraction distilling at 100C. The yield was 102 g of methyl oleate with IV of 85.3 (theory 85.61). Gas chromatography showed a single peak.

Methyl Elaidate

Methyl oleate (25 g) was heated with 40 mg of selenium at 200–210C for 0.5 hr under nitrogen. It was cooled to 90C, treated with 1 g carbon, and filtered. The filtered esters were dissolved in 900 ml of methanol and crystallized at −30C. The crystals were redissolved in 500 ml methanol each time and crystallized successively at −30C once and twice at −20C. The final crystal fraction was freed of methanol and distilled under vacuum to yield 11 g of methyl elaidate with IV of 85.3. This also gave a single peak on gas chromatographic analysis. A portion of this was converted into acid by saponification and acidification. The crude acid was crystallized from petroleum ether, and the crystallized acid had a melting point of 44.4C (Reference 12, 44–45C).

Methyl Stearate

Cosmetic grade stearic acid was esterified with methanol and sulphuric acid, and the methyl esters were distilled to yield a water-white product. Gas chromatography of these distilled esters through an Apiezon-L column (15%) at about 248C gave the

TABLE I

Analytical Characteristics of Hdyrogenated Cottonseed Oil Samples

Sample No.	Oil of fat: IV Wijs. 30 min	Oil of fat: $a_{10.38}$ μ	Methyl esters: Unsaturated %	Methyl esters: $a_{10.38}$ μ	Methyl esters: Methyl elaidate %
1	104	0.04761[a]	76.8[b]	0.03644[a]	4.9
2	91.6	0.1237	76.2	0.1155	22.8
3	71.6	0.2251	76.2	0.2110	44.4
4	67.1	0.2296	76.0	0.2188	46.1
5	66.4	0.2325	71.0	0.2111	44.4

[a] All values are averages of duplicate determinations.
[b] Values are shown by gas chromatography (oleate + linoleate).

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following composition: methyl laurate traces; methyl myristate, 1.1%; methyl palmitate 54.7%; methyl stearate, 44.2%. Since all samples of oils and fats usually contain these acids among their saturated components in slightly varying proportions compared with the above compositions, the infrared spectrum of this mixture of methyl esters was used to ascertain the contribution of saturated components to the background absorptions.

Gas Chromatography

All gas chromatographic analyses were done on a Perkin-Elmer Model 800 Gas Chromatograph, equipped with a flame ionization detector, with either butanediol succinate (8% on HMDS Chromosorb W) 2-meter or Apiezon-L (15% on HMDS Chromosorb W) 2-meter columns under isothermal conditions at 200C with former and 250C with the latter columns respectively.

Infrared Spectrophotometry

All runs were made on Perkin-Elmer Model 21 Infrared Spectrometer with sodium chloride optics. The samples were dissolved in carbondisulphide, and runs were taken between 9–11 μ. A typical run for a glyceride sample and its derived methyl esters (sample No. 5) are shown in Figure 1. It is noted that both *trans* peaks come at 10.38 μ, and all absorbance readings were taken at this wavelength. For methyl esters the baseline was drawn between 9.52 and 10.76 μ as all the methyl esters, including the primary standards, have humps at about these wavelengths. With the glyceride samples the baseline was drawn between 10.12 and 10.76 μ. The absorptivity ($a_{10.38\ \mu}$) for the primary standards are:

	$a_{10.38\ \mu}$
Methyl stearate (commercial)	−0.004
Methyl oleate	0.02
Methyl elaidate	0.462

Calculations

Methyl elaidate content of the methyl esters of all hydrogenated samples were calculated by the following equations:

1. $a_{10.38\ \mu} = \frac{A}{bc}$

   where A is the absorbance at 10.38 μ for the sample minus the absorbance at that point for the baseline
   b is the sample path length, 0.1 cm
   c is the concentration in grams per liter

2. $$\%\ trans\ \text{as methyl elaidate} = \frac{100[a_{10.38\ \mu}\ (\text{observed})] - [\%\ \text{unsaturates} \times a_{10.38\ \mu}\ (\text{methyl oleate})]}{a_{10.38\ \mu}\ (\text{methyl elaidate}) - a_{10.38\ \mu}\ (\text{methyl oleate})}$$

Results and Discussion

In the infrared spectrum the glycerides show a steadily decreasing background absorption between 9 and 10 μ while the methyl esters have humps and depressions. These humps facilitate the choice and drawing of a suitable baseline and serve as marks of distinction between methyl esters and glycerides. The *trans* peak for both occurs at 10.38 μ. The baseline 9.52 to 10.76 μ for methyl esters chosen in the present study has the added advantage that the saturated components have only a small relative negative absorption. Since the $a_{10.38\ \mu}$ value for purely (i.e., 100%) saturated methyl esters (−0.004) is less than 1% of that for pure methyl elaidate (0.462), it is obvious that correction for absorption because of these is unnecessary for most hydrogenation oil samples, where the saturated components are about 20–30% like most Vanaspati samples. Therefore in calculations of the methyl elaidate content of methyl esters of hydrogenated samples no correction is made for saturated components. Absorptivity obtained for pure methyl oleate (0.02) with this baseline is about half that (0.04 to 0.05) reported by others (11,12). All these contribute towards more accurate estimation of the *trans* component. When the $a_{10.38\ \mu}$ of the hydrogenated samples (baseline 10.12 to 10.76 μ) is

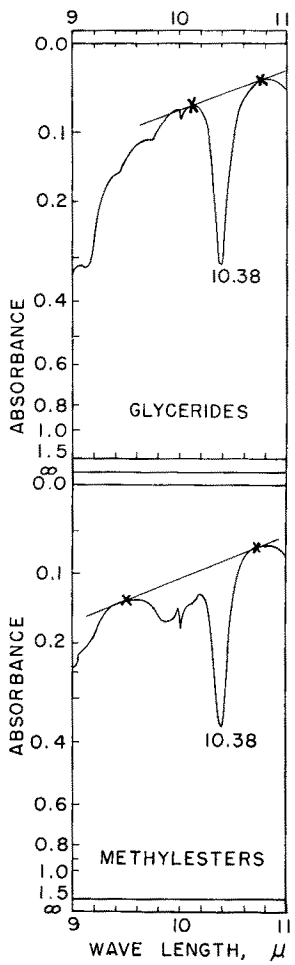

FIG. 1. Infrared spectrum (9–11 μ). Hydrogenated cottonseed oil (Table I, No. 5) and its methyl esters.

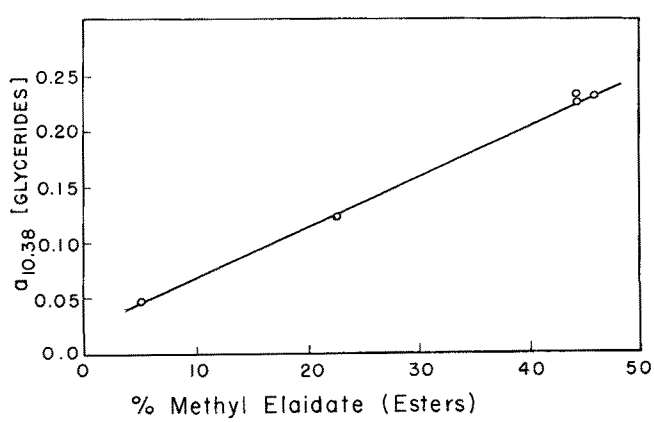

FIG. 2. $a_{10.38\ \mu}$ Glycerides *versus* % methyl elaidate of corresponding methyl esters.

plotted against the methyl elaidate content of the corresponding methyl esters, a straight line is obtained (Figure 2).

Thus all that is needed to obtain the *trans* isomer content of a hydrogenated oil or fat is to calculate its $a_{10.38}$ μ (*trans* peak) from its infrared spectrum and read from the graph the corresponding methyl elaidate percentage. It may be mentioned that only 15 minutes are required to complete this analysis after the sample is drawn at the plant. Thus, by examining samples by this method in the last hour of reaction, *trans* content of the products from batch to batch can be kept within a narrow range and more uniform quality ensured. Since the infrared absorptions vary from instrument to instrument, standard graphs (Figure 2) should be made for each instrument and should be checked periodically with methyl elaidate and methyl oleate. Points corresponding to hydrogenated samples Nos. 1 and 2, where linoleic acid is definitely present, are also on the same line as others though no correction for background absorption because of the methyl ester of this acid was made. An obvious conclusion is that, with this baseline, both methyl oleate and methyl linoleate have about the same absorption, but this needs confirmation with pure methyl linoleate. To check the validity of this method the infrared absorption of two samples of Vanaspati were taken, and the methyl elaidate content was read from the graph (Figure 2). These samples were converted into methyl esters, the infrared estimation was done on these, and the methyl elaidate content calculated as per the equation given earlier. The results are recorded in Table II. The agreement between the values read from the graph and those by actual determination is extremely good. Thus though these samples are blends of hydrogenated peanut, soybean, and cottonseed oils with the sesame oil added to fulfill statutory requirements, the method is valid.

TABLE II

trans Unsaturated Content of Vanaspati Samples

Sample			Methyl esters	
	$a_{10.38}$ μ	% Methyl elaidate from graph	$a_{10.38}$ μ	Methyl elaidate % calculated
Vanaspati No. 1	0.2229	44.2	0.2105	44.2
	0.2187	44.0	0.2083	43.7
Vanaspati No. 2	0.2153	42.5	0.2023	42.4
	0.2143	42.4	0.1995	41.8

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