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Direct Determination of *Trans* Unsaturation in Triglycerides by Infrared Spectrophotometry

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Abstract

A differential infrared spectrophotometric method is described for the determination of *trans* unsaturation in fats. The method utilizes absorption at 965 cm^{-1}, due to the C–H out-of-plane deformation vibrations of *trans* unsaturated compounds. The method is rapid, accurate, and directly applicable to the determination of *trans* unsaturation in triglycerides. It is applicable to samples which contain low concentrations of *trans* acids (down to 2%) and also to samples with fatty acids of mixed chain length.

THE I.R. ABSORPTION BAND at 965 cm^{-1} of *trans* unsaturated compounds has been widely used in recent years for the quantitative determination of *trans* fatty acids and their derivatives (1,2,3). O'Connor (4) reported anomalous absorptivities in the case of glycerides containing very short chains and mixtures of short and long chain fatty acids. He considered that determination of *trans* unsaturated acids in triglycerides could at best be only partly quantitative until more spectra of pure triglycerides became available. Kaufmann (5) investigated, in addition, glycerides composed of medium and long chain saturated acids and reported "apparent *trans* contents" for these compounds. Similar anomalous results were reported by the Spectroscopy Committee of the A.O.C.S. (6), but at that time they still recommended that *trans* determinations be made on the triglycerides rather than risk isomerism by converting to methyl esters.

As a result of collaborative studies (7,8) this committee developed a method for the determination of *trans* unsaturation using a "baseline" type of background correction.

Firestone and De La Luz Villadolman (9) discuss the problems in the analysis of triglycerides and how they can be solved by the use of this A.O.C.S. procedure.

The problem of obtaining a correct analytical result is at least partly caused by the strong background absorption of the triglycerides. McDonald (10) and Cleverley (11) published differential techniques similar to the one described in this paper for the estimation of *trans* unsaturation in fatty acids and esters prepared from naturally occurring lipids. However, the analysis of triglycerides was not discussed.

In this study, the unwanted absorption was cancelled out by a differential technique, using in the reference beam a solution of a fully saturated triglyceride. The reference compounds should ideally be the test sample fully hydrogenated, but in practice negligible error is introduced provided that the chain lengths of the component fatty acids of the reference triglyceride are reasonably similar to those of the sample.

The method is particularly advantageous when working with small routine type instruments such as Perkin-Elmer Infracord Model 137 Spectrophotometer with fixed slit width and fixed scan speed program. With these instruments the method recommended by the Spectroscopy Committee is inaccurate because of the steeply sloping baseline.

A further advantage of the proposed method is that it is possible to analyze samples with *trans* acids content as low as 2%, whereas other published methods require that the sample be converted to the methyl ester if the *trans* acids content is below 15%. Samples containing fatty acids of mixed chain length can also be analyzed if part of the sample is fully hydrogenated and used in the reference beam of the spectrophotometer.

In addition to the determination of spectral data, it is necessary to know the iodine value of the sample to allow calculation of the trans acids content. This is not considered to be a limitation of the method, since this analytical constant is almost invariably required as part of any fat analysis program.

Experimental

Materials and Apparatus

Fully hydrogenated beef tallow stearin—saponification value 203; I.V. 0.25. This was prepared from beef tallow stearin by exhaustive hydrogenation using an oil-free nickel catalyst supported by kieselguhr.

Partially hydrogenated whale oil—saponification value 195; I.V. 57.5.

Peanut oil—saponification value 193; I.V. 93.0.

Secondary standard triglycerides—*trans* content 51.9% as trielaidate; I.V. 67.3. Supplied by R. T. O'Connor of the A.O.C.S. Spectroscopy Committee.

Carbon disulphide—Univar.

All spectra were plotted on a Perkin-Elmer Model 137 Infracord double beam spectrophotometer. A

1 mm. fixed path-length cell was used in the sample beam and a variable path-length cell in the reference beam.

Method. Prepare 5% or 2% solutions of the triglycerides in carbon disulphide, the concentration selected depending on the expected *trans* acids content of the sample. Plot the baseline from 1050 to 900 cm^{-1} with the appropriate concentration of fully saturated reference triglyceride in both beams of the spectrophotometer. Adjust the variable path-length cell to compensate the fixed cell. Replace the solution in the fixed path-length cell with the test triglyceride solution, and plot the spectrum over the same range. The absorbance at 965 cm^{-1} determined from the above spectra should lie between 0.05 and 0.4. If it is outside this range the determination should be repeated with an appropriate concentration of sample and reference triglyceride.

Discussion

The calculation of the *trans* acids content from the differential spectrum is based on the following considerations:

$$A = bc_t a_t + bc_c a_c - bc_u a_s M_u/M_s \quad . \quad . \quad . \quad . \quad (1)$$

Where

M_u = mol wt of triolein

M_s = mean mol wt of saturated triglycerides

c_t, c_c, c_u = g/l concentrations of trielaidate, trioleate and total unsaturated glycerides

a_t, a_c, a_s = absorptivity (in g/l concentrations) of trielaidate, trioleate, and saturated triglyceride

$$c_u = c_t + c_c = c \cdot IV/IV_u \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Where

IV = iodine value of sample

IV_u = iodine value of trioleate

From equations (1) and (2)

$$c_t = A/b(a_t - a_c) - c \cdot IV(a_c - a_s M_u M_s)/IV_u(a_t - a_c) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and

$$\% \text{ trielaidate} = 100\, c_t/c \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Substituting the following numerical data:

$M_u = 885$

$M_s = 825$

$b = 0.1$

$IV_u = 86$

$a_t^* = 0.475$

$a_c^* = 0.084$

$a_s^* = 0.078$

* Taken from reference 2.

we get:

a) % trielaidate = $51.14A - 1.82 \cdot 10^{-2} \cdot IV^*$ for 5% sample solutions

b) % trielaidate = $127.85A - 1.82 \cdot 10^{-2} \cdot IV^*$ for 2% sample solutions

* With 5% or 2% solutions, respectively, of fully hydrogenated beef tallow stearin in the reference beam.

Results

The absorptivities used in the calculations above are those established by Shreve *et al.* (2). In order to confirm their applicability, a secondary standard triglyceride, from R. T. O'Connor, was used. This had an I.V. of 67.3 and a stated *trans* content of 51.9% trielaidate. The absorbance of its 2% solution in CS_2 (with a 2% solution of fully hydrogenated beef tallow stearin in the reference beam) was 0.416. The *trans* content calculated from equation (1) was 52.0%.

It can be seen that the use of the published absorptivity was justified in the case considered and with the particular spectrophotometer employed. However, it must be emphasized that different instruments may differ with respect to slit width, scattered light, electrical and mechanical inertia, etc., and therefore require the substitution of different absorptivities. It is suggested that other workers intending to use the method should check this point and make numerical adjustments to the equations if necessary.

The range of application and reproducibility of the method was tested by using various mixtures of a partially hardened whale oil with fully hydrogenated triglyceride or peanut oil. Results are shown in Table I.

The *trans* content of the whale oil was 50.5 ±1.7% (± 3.4% S.D.). Table I shows that *trans* contents ranging from 2 to 50% can be determined with a standard error of 3.4% and the results are not affected by the presence of a large excess of *cis* unsaturation.

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TABLE I

Sample No.	Composition of mixture: Whale oil %	Peanut oil %	Stearin [a] %	Concentrate in CS_2 %	I.V. of sample	A	% *Trans* (trielaidate) found	% Calculated	% Deviation
1	4	....	96	5	2.3	0.0390	1.95	2.02	−3.5
2	4	96	....	5	91.3	0.0747	2.12	2.02	+5.0
3	8	....	92	5	4.6	0.0804	4.02	4.04	−0.5
4	8	92	....	5	89.9	0.1176	4.35	4.04	+7.7
5	10	....	90	2	5.7	0.0419	5.25	5.05	+4.0
6	12	88	....	5	88.5	0.1510	6.09	6.06	+0.5
7	16	....	84	5	9.2	0.1562	7.81	8.08	−3.3
8	16	84	....	5	87.1	0.1910	8.16	8.08	+1.0
9	20	....	80	2	11.5	0.0802	10.04	10.10	+0.9
10	20	80	....	5	85.7	0.2267	10.01	10.10	−0.1
11	24	....	76	5	13.7	0.2312	11.56	12.12	−4.6
12	24	76	....	5	84.2	0.2702	12.26	12.12	+1.2
13	28	72	....	5	82.8	0.3135	14.50	14.14	+2.5
14	32	....	68	5	18.3	0.3220	16.13	16.16	−0.2
15	32	68	....	5	81.4	0.3341	15.58	16.16	−3.6
16	36	64	....	5	80.0	0.3671	17.29	18.18	−4.9
17	40	....	60	2	22.9	0.1617	20.25	20.20	+0.3
18	60	....	40	2	34.4	0.2492	31.22	32.32	−3.4
19	80	....	20	2	46.1	0.3175	39.74	40.40	−1.6
20	100	....	....	2	57.3	0.3987	49.91	50.50	−1.1

[a] Stearin = fully hydrogenated beef tallow stearin.