Accurate Determination of *trans* Isomers in Shortenings and Edible Oils by Infrared Spectrophotometry

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ABSTRACT

A procedure is described for the accurate analysis of the *trans* content of fats and oils in the range of 0.5-36% *trans*. This procedure is shown to be more accurate than other current infrared spectrophotometry methods. This increased accuracy is obtained by using a 2-component calibration standard mixture and measuring the samples as methyl esters. Good agreement between this method and the measurement of *trans* content by a gas chromatography procedure is demonstrated.

INTRODUCTION

The interest in the nutritional effects of *trans* unsaturated fatty acids and recent technological advances in the measurement of positional and geometric *trans* isomers by gas chromatography (GC) have made it important that reliable data and accurate methods for the determination of *trans* content be available.

Isolated (nonconjugated) *trans* double bonds, the predominant *trans* configuration, exhibit an infrared (IR) absorption band at $\sim$ 967 cm^{-1}. Measurement of this absorption band under controlled conditions is the basis for a quantitative method (1) adopted by the American Oil Chemists' Society (AOCS) for the determination of the *trans* content in vegetable oils. This method, which uses the baseline technique, is particularly useful for the analysis of vegetable oils which may have undergone some degree of isomerization during the hydrogenation process. However, it suffers from several drawbacks. The major drawback is that when a triglyceride sample is analyzed as a triglyceride, and then converted to methyl esters and reanalyzed as methyl esters, the triglyceride result is higher. This high bias of the triglyceride analysis is more severe at low *trans* levels (< 15% *trans*). The AOCS method prescribes conversion of the triglyceride sample to methyl esters at low *trans* levels (< 15%) for accurate results. A second drawback is that samples analyzed as methyl esters produce *trans* levels which are 1.5-3% low (2) for *trans* values from 1 to 15%. A third drawback is that conjugated species absorb very close to the isolated *trans* bond (3) and can interfere with the isolated *trans* measurement because of an uncertainty as to the correct location of the baseline.

The high bias of the triglyceride analysis was explained by Kaufmann et al. (4) and verified by Firestone and DeLaLuz Villadelmar (5) as a triglyceride absorption. Non-*trans* containing triglycerides exhibit a broad absorption at 967 cm^{-1} and the magnitude of this absorption is dependent on the nature of the triglyceride (2). This non-*trans* triglyceride absorption is equivalent to 3-4% *trans* (2) in the sample as determined by spiking triglyceride samples with trielaidin. At the same time, methyl ester analyses produce *trans* levels which are 1.5-3% low (2) as determined by spiking methyl esters samples with methyl elaidate.

Ideally, analysis of a sample as a triglyceride and as a methyl ester should give identical *trans* values. Firestone and LaBouliere (2) addressed this situation by proposing arithmetic corrections for the positive triglyceride absorption and negative methyl ester absorptions. Another remedy proposed by Huang and Firestone (6) included a differential IR measurement wherein a non-*trans* containing component is placed in the reference beam of the spectrophotometer to correct for the positive triglyceride absorption or the negative methyl ester absorption. This procedure gave results which were 1-2% high for both triglycerides and methyl esters.

Allen (7) described a rapid method for the determination of isolated *trans* unsaturation based on the principle that non-*trans* containing triglycerides show a large IR absorption at 1163 cm^{-1} due to the carbon-oxygen stretch of the ester group and a weak absorption at 965 cm^{-1}. The ratio of the absorbance at these wavelengths (A_{1163} cm^{-1}/A_{965} cm^{-1}) was found to be proportional to the amount of *trans* unsaturation. Huang and Firestone (8) compared the rapid method to the tentative AOCS procedure with samples containing 0-20% isolated *trans* components. The rapid method was found to be less accurate.

Currently, no simple procedure exists for the accurate determination of isolated *trans* levels in fats and oils below 15% *trans* by IR spectrophotometry. We have devised a procedure based on the use of non-*trans* containing methyl esters combined with methyl elaidate for calibration of the method. The *trans* content of samples determined by the IR procedure described here were further compared to *trans* content determined by GC and were found to be in good agreement.

EXPERIMENTAL PROCEDURES

Equipment

IR measurements were made with a Beckman IR-12 spectrophotometer using 0.10 cm fixed thickness liquid sampling cells with NaCl windows.

Reagents

Methyl elaidate, methyl stearate and methyl linoleate were purchased in 99+% purity from Nu-Chek-Prep, Inc., Elysian, MN. The methyl ester standards were checked for purity by using the AOCS official method (9). ACS reagent grade carbon disulfide was used as solvent.

Sample Solutions

Methylation. We placed 10-15 g of sample in a 150-mL beaker, added 50 mL of sodium methoxide solution and heated on a hot plate until clear. We added 25 mL of saturated NaCl in 0.5% HCl, removed it from heat, added 50 mL of hexane and decanted the top layer through Whatman 40 filter paper, which contains sodium sulfate, into a 50-mL beaker. Hexane was then evaporated.

Analyte. We weighed 0.5 ± 0.0001 g methyl ester into a 25-mL volumetric flask, dissolved it in carbon disulfide and diluted to volume.

Standard Solutions

Methyl elaidate stock solution. We accurately weighed 0.5000 ± 0.0001 g of methyl elaidate (actual weight was

recorded). We transferred this to a 50-mL volumetric flask and brought to volume with carbon disulfide (with slight heating when necessary). This solution was equivalent to 0.01 g/mL of methyl elaidate.

Methyl linoleate stock solution. We accurately weighed 5.000 ± 0.0001 g of methyl linoleate (actual weight was recorded). We transferred this to a 200-mL volumetric flask and brought to volume with carbon disulfide (with slight heating when necessary). This solution is equivalent to 0.025 g/mL of methyl linoleate.

PROCEDURE

We filled 2 cells with CS_2 solvent and measured absorbance from 900 cm^{-1} to 1505 cm^{-1}, which should be a reasonably straight baseline. We transferred a portion of the sample solution or calibration solution into the sample cell and scanned from 900 cm^{-1} to 1050 cm^{-1}. We then rinsed sample cells with 5 or 6 washings of carbon disulfide between all measurements, and drew a baseline tangent to the peak minima adjacent to the analytical peak at the normal wave number of 967 cm^{-1} as demonstrated in Figure 1. We measured the absorbance of the analytical peak and the absorbance of the baseline tangent at the same wave number as the peak maximum, then subtracted the absorbance of the baseline tangent from the absorbance of the analytical peak to obtain the baseline corrected absorbance (A) at 967 cm^{-1}. This was repeated for each sample.

Because this method involved a baseline corrected absorbance measurement, drawing the correct baseline was important. We found that the best results are obtained when the baseline for the sample spectrum is drawn exactly as the baseline in the standard spectrum. This is easily accomplished by overlaying the 2 spectra to draw the baseline.

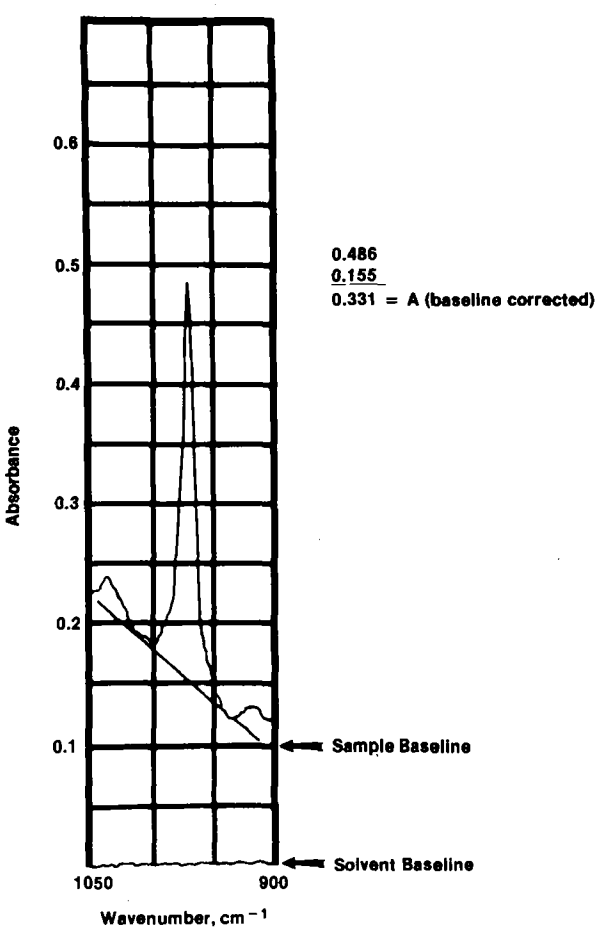

FIG. 1. Spectrum of *trans* unsaturation.

Preparation of Calibration Curve

Using the 2 stock solutions, known amounts of methyl linoleate and methyl elaidate were added into 25-mL volumetric flasks. The baseline corrected absorbance (A) at 967 cm^{-1} for each calibration standard was measured (Table I) and plotted against the g of elaidic acid equivalents.

TABLE I

Calibration of Standard Mixtures

Sample no.	Amounts measured (g)		Elaidic acid, equiv.[a]	Baseline corrected absorbance
	Me linoleate	Me elaidate		
1	0.500	0.000	0.000	0.000
2	0.490	0.010	0.0095	0.027
3	0.475	0.030	0.0286	0.069
4	0.460	0.040	0.0381	0.085
5	0.450	0.050	0.0477	0.108
6	0.425	0.080	0.0762	0.181
7	0.375	0.100	0.0953	0.219

[a]Elaidic acid equiv. = g Me elaidate X 0.953.
MW elaidic acid/MW Me elaidate = 282.45/296.45 = 0.953.

Calculation of Results

From the baseline corrected absorbance (A) for each sample, we determined the g of elaidic acid equivalents from the calibration curve. We calculated the *trans* content from Equation I:

$$\%\ trans\ \text{Fatty acid basis} = \text{g elaidic acid equiv.} \times 100/\text{sample wt, g} \quad [I]$$

This calculation reports the *trans* content as a wt percentage of elaidic acid/g of methyl ester sample.

RESULTS AND DISCUSSION

To demonstrate the effectiveness of this 2-component standard, the same samples were analyzed by 4 different methods—the proposed method described here, the AOAC method (10), and the AOCS method (1) and a GC procedure (D.C. Underwood and R. Houston, private communication). Underwood and Houston's GC procedure used a Hewlett-Packard 5880 instrument equipped with a flame ionization detection (280 C) and a 50M Silar 10C Quadrex glass capillary column. A 1-μL sample was injected (260 C) using programmed temperature conditions (150 C-190 C, 1.3 C/min) and helium carrier gas (0.5 mL/min). The results are summarized in Table II. A modified AOCS method was used for the work in that conversion of the triglyceride samples to their methyl esters was not performed for samples with less than 15% isolated *trans*. As the samples were not converted to their methyl esters, the recognized high bias of the adopted AOCS method is apparent in the results, and this bias is not obvious at low *trans* levels. The calculation factor suggested by Firestone and LaBouliere (2) and used in the AOAC method appears to overcome this high bias better at high *trans* levels than at low *trans* levels (< 5%). The calibration procedure prescribed in this proposed method corrects for the high bias of the AOCS method, eliminates the need for calculation factors in the AOAC method, and results in good agreement between the

TABLE II

Comparison of Methods—*trans* Isomers

Sample	AOCS Cd 14-61	AOAC 28.052 (1975)	Proposed method	Gas chromatography
Oils				
Winterized, partially hydrogenated soybean oil	12.4	13.7	11.2	11.0
Sunflower/soybean oil blend	1.7	2.6	NF	0.4
Corn oil	1.0	1.9	NF	0.3
Sunflower seed oil	1.6	3.1	1.3	1.1
Winterized, partially hydrogenated cottonseed/soybean oil blend	22.6	20.6	17.8	17.2
Shortenings				
All vegetable #1	20.3	18.3	14.9	14.4
All vegetable #2	21.4	19.4	15.3	15.3
Animal/vegetable	16.8	14.7	10.0	10.1
Margarines				
A	29.9	32.3	29.4	29.3
B	20.8	23.1	20.5	18.8

proposed IR method and a GC method. To demonstrate the accuracy of this method, the fatty acid methyl esters from 3 different vegetable oil samples were spiked with low levels of methyl elaidate to determine the recovery. These data are given in Table III. These spiking experiments show the applicability of this method for *trans* levels below 15% where existing methods have difficulty measuring the *trans* level accurately.

To demonstrate the comparability of this method with the analysis as triglycerides, selected samples were analyzed as triglycerides and then the samples were converted to their methyl esters and reanalyzed by this method. The results are given in Table IV. The triglyceride analysis is analogous to the methyl ester calibration procedure described here except that tristearin is substituted for methyl linoleate and trielaidin for methyl elaidate in the preparation of the standard solutions. The data in Table IV show the good agreement between the triglyceride and methyl ester analyses. As a note, the methyl ester values are con-

TABLE III

Results of Methyl Ester Samples Spiked with Methyl Elaidate

Sample	% *trans* Original + added	% *trans* Found
Winterized salad oil (C)	Unspiked	13.5
	15.5	15.8
	17.3	17.4
	22.0	21.9
Refined & bleached vegetable oil (F)	Unspiked	<0.1
	2.7	2.7
	5.3	5.4
	11.3	11.6
	15.3	15.5
Refined & bleached vegetable oil (G)	Unspiked	<0.1
	2.6	2.8
	5.5	5.6
	8.2	8.3

TABLE IV

Triglyceride and Methyl Ester Analysis of Identical Samples

Sample	% *trans* Isomer (elaidic acid equiv. measured as): Triglyceride[a]	Methyl ester
Oils		
Margarine oil	36.2	35.9
Winterized salad oil	13.8	13.6
Winterized, partially hydrogenated soybean oil	11.6	11.2
Sunflower/soybean oil blend	0.2	NF
Corn oil	NF	NF
Sunflower seed oil	2.2	2.0
Winterized, partially hydrogenated cottonseed/soybean oil blend	18.2	17.8
Shortenings		
All vegetable #1	15.8	14.9
All vegetable #2	16.9	15.3
Animal/vegetable	11.0	10.0
Margarines		
A	30.3	29.4
B	21.5	20.5

[a]Elaidic acid equiv. = g trielaidin X 0.9570.

sidered more accurate because no interferences are known to be present at the *trans* absorption. It is for this reason that a 2-component standard mixture using methyl esters was chosen as the preferred method of measurement by IR spectrophotometry. Further, the data in Table IV on intact triglycerides are the same samples analyzed in Table II and the use of prescribed calibration standards makes these *trans* values more accurate than in the AOCS procedure.

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Crystal Structure Modifications of Tristearin by Food Emulsifiers

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ABSTRACT

The effect of several emulsifiers as crystal structure modifiers of tristearin has been investigated. The less thermodynamically stable modification, named α, is preserved when 1-10% of sorbitan monostearate was added before allowing the molten tristearin to cool and crystallize. Several other emulsifiers have been tested and it has been found that the combination of bulkiness of the hydrophilic groups with the right lengths of the hydrophobic chains of a given emulsifier is necessary to preserve the α-modification. Liquid emulsifiers and those having a pronounced hydrophilic character are not efficient as modifiers. The emulsifier has been shown to be incorporated into the tristearin during crystallization from solvent without an immediate effect, but it affects subsequent behavior upon melting and resolidification.

INTRODUCTION

The addition of surface-active agents to chocolate to retard fat bloom has been done for many years. Emulsifiers such as lecithin and monoglycerides have been considered as both viscosity controllers and anti-bloom agents (1-3).

The development of tempering methods allowed an evaluation of the efficiency of a fat-bloom retardant and, as a result, a procedure has been proposed to test the effect of adding a single compound on the chocolate bloom. Sorbitan monostearate (Span 60) was found to be the only effective modifier in preventing blooming in an early study (4). Emulsifiers such as ethoxylated sorbitan esters (Tween 60, Tween 61, Tween 20) were reported to have fair protection of the fat. Several blends of emulsifiers such as Span 60-Tween 60 (40:60) were reported as excellent antibloomers. It has been concluded that the extent of protection provided by the emulsifiers is not fully known and should be further investigated (4).

The polymorphism of triglycerides composed of saturated fatty acids with an even number of carbons has been studied extensively by a number of investigators (5,6). Bailey was the first to compile comprehensive polymorphic data on fats (7) and Lovegren and Gray added significant data on those systems using differential scanning calorimeter to determine the changes that occur (8). Hoerr and Paulicka (9) have studied the various polymorphs using X-ray diffraction and explained the crystal structures of these compounds in terms of molecular orientation in the crystal lattices.

To the best of our knowledge, only a little information has, so far, been published concerning the role of the added surfactants on the crystal structure modification of the saturated fat. Recently, Kawamura (10) has shown the effect of sorbitan monostearate and sorbitan tristearate on the thermal behavior of palm oil using DSC techniques and has demonstrated typical phase transformations of the palm oil in the presence of these emulsifiers. Some other scientific work is connected with the study of the bloom phenomena in chocolate. On the other hand, increasing use is being made of these modifiers in the food industry where their effect is very much related to the modification of the crystal structure of the fat.

In a symposium paper presented by Krog, an attempt was made to find a correlation between the fat and the emulsifier crystal structures. However, no conclusions have been drawn (11).

In the course of our previous studies, we examined the effect of various food emulsifiers on the crystal structure and habit of stearic acid, and have shown the preservation of preferable modification of stearic acid in the presence of modifiers (12,13).

The purpose of this paper, therefore, is to study more carefully the effect of several food emulsifiers on the crystal structure modification of tristearin, to examine X-ray diffractions and thermal behavior (DSC) and to correlate between molecular structure as well as physical properties of emulsifiers and the fat modification formed when crystallization from melt and solvents was induced.

EXPERIMENTAL

Materials

Tristearin was purchased from Sigma and was of 99% purity